UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF MINERAL CONTENT OF LITTLE MILLET AND BARNYARD MILLET

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APPROVAL

This project entitled “Determination of mineral content between little millet and barnyard millet” was prepared by Harriet Anabelle Anak Manggon (188294) and submitted to the Faculty of Medicine and Health Sciences as a partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

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DECLARATION

I am hereby declared that this thesis report is based on my original work except for quotations and citations which have been acknowledged to the corresponded authors.

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ABSTRACT

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Harriet Anabelle Anak Manggon

Millet is a type of whole grain belonging to the family Poaceae. Millet can be categorised into two types; major and minor millets. In this study, two types of minor millets, namely barnyard and little millet are analysed for its mineral content. Barnyard and little millet are notable for its' high source of potassium and phosphorus. Being a whole grain and having high potassium content, millet has the potential to be a healthier alternative for combatting diseases such as diabetes and obesity in Malaysia. This study aimed to determine the mineral contents of barnyard millet and little millet. The millets were chosen through purposive sampling. Eight minerals were analysed which are copper, zinc, sodium, potassium, magnesium, iron, calcium and phosphorus. They were analysed using atomic absorption spectrometry (AAS). The results showed that both millets are abundant in potassium, phosphorus, magnesium and sodium, while lesser minerals found were copper, calcium, iron and zinc. There was a higher content of copper, phosphorus and potassium in little millet whereas for barnyard millet there was a higher content of sodium, magnesium, zinc, calcium and iron. The differences in mineral content may be attributed by cultivars, soil pH and mineral, and type of reagents used. The high potassium content in these millets proves that millet can be a potential healthy alternative for Malaysian consumption as potassium is inversely related to obesity, blood pressure and metabolic syndrome. Both millets are found to be nutritionally superior to white rice in all minerals except calcium. With further research, millet can be proved to be a potential healthy alternative in place of white rice for Malaysian consumption because of its nutritional advantage.

ABSTRAK

Penentuan Kandungan Mineral antara Milet *Barnyard* dan Milet *Little*

Harriet Anabelle Anak Manggon

Milet adalah sejenis bijirin penuh yang tergolong dalam keluarga Poaceae. Milet boleh dikategorikan kepada dua jenis; millet utama dan minor. Dalam kajian ini dua jenis millet minor iaitu milet *barnyard* dan milet *little* dianalisis untuk kandungan mineralnya. Milet *barnyard* dan milet *little* terkenal kerana sumber potasium dan fosforus yang tinggi. Sebagai bijirin penuh dan mengandungi sumber potasium yang tinggi, bijirin ini berpotensi menjadi alternatif yang lebih sihat untuk memerangi penyakit seperti diabetes dan obesiti di Malaysia. Kajian ini bertujuan untuk menganalisis kandungan mineral milet *barnyard* dan milet *little*. Milet dipilih melalui persampelan bertujuan. Lapan mineral dianalisis iaitu kuprum, zink, natrium, kalium, magnesium, besi, kalsium dan fosforus. Mineral tersebut dianalisis menggunakan spektrometri penyerapan atom. Hasil kajian menunjukkan bahawa kedua-dua milet mengandungi kalium, fosforus, magnesium dan natrium, dalam tahap yang tinggi sementara mineral dalam kuantiti lebih sedikit yang ditemui adalah kuprum, kalsium, besi dan zink. Kandungan kuprum, fosforus dan kalium lebih tinggi dalam milet *little* berbanding milet *barnyard* yang tinggi kandungan sodium, magnesium, zink, kalsium dan zat besi. Perbezaan kandungan mineral mungkin disebabkan oleh kultivar, pH tanah dan mineral, dan jenis reagen yang digunakan. Kandungan kalium yang tinggi dalam milet ini membuktikan bahawa milet boleh menjadi alternatif yang berpotensi diamalkan rakyat Malaysia kerana kalium dapat mengurangkan obesiti, tekanan darah dan sindrom metabolik. Kedua-dua milet didapati mempunyai kandungan nutrien yang lebih tinggi daripada beras putih dalam semua mineral kecuali kalsium. Dengan penyelidikan lebih lanjut, milet dapat dibuktikan sebagai alternatif sihat yang berpotensi sebagai pengganti beras putih untuk penggunaan rakyat Malaysia kerana kelebihan kandungan nutriennya.

CHAPTER 1

1.0 Introduction

1.1 Background

Millets comprise of a group of small-seeded annual grasses in the family Poaceae. Millets were thought to be cultivated in Asia more than 4000 years ago, and they were the major crop in Europe during the Middle Ages (Encyclopædia Britannica, 2019). The six major types of millet are pearl millet (*Pennisetum glaucum),* finger millet (*Eleusine coracana)*, barnyard millet (*Echinocloa frumentaceae),* proso (*Panicum miliaceum),* teff millet (*Eragrostis tef)* and foxtail millet (*Setaria italica)*. Out of these, pearl millet is the most widely grown accounting about 40% of the world's production.

Millet stalks range in height from 30 to 130 cm (1 to 4 feet), with the exception of pearl millet (*Pennisetum glaucum*), which has stalks 1.5 to 3 metres (5 to 10 feet) tall and about 2.5 cm (1 inch) thick. Millet stalks grow to an open, branched, compact or unilateral panicle, accompanied by wide, long and pointed light green leaves. The leaves contain some spikes among which the seeds or millet grains can be found. The grain size is generally about 3 millimetres, with an average of 5g for a handful of grain (thousands). The grain is round, yellowish in colour, and edible.

Millets are generally grown for local food consumption in most parts of the world, accounting for 80% of millet grown for this purpose, with over 95% of the production in Asia and Africa. Other uses of millet include animal feed (7%), beer brewing, seed production and waste (Food and Agricultural Organisation of the United Nations, 1996).

They are a major crop in less-developed countries especially in Asia and Africa. In Asia, millet are mainly grown in India and China, with Pakistan, Myanmar and Nepal producing small quantities. India is the largest producer of millet, producing 11 million tons per year, accounting of about 40% of the world's production. Pearl and finger millet are the main crop grown there. Whereas China produces 3.7 million tons of millet annually, focussing on foxtail millet production. In Africa, millet production are distributed in several countries including Nigeria, Niger, Burkina Faso, Mali, Senegal and Sudan. They constitute as a staple food for a large segment of the population in these countries. Millet is prepared as part of traditional dishes, flat bread, porridge and other forms of meals that varies between cultures and countries. Meanwhile, in developed countries, limited quantity of millets are produced for the purpose of marketing a high-value specialty of bird seed (Food and Agricultural Organisation of the United Nations, 1996).

Millets thrive better than other crops under extreme conditions such as semiarid tropics, high temperatures and infertile soils or acidic soils with low water-holding capacity (Food and Agriculture Organization, 1996). This is because most millet has strong, deep rooting systems and short life cycles. It can survive and produce small quantities of grain with as little water quantity of mean annual precipitation of 300 mm, compared to 400 mm for sorghum and 500-600 mm for maize. Additionally, millets are thermophilic where it can grow at relatively higher temperatures (Saxena et al., 2018). Currently there is no study regarding the growth of millet in Malaysia.

In regards to nutrient content, millet is superior compared to other common cereal grains. Millet is considered as functional foods due to high content of health-promoting phytochemicals and antioxidants (Kumar, Tomer and Kaur, 2018). This includes minor millets such as little millet and barnyard millet. According to Sarita and Singh (2016), these millets are categorised as ‘minor’ due to them being less known and cultivated compared to ‘major’ millets. Major millets include pearl millet, foxtail millet, proso millet and finger millet whereas minor millets are barnyard millet, little millet, kodo millet, guinea millet, browntop millet, teff, fonio, sorghum and Job’s tear. Although they are not as heavily researched as major millets, notable findings showed that little millet and barnyard millet are high in nutrients (Saleh, Zhang, Chen and Shen, 2013).

Therefore, the objective of this study is to investigate the mineral content of little millet and barnyard millet, which includes iron, zinc, magnesium, copper, calcium, potassium, sodium, chromium, and phosphorus. Little millet and barnyard millet are selected because there is little data found on these millet. Currently, there is no data on millet consumption in Malaysia. The contribution of these mineral properties to our overall health should not be underestimated and may improve the nutritional status among Malaysians.

1.2 Problem Statement

Since the 1990s, Malaysia has been facing the issue of mineral deficiency, specifically calcium and iron. Recently, Malaysian Dietary Supplement Association (MADSA) released a report that stated Malaysians do not consume enough legumes, fruit, milk and vegetables, which are rich in nutrients such as calcium and iron but have a fondness for sugary drinks. (The Malaysian Dietary Supplements Industry Status and Outlook Report 2019-2020). Overconsumption of sugar contributes to various non-communicable diseases, such as diabetes, obesity, and cancer (Lustig et al., 2012). An absence or chronic deficiency of calcium can lead to bone mass loss and osteoporosis. Similarly, iron deficiency affects all ages, from poor growth and brain development in foetus and children, to anaemia in adolescents and adults. World Health Organisation (WHO) reported that the prevalence of anaemia among women of reproductive age (% of women ages 15-49) in Malaysia was 24.90 as of 2016. Meanwhile, according to Annual Report Ministry of Health Malaysia 2017, it was found that 11.6% of Form 4 students were suspected detected as having Iron Deficiency Anaemia (IDA), where female students (18.5%) had a markedly higher incidence compared to male students (2.5%). The prevalence of iron deficiency is decreasing in Malaysia, however due to its severe consequences and comorbidities, it has become a health issue to be addressed urgently.

Thus, millet can be a possible solution to combat these specific mineral deficiencies. Additionally, little millet and barnyard millet have not been studied for their potential health benefits (Saleh, Zhang, Cheng & Shen, 2013) and its potential to provide food and nutritional security (Sood, Khulbe, Gupta, Agrawal, Uphadhyaya & Bhatt, 2015).

Currently, there is not many studies or researches that has been conducted on millets in Malaysia. Unlike rice, millet is not a household name and generally unknown to the public especially in Malaysia. Millet is mainly known to the Indian community, for it is their traditional food, and millet is also widely consumed in India. Its consumption and health benefits are also mostly known to those who frequent or work in organic shops. Thus, the variety of millet are generally limited in Malaysia. Most commonly found millet in shops for consumption purposes comes in the form of hulled millet, organic millet, and glutinous millet.

In Nutrition Composition of Malaysian Foods (1997), there is a limited available database on millet. Consequently, the general public are unaware of the nutrient contained within millet, which may potentially benefit them or harm their health. Furthermore, medical experts especially in the nutrition field would have difficulties in advising their clients or clients to select healthier food choices without the knowledge of this data. Filling the gap in Malaysian nutrient database is essential to inform the public of healthier food choices available in the market.

1.3 Significance of the Study

In Malaysian household, the staple food is white rice. White rice is a highly processed grain where it is stripped of its hull, its hard protective coating; bran, the outer layer; and nutrient-rich core, germ (Bhosale & Vijayalakshmi, 2015). Due to its processing, white rice is nutritionally lower in value because it lacks vitamins and minerals.

However, as time changes, there is a growing awareness and consumption of healthier carbohydrate alternatives such as brown rice and even other grains including quinoa and chia seed. This is due to the increasing awareness of non-communicable diseases (NCD) in Malaysia. NCD comprised of chronic diseases such as cardiovascular diseases, cancer, diabetes, and obesity. According to the 2019 National Health and Morbidity Survey (NHMS), about 9.9% of Malaysians (3.4 million people) have at least two of three NCDs, i.e. diabetes, high blood pressure or high cholesterol levels.

Therefore, this study aims to introduce little millet and barnyard millet as another alternative in healthier food options. Compared to white rice, these millet has a much higher content of minerals such as potassium, calcium, iron, magnesium and others that can address the need of mineral deficiency in Malaysia (Shobana, Krishnaswamy, Shuda, Malleshi, Anjana, Palaniappan and Mohan, 2013). Furthermore, millet does not contain gluten, which makes it safe for people suffering from gluten allergy or celiac disease.

Through this study, the mineral content between little millet and barnyard millet will be able to be identified to help the public make healthier food choices. With millet, the options and choices for healthier alternatives to white rice becomes wider. In addition, millet is proven to be a sustainable crop due to its resilient nature and versatility to survive under extreme conditions.

Additionally, by identifying the mineral content, this would generate data on nutrient composition of millet. The result generated from the study can fill up the gap of previous database and make it accessible for future studies on millet.

1.4 Objectives

1.4.1 General Objective

To determine the mineral content of little millet and barnyard millet.

1.4.2 Specific Objectives

1. To determine the level of iron, zinc, magnesium, copper, calcium, potassium, sodium, and phosphorus content of little millet and barnyard millet using flame atomic absorption spectrometer.

2. To compare the level iron, zinc, magnesium, copper, calcium, potassium, sodium, and phosphorus content between little millet and barnyard millet flame atomic absorption spectrometer.

1.5 Null Hypothesis

There is no significant difference in the mineral properties between little millet and barnyard millet.

CHAPTER 2

2.0 Literature Review

2.1 Whole grains Consumption and Health Benefits

Whole grains are defined by the American Association of Cereal Chemists International and the FDA as consisting of the "intact, ground, cracked or flaked fruit of the grain whose principal components, the starchy endosperm, germ and bran, are present in the same relative proportions as they exist in the intact grain (Ferruzzi et al., 2014). Whole grain consist of three parts: the bran, endosperm and germ. Bran is the fiber outer layer that contains B vitamins, phytochemicals, antioxidants, zinc, iron, magnesium, and copper. Germ is the nutrient-rich core that is high in phytochemicals, antioxidants, healthy fats, vitamin B and E. It is also the embryo of the seed which has the potential to sprout into a new plant. Meanwhile, endosperm is the largest part of all three components, and it supplies nutrients for the growth of the germ. It contains starchy carbohydrates, proteins and small amounts of vitamins and minerals.

In the processing of grains, the bran and germ are removed. Thus the end product is called refined grains. The purpose of processing the grains is to decrease cost production and increase profit. Without the bran, the grain is easier to chew, making it more palatable for consumers. Furthermore, removal of germ increases its shelf life due to germ containing high in fat which oxidises rapidly. Additionally, refining grains produces fluffy flour, which contributes to airy features of bread and pastries. However, compared to whole grains, refined grains are significantly lower in nutritional value. According to Harvard, refining grains removes more than half of the wheat’s B vitamins, 90 percent of the vitamin E, and virtually all of the fiber (“Whole Grains”, Harvard, n.d.). Occasionally, food industries add the nutrients back into the grain by fortification, but not all can be replaced such as phytochemicals and not all are replaced in their original quantity.

There have been many prospective studies linking high consumption of whole grains to a reduced risk of cardiovascular diseases, coronary heart disease, stroke and type 2 diabetes. Additionally, a cohort study in Scandinavia involving more than 120000 adults spanning from 1992 to 1998 including follow ups until 2009, shows that consumption of whole grains decreases risk for all causes of mortality. In the same cohort, it was also found that it is associated with a lower incidence of colorectal cancer.

Due to its health benefits, there has been a prominent emphasis on whole grain incorporation in diet nowadays. However, there is no standard or specific across countries for the recommendation of whole grain serving. Generally, it has been advised to make half of the total grains and cereals serving per day as whole grains. In America, 2015 Dietary Guidelines for Americans recommend 3 to 5 servings or more of whole grains daily for individuals age 9 and above. Another variation of whole grain diet is Dietary Approaches to Stop Hypertension diet (DASH), where it recommends 6-8 serving of grains, focusing on whole grains. This diet is designed primarily to help prevent or treat hypertension, but it also offers health benefits to reduce the risk of osteoporosis, cancer, heart disease, stroke and diabetes. Seal, Nugent, Tee and Thielecke (2016) conducted a survey of current whole-grain dietary recommendations, reviewing websites and personal communication with national governmental and professional health agencies/organisations using a network of nutritionists available. The findings of the survey revealed that there is no consistent health messages and dietary recommendations although its health benefits were confirmed and touted. Inconsistencies were found where in some countries, non-government organisations have different recommendations than their government. Only Denmark and USA governments implemented specific recommendations for whole grain intake. It was suggested that this gap need to be addressed, where it is necessary. This is especially encouraged in countries without whole-grain intake recommendations to incorporate them into dietary guidelines (Seal et al., 2016).

Meanwhile in Malaysia, MyBreakfast study, a national cross-sectional study was conducted in 2013 to assess whole grain intakes and dietary source in Malaysian children and adolescents. Due to Malaysian dietary guidelines do not have a specific recommendation for whole grain intake but advise that "half of grain servings should come from whole grain", the researchers set the recommendation for whole grain intake at least 2 servings per day and referring to the US quantitative recommendation of 48 g/day. Findings revealed that only 25% of children and 19% of adolescents were whole grain consumers and less than 3% of the children and adolescents reached the recommendation of 48g/day.

2.2 Millet

2.2.1 Little millet

Little millet *(Panicum sumatrense)* is a cereal native to India. It is also widely cultivated across Nepal, Pakistan, Sri Lanka, eastern Indonesia and western Myanmar (Food and Agricultural Organisation, 1996). In India, it plays an important role as part of tribal agriculture, particularly in Eastern Ghats of India. Tribal agriculture can be defined as traditional agriculture that encompasses local knowledge, beliefs, technology adaptation and other customary practices (Behera, 2010). According to Britannica, Eastern Ghats are geographical areas consisting of discontinuous low ranges found parallel to the coast of the Bay of Bengal. Villages in Eastern Ghats have a local practice of tribal agriculture, such as the tribe of Kandhas (Behera, 2010).

There are two races of little millet; *nana* and *robusta. Nana* can grow from 60 to 170 cm in height whereas *robusta* can grow from 120 to 190 cm tall. Little millet seed looks similar to common millet seed, but little millet is smaller in the size of their seeds (about 1.8 to 1.9 mm long; round and smooth) and panicles. It is an annual plant, with a growth duration of 80 to 100 days. This crop can withstand extreme conditions at both ends – may it be drought or water logging. Additionally, it can be planted up to 2100 m above sea level (Food and Agriculture Organization, 2001).

Figure 2.1 Little millet

(Source: Rao et al. 2017)

Little millet is one of the minor millets because it has not undergone vast research in terms of agriculture and nutrition. Other minor millets include foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum scrobiculatum*), and barnyard millet (*Echinochloa colona*). According to Food and Agriculture Organisation (2001), little millet is a crop that is unrecognised in its value and potential to provide nutritional security. Compared to other millets such as pearl and finger millet, there are few kinds of research on the potential of little millet. However, notable studies on little millet do highlight its advantages such as reported by Manimozhi Selvi, Nirmalakumari and Senthil (2015). In this study, little millet and 110 of its genotype were studied to determine sources and improve zinc, iron and calcium contents in little millet which would be a potential approach to combat widespread malnutrition. From the study, it was found that several genotypes contain a higher content of zinc, iron and calcium compared to other genotypes (%) and other major crops. Similar to other studies, it is suggested that the superior little millet genotypes undergo hybridisation with agronomically superior accessions / breeding lines to combine grain nutrients (zinc, iron and calcium) and grain yield (N. Patel et al., 2018).

2.2.2 Barnyard millet

Barnyard millet consists of two major cultivated types, which are *E. frumentacea* (Indian barnyard millet) and *E. esculenta* (Japanese barnyard millet). Both falls under the genus *Echinochloa*, which in total has 35 species. Besides that, *E. crus-galli* (Lijiang millet) and *E. oryzicola* (Mosou barnyard millet) are grown in China (Yabuno 1966, Yuichiro et al. 1999, Yamaguchi et al. 2005).

Both Japanese and Indian barnyard millet are mainly cultivated for human food consumption purposes as well as fodder for animals. Barnyard millet is grown in areas unsuitable for rice paddy, thus it has become a replacement as a staple food for the tribal communities that plants this millet, mainly tribal communities from India, Japan and China. Like most millet, barnyard millet is also a versatile crop where it can thrive in drought and waterlogged condition. It can be grown in marginally fertile soil such as partially waterlogged soils and sandy loam (a soil with roughly equal proportions of sand, silt, and clay) (Singh, 1983).

Indian barnyard millet is called by other names such as sawa millet, or billion dollar grass. The origin of Indian barnyard millet can be traced back to its wild ancestor *Echinochloa colona,* commonly known as jungle rice, deccan grass, or Awnless barnyard grass. However, its exact date of domestication is unknown. Indian barnyard millet has always been cultivated along the regions of India, Central African Republic, Tanzania and Malawi (Doggett 1989). Meanwhile, Japanese barnyard millet is annually cultivated in the countries of Japan, Korea, China, Russia and Germany. Its wild ancestor is E. crus-galli or barnyard grass, and it was believed to be first cultivated in Japan dating back to about 4000 years ago (Doggett, 1989). Watanabe (1970) reported that it was grown in Japan as early as Yayoi period, dating back some 4–5 millennia. However, another finding suggested that the domestication of this millet dated back to the Jomon period of Japan in 2000 B.C. (Nesbitt 2005).

Barnyard millet is an annual grass and its plant can grow up to 220 cm. Among all types of millet, barnyard millet has the fastest growth duration with 45-60 days, taking into consideration the accession and environment, where it may take a longer duration when grown under northern hill ecosystem. Hulse et al. (1980) reported that *Echinocloa* millets grow well in different seasons but at high elevations may require 3–4 months to mature.

Figure 2.2 Japanese barnyard millet
(Source: FAO, 2001)

2.3 Mineral Properties of Little Millet and Barnyard Millet

2.3.1 Magnesium

Magnesium is an essential nutrient – meaning it needs to be obtained from external sources because the body is unable to produce it or does not produce a sufficient amount. According to Recommended Nutrient Intake (2017) for Malaysia, an adult male requires 400 mg per day whereas an adult female requires 310 mg per day. Magnesium is naturally present in many foods such as spinach, kale, avocado, legumes, salmon and whole grains. Although there is no whole food that meets 100% of RNI for magnesium, a cup of spinach provide 39% (157 mg) of the RNI whereas a half-cup of pumpkin seeds can provide up to 37% (150 mg) of the RNI. As for whole grains cereals, a cup of quinoa and whole wheat flour can provide up to 26% (118 mg) and 40% (160 mg) of magnesium, respectively (United States Department of Agriculture, 2019). Additionally, magnesium is available in fortified food and comes in the form of supplement.

Magnesium is needed for more than 300 biochemical reactions in the body. According to the National Institutes of Health, this includes regulating blood pressure, nerves and muscle functions, heartbeat, blood glucose and many more (National Institutes of Health, 2018). Therefore, any impairment of magnesium intake or the occurrence of magnesium deficiency will lead to interruption of these metabolic processes.

Magnesium deficiency, or, hypomagnesemia is typically caused by poor absorption of magnesium in the gut or increased magnesium excretion in the urine. In healthy individuals, hypomagnesemia rarely occurs because magnesium is regulated by the kidneys. Certain groups of people such as people with gastrointestinal diseases, type 2 diabetes, chronic alcoholism and the elderly are more susceptible to hypomagnesemia. Hospitalised people are also at an increased risk of magnesium deficiency, which may be due to interference from their chronic conditions, intake of certain medications such as diuretics and treatment with certain chemotherapies (Hansen & Bruserud, 2018).

Magnesium loss can also occur from food preparation methods. A study by Swaminathan (2003) found that boiling of magnesium-rich foods will result in significant loss of magnesium. Additionally, refining or processing of food such as refined grains have significantly lower magnesium content compared to its whole grain counterpart. This is due to the removal of nutrient-rich germ in refined grains (Michigan State University, 2012).

Long term magnesium deficiency is damaging to our health. The initial symptoms of magnesium deficiency are nausea, vomiting, weakness and decreased appetite. Later stages may show signs of muscles cramps, numbness, tingling and abnormal heart rhythms. Severe hypomagnesemia can lead to life-threatening complications including seizures, cardiac ischemia, coma, and death (Gragossian & Friede, 2020).

Magnesium intake has been associated with several health-promoting effects. A study by Schwalfenberg and Genuis (2017) reported that sufficient intake of magnesium improves breathlessness for asthmatic individuals, as well as bone mineral density. This may reduce the risk of osteoporosis and bone fractures. Additionally, people with higher intake of magnesium have lower risk of type 2 diabetes. A meta-analysis of 13 cohort studies found that there is a significant inverse association between magnesium intake and risk of type 2 diabetes (Dong, Xun, He & Qin, 2011).

2.3.2 Potassium

Potassium is the third most abundant mineral in the body. It is required for normal cell functioning and is especially important to manage high blood pressure. Potassium can be found in various type of food such as fruits, vegetables, certain soybeans, poultry and nuts.

Potassium is regulated by the kidneys. Therefore, it is imperative those with chronic kidney diseases to look out for excessive potassium intake as it may have a detrimental effect on their kidney and overall health. In severe cases, high potassium level can lead to severe muscle weakness, irregular heartbeat and/or heart attack.

High potassium intake is associated with lower incidence of stroke and has been shown to hinder the formation of calcium stones or kidney stones. Furthermore, it has been shown to support bone mineral density. The mechanism is unclear, but studies from a few clinical trials and observational studies suggested it has to do with acid-base balance. Potassium intake has an inverse relationship with the rate of fasting glucose and insulin resistance. Nurses' Health Study reported that intake had a 38% lower risk of developing type 2 diabetes over 6 years of follow-up than those in the lowest quintile (National Institute of Health, 2019).

Potassium has been recognised as a shortfall nutrient in the United States. Majority of Americans do not consume the required amount for proper functioning and maintenance of health (Whelton, 2018). One of the factors is the heavily refined and processed food in Western diet which hinders the bioavailability of potassium in the body. Severe potassium deficiency can cause hypokalemia. Hypokalemia is defined as serum potassium level less than about 3.6 mmol/L (Viera and Wouk, 2015). Hypokalemia can result low dietary potassium intake, potassium losses in stool during diarrhea, and potassium losses in kidneys through vomiting (metabolic alkalosis). Moreover, it can be caused by the use of diuretics, laxative abuse, chronic drinking and heavy sweating (National Institute of Health, 2019).

The signs and symptoms of hypokalemia depend on the severity of the condition. Symptoms will usually manifest when serum potassium is below 3.0 mEq/L, or had a rapid decrease from normal level. Symptoms range from affected nervous system (numbness and cramps of the limbs), constipation, and intestinal paralysis and impaired functioning of cardiovascular systems such as cardiac arrhythmias and heart failure (Kardalas et. al, 2018).

On the other end of the spectrum, excessive intake of potassium results in hyperkalemia. Hyperkalemia occurs when serum potassium exceeds 5.5 mmol/l. Renal excretion, excessive intake or leakage of potassium from the intracellular space are the causes of hyperkalemia (Lehnhardt and Kemper, 2011). Symptoms are non-specific; it may be absent but in severe cases it may manifest as muscle weakness, paralysis, heart palpitations, paresthesias (a burning or prickling sensation in the extremities), and lethal cardiac arrhythmias (Viera and Wouk, 2015).

2.3.3 Zinc

Zinc is a trace element that is required for proper functioning of body cells. Zinc is a precursor for DNA and protein and helps our immune system to fight off pathogens. Additionally, zinc is needed for proper development of foetus and infant (Ackland & Michalczyk, 2016).

Zinc supplement has been associated with reducing the symptoms and duration of diarrhoea of children in developing countries, many of whom are having zinc deficiency or malnourished (Bajait and Thawani, 2011). However, it is unclear whether the supplement will have the same effect for healthy children with sufficient intake of zinc. Additionally, a study involving elderly with age-macular degeneration (AMD) found that with the intake of multi-mineral supplement that includes zinc, for 6 years had a lower risk of developing AMD and less vision loss compared to those who did not consume the supplement. In the same study, people at high risk of the disease who took dietary supplements containing only zinc also had a lower risk of getting advanced AMD than those who did not take zinc dietary supplements (Chew et al., 2013).

Protein is a rich source of zinc. Meat (pork, beef), poultry, organ meat, fish and shellfish, and lesser amounts in egg and dairy products. Moreover, zinc can be found in nuts, legumes, and whole grain cereals whereas refined wheat products, tubers, fruits and vegetables have a lesser amount. Zinc from animal sources have a higher bioavailability compared to zinc from plant sources due to the presence of fibre and phytic acid in these sources.

For Malaysians, the recommended daily intake of zinc was referred from Food and Agriculture Organization and World Health Organization (2002) guidelines, which states that the estimated physiological requirements for absorbed zinc in adult men and women are estimated to be 1.4 mg/day and 1.0 mg/day, respectively (Recommended Nutrient Intakes for Malaysia, 2017).

Zinc deficiency can occur through increased requirements, malabsorption, inadequate intake, increases losses and impaired utilisation. This will lead to impaired functioning of the immune system that increases the risk of childhood infection. Due to its importance in cell growth and development, the effect of zinc deficiency is more apparent and severe for certain populations such as lactating and pregnant women, children, adolescents and infants. Caulfield (1998) reported that maternal zinc deficiency results in retarded growth and development of growing children. The elderly are also at a higher risk due to inadequate intake and less efficiency of zinc absorption. Additionally, individuals suffering from gastrointestinal disorders such as Crohn's disease, Sprue and short bowel syndrome. Excessive zinc consumption or zinc toxicity are usually caused by excessive intake of zinc dietary supplements, rarely from consumption of natural food. Effects include nausea, vomiting, diarrhoea, suppression of the immune system, and copper deficiency (Plum, Rink & Haase, 2010).

2.3.4 Calcium

Calcium is the most abundant mineral in the body. It can mainly be found in our bones and teeth where it plays its major role in supporting the structure and functions. Furthermore, these bone tissues act as a reservoir and source of calcium to maintain normal calcium concentrations in blood, muscle and intercellular fluids. Meanwhile, the remaining calcium, (serum calcium) is responsible for vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signalling and hormonal secretion (National Institutes of Health, 2019). Less than 1% of total body calcium is required for these functions because serum calcium is tightly regulated.

RNI for Malaysia (2017) recommended 800mg per day for female and male adults. This can be achieved from consumption of food such as a glass of milk and dairy products such as yogurt and cheese which contain the highest amount of calcium. Additionally, non-dairy products that contain a significantly high amount of calcium include spinach, Chinese cabbage and broccoli. Dietary calcium also is provided through supplements and certain fortified food.

Over the past decade, several dietary studies in Malaysia found consistent reports of calcium intakes below 500mg per day, and only 200-300mg per day amongst elderly women in rural areas. Medical Journey of Malaysia (2015) reported that the median intake for calcium was 357 mg/day or 43% of the RNI. It was also found that all socio-demographic groups did not meet 50% of the recommended level for calcium. Moreover, a study conducted among 289 adolescents in Peninsular Malaysia to assess their dietary intake of calcium and vitamin D found that majority of the adolescents failed to meet the daily requirement intake. (Suriawati, Majid, Al-Sadat, Mohamed and Jalaludin, 2016).

The main consequence of calcium deficiency, hypocalcemia (total serum calcium concentration < 8.8 mg/dL), is reduced mineralisation of bone. This will lead to rickets, osteomalacia and osteoporosis. These disorders are characterised by soft, pliable bones and loss of bone mass. Additionally, this can result in tetany, a condition of involuntary muscles cramps especially in hands and feet.

Meanwhile, abnormally high level of serum calcium is called hypercalcemia. It is usually a result of overactive parathyroid glands. Hypercalcemia leads to formation of kidney stones, renal insufficiency, vascular and soft tissue calcification and hypercalciuria (high levels of calcium in the urine). It can also cause constipation. Formation of kidney stones are usually caused by excessive intake of calcium supplements which have also been linked to increased risk of cardiovascular diseases (Tankeu, Agbor and Noubiap, 2017).

2.3.5 Phosphorus

Phosphorus is the second most abundant mineral in the body. It makes up about 1% of the body weight and it can be found in every part of the body. Phosphorus helps to strengthen teeth and bones, waste filtration in kidneys, cellular growth and development, and plays a role in energy metabolism (Recommended Nutrient Intakes for Malaysia, 2017). Phosphorus deficiency, known as hypophosphatemia, is rarely caused by insufficient dietary intake. It is more likely to occur due to non-dietary metabolic disorders. Examples of food rich in phosphorus are mainly protein sources such as poultry, meat, milk, egg, nuts and beans. Carbonated drinks also contain a high content of phosphorus because phosphoric acid is used for the carbonation process. Marcus (2013) reported that ample intake of calcium-rich food should go hand in hand with the consumption of phosphorus-rich food due to excessive phosphorus intake will lead to calcium excretion. High level of phosphorus can lead to diarrhoea, hardening of organs and tissues, kidney damage and affects the bioavailability and interaction of other minerals in the body such as calcium, magnesium, zinc and iron.

Phosphorus deficiency, known as hypophosphatemia, is rarely caused by insufficient dietary intake. It is more likely to occur due to non-dietary metabolic disorders (Erdman, Macdonald and Zeisel, 2012). The complications of hypophosphatemia includes muscle weakness, rickets, anorexia, ataxia, anaemia and in severe cases, death (Erdman et al, 2012). However, hypophosphatemia induced from dietary treatment (refeeding with calorie-rich sources) do occur in exceptional cases of recovery from diabetic ketoacidosis and alcoholic bouts, and respiratory alkalosis (Knochel, 1985).

On the other end of the spectrum, hyperphosphatemia, an excessive level of phosphorus in the blood will lead to calcification of the kidneys and coronary arteries. Similar to hypophosphatemia, it is a rare occurrence which is due to renal failure instead of dietary causes (Erdman et al, 2012).

2.3.6 Iron

Iron is one of the most important mineral for human health maintenance and proper functioning. Iron can be found mostly in red blood cells. Red blood cells transport oxygen throughout the body and remove carbon dioxide to be exhaled. Additionally, red blood cells stores iron. Iron is also involved in muscle metabolism, neurological development, cellular functioning, and synthesis of connective tissue and some hormones (Recommended Nutrient Intakes for Malaysia, 2017). Iron can be found in four major forms, where each form caters for different purpose. First, iron containing heme proteins which function to transport, store oxygen and electron transport. Next, iron sulphur enzymes that are involved in energy metabolism. Another form is iron storage and transport proteins, where it is involved in iron uptake, storage and transport. Furthermore, there are other iron forms which are mainly enzymes.

Iron comes in two forms: heme and non-heme. The difference between these two forms of iron is their mechanism and dietary sources. Another significant difference is that heme iron has a higher bioavailability compared to non-heme iron. This is because heme is soluble at the alkaline pH condition of the small intestine (West and Oates, 2008).

RNI for Malaysia 2017 recommends 14mg/day for male adults, and 29 mg/day for premenopausal women, and 11% for postmenopausal women, accounting for 10% bioavailability. However, the majority of Malaysian adults do not meet this requirement. Medical Journal of Malaysia (2015) reported that the median intake of iron was about 9.9% mg/day with men (10.8 mg/day) whereas women having lower intake (9.0 mg/day).

Chronic iron deficiency causes abnormally low level of red blood cells (RBC) circulating. Lack of RBC contributes to lack of haemoglobin, which is required to transport oxygen throughout the body tissues. This leads to iron-deficiency anaemia. Milman (2015) reported that iron deficiency anaemia is still a prevalence health disorder in Malaysia, especially among pregnant women. As of 2016, the prevalence of iron-deficiency anaemia among women of reproductive age (15-49 years old) is 24.9% (Index Mundi Malaysia, 2016).

Iron deficiency may be caused by inadequate iron intake, increased iron requirements especially during pregnancy, lactation or recovery from illnesses, and chronic blood loss (eg. menstrual bleeding). The symptoms and signs of anaemia include weakness, fatigue, pale skin pallor, irregular heartbeats, headaches and shortness of breath.

Iron absorption is affected by several factors. Enhancing factors such as ascorbic acid found in food, meat, fish and fermented vegetables and sauces increases the uptake of iron in the small intestine. Meanwhile inhibiting factors such as soya, phytate, iron-binding phenolic compounds found in coffee, tea, cocoa, calcium-rich products such as cheese and milk. Heme iron can be found abundantly in lean beef meat, ox liver, chicken, cockles, mackerel and anchovies, whereas rich sources of non-heme iron are chickpea, bitter gourd, kangkung, and spinach.

2.3.7 Copper

Copper is one of the essential trace minerals present in all human body tissues, mainly in the liver, brain, heart, kidneys and skeletal muscles. Copper plays a major role as oxidases, thus it is found abundantly in enzymes in the human body. Additionally, copper is needed for adequate growth, cardiovascular integrity, lung elasticity, neovascularization, neuroendocrine function, iron metabolism and connective tissue (National Research Council (US) Committee on Copper in Drinking Water, 2000).

According to Recommended Nutrient Intake (2017) for Malaysia, an adult regardless of gender requires 900 μg/day of copper. Currently, there is no data on the dietary intake of copper in Malaysia. This is mainly due to the non-availability of information on copper content for most foods in the local food composition database (Tee et al.,1997).

Copper deficiency is rare because it is naturally present in many foods. Moreover, it is available in fortified food and comes in the form of supplement. Cases of copper deficiency may be due to excessive intake of zinc (inhibiting factor of copper absorption), impaired copper absorption, gastric surgery including gastric bypass or gastrectomy, diet low in copper, conditions such as cystic fibrosis and inflammatory bowel disease (Wazir & Ghobrial, 2017).

Copper deficiency will contribute to anaemia, hypopigmentation, osteoporosis, hypercholesterolemia, connective tissue disorders, and other bone defects, abnormal lipid metabolism, ataxia, and increased risk of infection (National Institutes of Health, 2019). Copper toxicity is also a rare occasion which may occur with excessive intake of copper supplements, consumption of drinking water containing high level of copper (from water in copper pipes) and usage of copper pots during cooking. Copper toxicity causes symptoms and complications such as abdominal pain, dizziness, liver damage, kidney failure, coma and death. People with a high risk of contracting copper toxicity are people with immature liver functions and with genetic disorders. These conditions affect copper metabolism such as the case of Wilson's disease, an autosomal recessive disorder that causes deficiency of copper export pump ATP7B cause by gene mutation. Without clearance of copper in the body, this will lead to liver cirrhosis, acute hepatitis, haemolytic crisis, and liver failure and neuro logical symptoms such as dystonia, tremor and cognitive and mood disorders (Chen et al., 2015).

Two main sources of copper are organ meats and shellfish. Plant-based sources of copper include nuts and seeds, wheat bran cereals and whole-grain products. Poor sources of copper are milk and dairy products. Drinking water can also be a source of copper if the copper concentration exceeds range of 1-2 mg/L (European Food Safety Authority, 2015). According to Azlan et. al (2012), drinking water in Malaysia is not a source of copper due to its low concentration (2.99 µg/L in drinking water, 12.77 µg/L in mineral water, and 8.54 µg/L in tap).

2.3.8 Sodium

Sodium (Na) is the sixth most abundant element in the Earth's crust and it occurs naturally in most food, mainly in the form of sodium chloride (table salt). 1.0 g sodium is equivalent to 2.55 mg NaCl (NaCl consists of 40% Na).

Due to its abundance and osmotic pressure, sodium plays an important role in electrolyte fluid balance and fluid balance. Additionally, sodium is essential in the regulation of acid-base balance, nerve impulses transmission, blood and normal cell functions (Recommended Nutrient Intakes for Malaysia, 2017).

World Health Organisation (WHO) recommends that adults consume less than 5 g (just under a teaspoon) of salt per day as a prevention step to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease. In Malaysia, sodium intake is higher than the WHO recommendations. In 2019, Malaysian Community Salt Survey released a report that about 79% Malaysian adults consumed more than 2000 mg per day. Through 24-hour urinary results, it was found that sodium intake was 3167 mg/day, which corresponds to 138 mmol/day or 7.9 gram of salt or 1.6 teaspoon of salt. This contributes to the increasing prevalence of hypertension in Malaysia from 34.6% in 2006 to 35.3% in 2015 (National Health and Morbidity Survey, 2006; 2015). Although it is not a significant increase, this increment of about 0.7% accounts for around 184,000 Malaysians. Compared to China and Singapore, the prevalence of hypertension in Malaysia is higher and remains above 30% (Nur Liana et al., 2018).

Besides its main form, table salt, sodium is also added in many food products. In these food products, sodium comes in the form of monosodium glutamate (MSG), sodium nitrite, sodium saccharin, baking soda (sodium bicarbonate), and sodium benzoate. Furthermore, high level of sodium can be found in processed food such as condiments and canned foods. According to Malaysian Adult Nutrition Survey (MANS, 2014), Malaysian foods sources of high sodium content are local kuih (79%), breads (76.9 %) , mee hoon, kueh teow, laksa, laksam, lohsi fun(76%), ketchup (75.6%) and followed by mee (75.2 %).

2.4 White rice

Rice (*Oryza sativa*) is an annual grass belonging to the Gramineae family. The harvested grain is enclosed by a protective coating called the hull or husk. Milling usually removes both the hull and bran layers of the grain, which results in white rice.

White rice is a staple food for about one-half of the world, especially prominent in East and Southeast Asia. Malaysia is not an exception. In Malaysia, white rice is often eaten as part of the three main meals (breakfast, lunch and dinner). Furthermore, rice is a common ingredient in many local dishes and snacks either as cooked rice or indirectly in the form of rice flour. Examples of local delicacies are *nasi lemak, kuih seri muka, ang ku kueh* (red tortoise cake), *bihun* and so on. At an estimation of 87.9kg/person of white rice consumption, white rice accounts for about 26% of total caloric intake per day (Khazanah Research Institute, 2018). This is further proved by the findings that Malaysians consume almost three times more white rice compared to wheat over the span of 26 years (1990 to 2016).

Having said this, Malaysia's percentage share of total caloric supply from rice has been steadily declining since the 1960s. This is in line with Bennett's Law, which state that: as populations become wealthier, there is a shift from simple starch to a more diversified diet that includes a range of vegetables, fruit, dairy products, and especially meat (Charles & Godfray, 2011). Numerous studies have found similar findings that dietary habits changes reflect the health outcomes of the population (Turner and Thompson, 2013; Huisenovic et al., 2019; VanHeuvelen, 2019).

National Health and Morbidity Survey (2019) have shown an increase in prevalence of non-communicable diseases (NCD) in Malaysia. The current estimation of Malaysian stricken with NCD are 3.9 million adult diabetic Malaysians, 6.4 million with hypertension, and 8 million with hypercholesterolemia. To combat this epidemic, Ministry of Health Malaysia had constructed a 10-year National Strategic Plan for Non-Communicable Diseases (NSP-NCD) 2016-2025. This plan provide the over-arching framework for strengthening NCD prevention and control in Malaysia. This Strategic Plan is in-line with the Global Action Plan for the Prevention and Control of Non-Communicable Diseases 2013-2020.

There are various factors that contribute to the NCD epidemic including genetics, sedentary lifestyle, detrimental eating habits and smoking. In terms of dietary habit, it is worth noting overconsumption of high caloric food contributes to this increasing trend of NCD. According to a meta-analysis study by Harvard School of Public Health (HSPH), it was found that people who consumed three to four servings of white rice per day – were 1.5 times more likely to have diabetes than people who ate the least amount of rice. This may be due to white rice having a high glycaemic index which spikes blood glucose quickly. Previous studies have shown that high glycaemic index food increased the risk for type 2 diabetes (Bhupathiraju et al., 2014).

On that note, as Malaysia shifts its dietary habits to a more Westernised diet (high in fat and calorie), this provides a chance for food industries and relevant health-based organisations to introduce and advocate for more options of healthier alternatives, in place of white rice. This study aims to investigate the potential of little millet and barnyard millet as part of the strategy to combat NCD epidemic in Malaysia, along with comparing their micronutrient composition with that of white rice.

CHAPTER 3

3.0 Methodology

3.1 Sample Selection and Sampling Method

Two selected millets in this study were little millet and barnyard millet. The samples were collected by purposive sampling, purchased from Uyir Inspired Sdn Bhd. Purposive sampling was chosen by taking into consideration that there are little studies on the mineral content of barnyard millet and little millet. The Preparation methods of the two samples were listed in Table 3 according to the method of Momoh, Olaniyi & Raphael (2017).

Table 3: Methods of Preparation of Millet

Millet	Weight (g)	Methods of preparation
Raw little millet	24	● Grinded to a fine powder using stainless steel blender ● Sieved using 5 mm sieve ● Stored in freezer at -20°C until further use
Raw barnyard millet	24	● Grinded to a fine powder using stainless steel blender ● Sieved using 5 mm sieve ● Stored in freezer at -20°C until further use

3.2 Reagents and Chemical

All reagents were of analytical reagent grade. Below is a list of reagents and chemicals used (AOAC International, 2000). All acids obtained were supplied by J.t Baker (Central Valley, Thailand):

a) Water – deionised

b) Nitric acid (HNO_3) – Concentration 65%

c) Nitric acid (HNO_3) – 0.1M, made with diluted 7 mL HNO3,(**b**), with water, (**a**), to 1L

d) Hydrochloric acid – Concentration 50%

3.2.1 Solution Preparation

All standard solutions, working standards and blanks were prepared according to Journal of AOAC International (2000).

a) Standard Solution Preparation

All stock solutions were prepared in 1000ppm. For each element, 1g of each mineral is diluted in a solution of 14 mL water and 7 mL 65% nitric acid in a 1 L volumetric flask. It was then diluted to volume with water.

b) Working Standard Preparation

Standards of all minerals were diluted with 0.1M HNO_3 to a range of standards that covers the concentration of the elements to be determined.

3.3 Dry Ashing

The millet powder was then undergone a dry ashing method, which was introduced in AOAC Official Method 999.11 (AOAC International, 2005).

First, the crucibles were pre-heated in the air oven for 30 minutes at 105°C, and then cooled in the desiccator for about 1 hour. Once the crucibles reached room temperature, it was weighed (W1). 3g of each millet powder were added into the crucible, with a new weight (W2). Next, they were placed in the muffle furnace for ashing at 550°C overnight until the content become whitish in colour with no black particles. After that, they were removed and cooled in the desiccator. The new weight (W3) was recorded.

$$\% \, Ash = (W2-W3)/(W2-W1)$$

Where
W1 = weight of crucible
W2 = weight of crucible + sample before ashing
W3 = weight of crucible + sample after ashing

3.4 Analysis of Mineral Content

The content of iron, zinc, magnesium, copper, calcium, potassium, sodium, and phosphorus content of little millet and barnyard millet were determined using flame atomic absorption spectrometry according to the method of Amalraj & Pius (2015) and Sanusi, Sulaiman & Bako (2019).

The ashes of both samples were dissolved in 10 mL of 50% HNO_3 solution. The mixture was heated over a water bath and was allowed to cool. It was then transferred into 100 ml volumetric flask and made up to volume with distilled water. After that the minerals were analysed separately, using A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany). Air/acetylene flame and respective hollow-cathode lamps were used for absorbance measurements. Calibration curve for the minerals were prepared. At least 3 readings were obtained.

Calculation

The calculations were obtained from the printed report of the instrument. The concentration of the element in the sample was expressed in ppm. The results were converted to mg per 100 g sample as follows:

$$Mg/100\ g\ sample = Ppm \times (1/1000) \times 100$$

3.5 Experimental Design

The figure below is a summary of the methods used in this study (AOAC International, 2005; Amalraj & Pius, 2015; Momoh, Olaniyi & Raphael, 2017; Sanusi, Sulaiman & Bako, 2019).

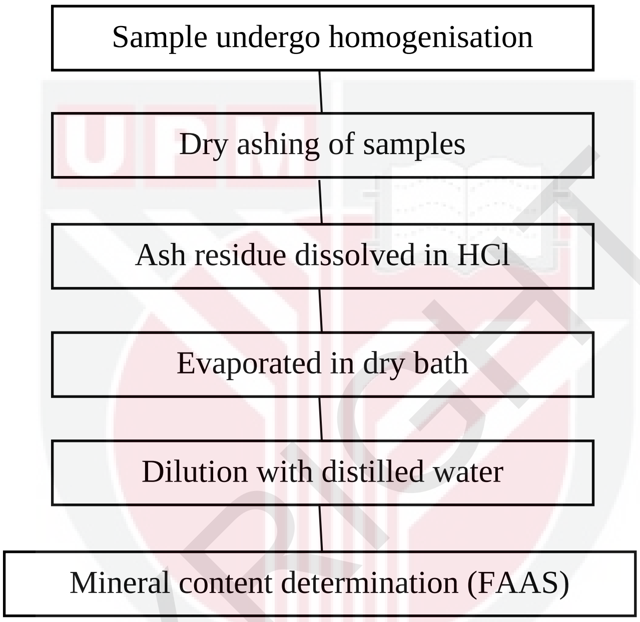

Figure 3.1: Experimental design used for determination of mineral content of little millet and barnyard millet

3.6 Statistical Analysis

The results obtained were expressed as means and standard deviation (SD). The statistical analysis was performed using IBM SPSS version 24. The difference in the means of mineral content between barnyard millet and little millet were analysed by independent t-test. Independent T-Test was chosen to compare the means of 9 minerals between two groups (barnyard millet and little millet). The significant level was set at $p < 0.05$.

3.7 Validity and Reliability

In this study, mineral content of the samples was determined using Flame Atomic Absorption Spectrometry (FAAS) in accordance to the method published in Journal Of AOAC International (2000). FAAS is an analytical method that is based on the absorption of UV-visible radiation by free atoms in the gaseous state. The use of this method is widely used in analytical laboratories for determination of inorganic elements in a variety of samples. This is due to its attributes such as relative simplicity in operation, high element specificity and detection, adaptability to measuring various elements and reasonable cost (Ihnat, 1987). Meanwhile, the determination of ash content and dry ash procedure were carried out from the guidelines of AOAC International (2005). Therefore, the official analytical methods are accurate and reliable methods.

Moreover, all analytical methods were undergone in triplicates to ensure accuracy of data. The mean values of data obtained were calculated into average values. Additionally, each step of the procedure were taken accordingly with proper calibration of instrument to reduce biases and errors.

CHAPTER 4

4.0 Results and Discussion

4.1 Total Mineral Content

The results of total mineral content of little millet and barnyard millet are presented in Table 4.1.

Table 4.1 Mineral levels for barnyard millet and little millet

Minerals	Barnyard millet (mg/g) n = 5	Little millet (mg/g) n = 5
Potassium (K)	77.22 ± 20.02	78.22 ± 36.51
Phosphorus (P)	49.69 ± 5.02	51.34 ± 24.73
Sodium (Na)	11.80 ± 5.46	7.56 ± 3.85
Magnesium (Mg)	6.06 ± 0.04	6.05 ± 0.39
Zinc (Zn)	1.71 ± 0.27	1.43 ± 0.61
Iron (Fe)	0.46 ± 0.55	0.35 ± 0.54
Copper (Cu)	0.2 ± 0.02	0.24 ± 0.11
Calcium (Ca)	0.037 ± 0.0005	0.037 ± 0.0007

Values are expressed as mean ± SD, n = 5. There is no significant difference between little millet and barnyard millet.

4.1.1. Comparison of minerals between millets

The results contradicted the hypothesis where it has no significant difference ($p<0.05$) between mineral content of barnyard millet and little millet. For both millets, the most abundant element is K while Ca is the least. In descending order, the content of mineral for both millets is K, followed by P, Na, Mg, Zn, Fe, Cu and Ca.

As presented in the table[1], barnyard millet is rich in K (mean 77.22 ± 20.02 mg/g), followed by P (49.69 ± 5.02 mg/g), Na (11.80 ± 5.46 mg/g) and Mg (6.06 ± 0.04 mg/g). Little millet is abundant with the same elements with K (78.22 ± 36.51 mg/g), followed by P (51.34 ± 24.73 mg/g), Na (7.56 ± 3.85 mg/g) and Mg (6.05 ± 0.39 mg/g).

The Fe content of barnyard millet and little millet is 0.46 ± 0.55 mg/g and 0.35 ± 0.54 mg/g respectively. However the level of Fe is lower compared to other studies, particularly in little millet. Shobana et al. (2013) and Food and Agriculture Organisation (1990) reported that Fe level of little millet is 9.3 mg/g compared to this study where little millet contained 0.35 mg/g. In barnyard millet, this study showed a similar result with other studies with Fe level averaging at 0.5mg/g.

Barnyard millet has comparable Zn content (1.71 ± 0.27 mg/g) to little millet (1.43 ± 0.61 mg/g). Meanwhile, little millet has higher content of Cu (0.24 ± 0.11 mg/g) than barnyard millet (0.2 ± 0.02 mg/g). The result is aligned with Pasha, Ratnavathi, Ajani, Raju, Kumarc and Beeduc (2018) and Renganathan, Vanniarajan, Karthikeyan and Ramalingam (2020) with Zn as one of the highest element contained in both millets. Zn is higher than the ones reported in Shobana et al. (2013) and FAO (1990). In these studies, Zn was determined at a range of

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[1] In this study, there is no significant difference between little millet and barnyard millet.

0.06 to 0.4 mg/g whereas in this study, Zn content was recorded at 1.43 mg/g and 1.71 mg/g for little millet and barnyard millet, respectively.

Next, the Mg content of both millets are lower compared to the study reported by Shobana at el. (2013) and FAO (1990). These studies recorded Mg level at 8.2 mg/g and 13.3 mg/g for barnyard millet and little millet respectively. However it is higher than the study reported by Pasha et al. (2018), where barnyard millet has 2.74 mg/g and little millet has 2.97 mg/g of Mg.

Na level in this study is found to be higher than other studies. Shobana et al. (2013) and Pasha et al. (2018) reported Na level of 0.81mg/g and 0.61 mg/g respectively in little millet. Meanwhile for barnyard millet, only Pasha et al. (2018) reported the level of Na (0.69 mg/g) as there is no other studies on Na content of barnyard millet.

Another abundant mineral is P. Both millets in this study recorded a high level of P compared to other studies. In FAO (1990) and Shobana et al. (2013), the level of P is higher in barnyard millet than little millet. Meanwhile, this study is consistent with Pasha et al. (2018) where P is higher in barnyard millet, although this study has a markedly higher P level at 49.68 mg/g compared to 5.75 mg/g in Pasha et al. (2018).

As for K content, barnyard millet has a slightly lower level than little millet with 77.22 ± 20.02 mg/g and 78.22 ± 36.51 mg/g respectively. The result is consistent with Pasha et al. (2018) where it stated that K content of small millets is the highest of all minerals, within the range of 4.5 to 9.82 mg/g.

Ca was detected at trace level averaging at 0.037 mg/g in both millets. Other studies reported Ca range of 0.21 to 2.1 mg/g (FAO, 1990; Shobana et al. 2013; Pasha et al. 2018).

4.1.2 Factors contributing to differences in mineral content

There are several reasons contributing to the differences in the mineral content of the millets. First, it can be affected by pH of the soil the millet was grown in. pH of the soil can be defined as the measure of acidity or alkanity of in the soil. pH of the soil affects the soil biogeochemical processes. This includes soil biological, chemical, and physical properties and processes that affect plant growth and biomass yield (Neina, 2019). According to Brady (1990), generally pH 6.0 – 7.0 (neutral) is ideal for most plant. However, the optimal soil pH varies for crops (McCauley, Jones, Olson-Rutz, 2017). High pH or alkaline soil is associated with high content of phosphorus, potassium and magnesium (Murphy, Hoagland, Reeves and Jones, 2008; Pasha et al. 2018). There were other studies that reported acidic soil (pH 5.6) is ideal for millet growth, especially for pearl millet (Oelke et al. 2011).

Next, mineral content of soil affects the uptake of micronutrients of whole grain plants. This is due to the differences in chemistry and composition of soils. In certain soils, nutrients may not be present or available in forms that the plants cannot utilise. Moreover, the usage of fertilisers and types of farming system (i.e. conventional, organic) plays a role relating micronutrient availability in soil to micronutrient concentration in grains (Murphy et al. 2008).

Furthermore, the differences can be attributed to different cultivars of barnyard millet and little millet. Pasha et al. (2018) studied the mineral analysis (potassium, sodium, magnesium, phosphorus and calcium) of traditional small millets which were cultivated and consumed in Rayalaseema region of South India. Two cultivars of barnyard millet and three cultivars of little millet were included in the study. For barnyard cultivars, the mineral content ranges from 0.21 mg/g to 7.63 mg/g, whereas for little millet, the mineral content ranges from 0.21 mg/g to 4.5 mg/g. Genetic variability caused cultivars to have varying levels of nutrients (Murphy et al. 2008; Marles, 2017).

Additionally, mineral yield after running the samples through atomic absorption spectrometry (AAS) may be affected by acid solvent used in sample preparation. Different acid used may produce varying analyte recovery due to incomplete acid digestion. This was shown in a study by Uddin, Khalid, Alaama, Abdualkader, Kasmuri and Abbas (2016). In this study, herbal medicine samples were digested with three different digestion methods, namely method A (a combination of nitric-perchloric acids HNO_3–$HClO_4$ in a ratio 2:1), method B (only nitric acid HNO_3), and method C (a mixture of nitric-hydrochloric acids HNO_3–HCl in a ratio 1:3). Out of all three methods, method C produce the highest analyte recovery rate for all metals. This is due to the ability of the mixture to release the metal ions from complex matrices of the samples (Uddin et al. 2016). However in this study, millet ashes were digested using hydrochloric acid alone which may not yield an optimum analyte recovery.

4.1.3 Comparison of millets with white rice

The mineral contents of three varieties of white rice (Fragrant, Basmati, Siam) can be seen in Table 4.2.

Table 4.2 Mineral levels for fragrant rice, basmati rice and siam rice

Minerals	Fragrant rice (mg/g) n = 6	Basmati rice (mg/g) n = 6	Siam rice (mg/g) n = 6
Sodium (Na)	0.25 ± 0.13	0.39 ± 0.05	0.36 ± 0.07
Magnesium (Mg)	0.92 ± 0.11	1.25 ± 0.35	1.39 ± 0.36
Zinc (Zn)	0.11 ± 0.03	0.11 ± 0.01	0.12 ± 0.01
Iron (Fe)	0.26 ± 0.29	0.25 ± 0.35	0.03 ± 0.02
Copper (Cu)	0.013 ± 0.02	0.002 ± 0.001	0.004 ± 0.0005
Calcium (Ca)	0.45 ± 0.08	0.58 ± 0.10	0.5 ± 0.06

Values are expressed as mean ± SD, n = 6.

(Adapted from: Fairulnizal et al., 2015)

The data for mineral content of white rice was obtained from Fairulnizal et al. (2015) which form the basis for the data in Malaysian Food Composition Database (FCD 2015). In these sources, three types of rice were analysed, namely Basmati, Siam and Fragrant rice. These varieties were chosen due to its high consumption in Malaysia. Minerals analysed in this study includes Mg, Ca, Na, Fe, Zn, and Cu. Meanwhile, data for P was obtained from Malaysian

Food Composition Database (FCD 2015). Other analyses include proximate analysis, vitamins, total sugar, fatty acids, trans fatty acids, and cholesterol.

According to Malaysian Food Composition Database (FCD 2015), white rice is most abundant in P, ranging from 6.1 mg/g to 7.1 mg/g. The second highest element is Mg, with Siam rice having the highest content (1.39 ± 0.36 mg/g), and fragrant rice having the lowest (0.92 ± 0.11 mg/g). This is followed by Ca (0.45 ± 0.08 mg/g to 0.58 ± 0.10 mg/g). The fourth most highest mineral is Na, with Basmati and Siam rice averaging at 0.39 mg/g, while Fragrant rice having 0.26 mg/g. Zn comes in next with an average of 0.11 mg/g, and finally Cu with a range of 0.002 mg/g (Basmati) to 0.013 mg/g (Fragrant).

To summarise, the mineral content of Fragrant and Basmati rice follows as order, from highest to lowest: P, Mg, Ca, Na, Fe, Zn and Cu. Whereas for Siam rice, P is found to be the highest, followed by Mg, Ca, Na, Zn, Fe and Cu. Potassium was not analysed as potassium is not a major mineral in white rice.

From this finding, the major difference between millets and white rice is the lack of potassium in the refined grain. This is common as white rice is not a source of potassium. White rice is considered as a low potassium food.

Another major difference is the level of calcium. Calcium is the third most abundant element in white rice whereas for little millet and barnyard millet calcium is the least abundant mineral. In this study, the millets are nutritionally more superior to rice in all minerals except calcium. However, in comparing the millets from other studies with white rice, millets are nutritionally more superior in all minerals, including calcium, to white rice (FAO 1990; Shobana et al. 2013; Pasha et al. 2018).

The main reason in the difference of micronutrient composition is the processing effects that both grain types (millet and white rice) has undergone. Millets are typically dehulled, the process where the inedible husk is removed. Removal of the husk enables millets for human consumption. Meanwhile, white rice typically undergone milling to remove the husk, bran and germ. This results in altered flavour and texture of the rice, along with extended shelf life. After that, rice will generally be polished to form a white, bright and shiny appearance.

The processing effects plays a vital role in micronutrient composition of the grains because minerals in the grain are located varyingly in the seed. During polishing and hulling, the elements concentration in different locations of the seed experienced a major loss (Lamberts et al. 2007). A study by Lu et al. (2013) analysed the concentrations of mineral in different parts of rice grain. The study reported that bran contained the highest concentrations of Zn, Fe and K. This is followed by the hull and polished rice. Meanwhile the concentration of Ca is heavily concentrated in the hull, bran and finally polished rice.

A closer look into the mineral concentration revealed Zn in bran is 3 times more than Zn in the hull and polished rice. Meanwhile, Fe concentrations in the bran were 7 times more than in polished rice. Similarly, a significantly higher value of K is found in the bran compared to the polished rice (32 times the concentrations). This corresponds to another study by where it was reported that Mg, Fe and Ca can mainly be found in the hull, while K is heavily concentrated in the bran (Govarethinam, 2014). Meng et al. (2005) stated that Fe content in rice is affected by the iron absorption from soil, and the transportation and accumulation of Fe in rice. Additionally, the variation of Fe content depends on rice cultivars or genotypes. The findings of these studies revealed that micronutrients content decreased as it moves further away from the outer layer, and towards the centre of the grain (Hansen et al. 2012).

As for millet, the process of dehulling was found to greatly lower the content of mineral. However, the percentage of loss varies according to the type of millet species It was reported that Ca, Mg and Na experienced significant losses when milling pearl millet to flour with an extraction rate of 67%, but losses did not occur with Fe and K (Himanshu et al. 2018).

The findings from this study did not reflect the problem statements regarding iron and calcium deficiency experienced by Malaysians. Although the level of iron in both millets were higher than in white rice, it is not considered as a rich source of iron. Rich sources of iron (18 mg and more) includes food such as shellfish beans and lentils, spinach, liver and other organ meats. Similarly, calcium content in this study found is significantly lower than reported in other studies. The calcium level is also lower than in white rice.

However, in line with other studies, both millets in this study are abundant in potassium. Adequate intake of potassium has been proven to have a protective effect on obesity (Cai et al., 2016). The same study found a protective effect of adequate potassium intake on metabolic syndromes. Meanwhile, other studies found that high intake of potassium, as opposed to adequate, is associated with a lower risk of obesity (Murakami et al., 2015) and metabolic syndromes in females (Shin et al., 2013).

These studies and other studies have not found a clear mechanism between potassium intake and obesity and metabolic syndrome. Possible explanations may be related to the role of potassium on carbohydrate accumulation and glucose homeostasis (Cai et al, 2016). According to Chatterjee et al. (2011), low serum potassium can lead to low production of insulin, which may result in an increase of blood glucose. This increases the risk of diabetes 2. However, there is not yet an intervention on whether an increased intake of potassium will reduce the risks of diabetes 2.

Additionally, potassium intake plays an important role in blood pressure regulation. Findings from Houston (2011) reported that an increased potassium intake of 4.7 g per day reduces the risk for cardiovascular diseases, particularly in myocardial infarction and cerebrovascular accident. In another study, participants in a randomised controlled trial showed reduced cardiovascular mortality when consuming high-potassium food, and decreased sodium consumption (Staruschenko, 2018).

Therefore, potassium in these millets can be utilised to be a healthier alternative in place of rice. Rice contains little to none potassium, and lower in minerals compared to millets. As a healthier alternative, millet consumption is in line with the National Health and Morbidity Survey strategy to fight against the upward trend of NCD occurring in Malaysia.

However, many Malaysians are not familiar with millet. On that note, there may be difficulties in incorporating millet into the local Malaysian food scene. To overcome this challenge, we can look into how other countries consumed millet.

Millets are usually consumed by tribal communities in India and Africa. In these regions, millets are traditionally prepared and consumed as porridge, chapati, noodle and various other local ethnic dishes. Furthermore, millets are suitable to grow in these regions due to their hardy and versatile nature to thrive in hilly areas, which may not be suitable for other crops. Similarly, Malaysia could look to these countries for examples and reference to adopt millet in our local cuisines. As an example, millet flour can be used as a substitute for rice flour or refined flour.

Another approach is by consuming ready-to-eat millet products. There are various millet products that are sold in the market. For example, there are millet cereals, biscuits, and noodles. Some of these products may be fortified with minerals and vitamins, commonly iron or zinc-fortified (Tripathi, Prakash and Platel, 2011; Tripathi and Platel, 2010).

With the findings from this study, we are able to provide micronutrient composition of millet in Malaysia Food Composition Database. This will be the first of its kind, particularly small millets. As a result, Malaysian will now have a wider and informed choice of whole grains options. The existence of millet can now be realised and its health benefits to be spread and made aware to the public.

CHAPTER 5

5.0 Conclusion, Limitations & Future Recommendations

5.1 Conclusion

This study had determined and compared the mineral contents of little millet and barnyard millet. The minerals analysed were potassium, sodium, magnesium, phosphorus, iron, copper, zinc, and calcium. There is no significant difference in mineral content between both millets. The findings revealed that there is a higher content of copper, phosphorus and potassium in little millet than barnyard millet, whereas there is a higher content of sodium, magnesium, zinc, calcium and iron in barnyard millet. For both millets, the mineral levels in descending order with potassium being the most abundant element, followed by phosphorus, sodium, magnesium, zinc, iron, copper and finally calcium.

Comparison of this study with other studies revealed differences in mineral contents in millets. The differences can be accounted for by various factors such as cultivars, pH of soil and its mineral contents, and solvent used in acid digestion.

Refined grains such as white rice has lower mineral content compared to little millet and barnyard millet. Processing of grains plays a vital role in affecting micronutrient levels due to localisation of elements in specific areas of the grain kernel. Both millets from this study are nutritionally more superior to rice in all minerals except calcium.

Although the findings of this study did not reflect the problem statements on addressing iron and calcium deficiency in Malaysia, it points to the potential of millet as nutritionally beneficial grain to combat NCD as it has high potassium content. Potassium is significantly related to lower risk of blood pressure, obesity and metabolic syndrome. With further research, millet can be proved to be a potential healthy alternative in place of white rice because of its nutritional advantage.

5.2 Limitations and Recommendations

There are several limitations in this study. Firstly, there needs to be further analysis on the effect of processing on millets. Processing and preparation of millet, from the moment it is marketed to the cooking method will affect micronutrients of grains.

Furthermore, determination of anti-nutrients in millet needs to be conducted in order to assess its effect on mineral content. Anti-nutrients are natural compounds typically found in plant crops that blocks the absorption of nutrients. They may form insoluble complexes with certain cations such as calcium, magnesium, copper, zinc, iron and copper, thereby limiting the bioavailability of these minerals. Examples of anti-nutrients include phytic acid, oxalates, saponins and tannins. Moreover, the presence of dietary fibre in grains may also hinder mineral absorption.

Additionally, there needs to be further discussion on marketing and incorporation of millet into Malaysian food scene and industry. There are needs to have more ideas on adopting millet into local Malaysian dishes and context.

Another limitation is the usage of hydrochloric acid as a reagent for acid digestion of millet ash. Variation in mineral content of millet may be caused by incomplete or partial acid digestion. According to Uddin et al. (2016), the most efficient digestion method is using a mixture of nitric-hydrochloric acids HNO_3–HCl in a ratio 1:3. In this study, the other digestion of methods of using nitric-perchloric acids HNO_3–$HClO_4$ and B nitric acid HNO_3 alone are not as effective in producing the highest analyte recovery.

Next, this study did not use mineral tablets in order to assess the effectuality of the millet sample preparation. Usage of mineral tablets is performed to ensure that the analytical method and sample preparation is accurate, reproducible and specific. This will minimise errors that are preventable in the first place. Furthermore, future study can include other types of millet commonly found in the market such as finger millet or pearl millet.

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