Promoting Dietary Diversification in the ASEAN Region: Exposing Food Taboos, and Exploring the Nutrient Profiles of Underutilized, Indigenous Food Resources

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This work, made possible by
buckets of sweat, cups of tears, and a few drops of blood,
is dedicated to
my family in the Philippines and here in Germany,
especially to my daughter,
Elisabeth.

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Acronyms and Abbreviations

AA	Amino Acids
AAS	Atomic Absorption Spectrometry
AI	Adequate Intake
APHDA	ASEAN Post-2015 Health Development Agenda
ASEAN	Association of Southeast Asian Nations
AVRDC	World Vegetable Center
BioFoodComp	Food Composition Database for Biodiversity
BMI	Body Mass Index
COVID-19	Coronavirus Disease 2019
DR-NCDs	Diet-related Non-communicable Diseases
FA	Fatty Acids
FAO	Food and Agriculture Organization
FID	Flame Ionized Detection
FSSP	Food Staples Sufficiency Program
GC	Gas Chromatography
GHI	Global Hunger Index
GI	Glycemic Index
GRiSP	Global Rice Science Partnership
HHI	Hidden Hunger Index
HIV/AIDS	Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
IDA	Iron Deficiency Anemia
INFOODS	International Network of Food Data Systems
IR	International Rice
IRRI	International Rice Research Institute
lb	Live Births
MDGs	Millennium Development Goals
PDR	People’s Democratic Republic
PhilRice	Philippine Rice Research Institute
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RAE	Retinol Activity Equivalent
RFI	Reference Nutrient Intake
RNI	Recommended Nutrient Intake
RSI	Recommended Safe Intake
SDGs	Sustainable Development Goals
TE	Tocopherol Equivalent
UN IGME	United Nations Inter-Agency Group for Child Mortality Estimation
UNICEF	United Nations International Children's Emergency Fund
USDA	United States Department of Agriculture

VAD	Vitamin A Deficiency
WHO	World Health Organization
y.o.	years old
ZnD	Zinc Deficiency

Chapter 1 Introduction

1.1 Malnutrition in the ASEAN Region

The Association of Southeast Asian Nations or ASEAN was founded in August 8, 1967 by the foreign ministers of Indonesia, Malaysia, Philippines, Singapore, and Thailand. Currently, it is composed of ten member countries with the inclusion of Brunei Darussalam in 1984, Vietnam in 1995, Lao PDR and Myanmar in 1997, and Cambodia in 1999 (ASEAN Secretariat, 2019).

Although the members of the ASEAN are very diverse in terms of history and culture, economy, demography, geology, and biodiversity, they do face the same challenges when it comes to public health and nutrition. The ASEAN region with a total land area of 4.5 million km^2 (ASEAN Secretariat, 2019), is home to around 660 million people (World Population Review, 2019). Brunei Darussalam is the least populated member with 433,285 inhabitants, while Indonesia has the biggest population at 270,625,568. In the latest Global Hunger Index or GHI (von Grebmer et al., 2019), with the exception of Brunei Darussalam and Singapore with no GHI scores, the rest of the member states are suffering from hunger (Figure 1). The GHI score (Table 1) takes into the account the percentages of the undernourished in the population, wasting and stunting in children under five years old, as well as the under-five mortality rate of a country. Unfortunately, the GHI scores of Brunei Darussalam and Singapore were not calculated either because of unavailable data or because of their small population, the latter being the most probable reason. Thailand, having the lowest GHI score of 9.7 is suffering from "low" hunger. Malaysia, Vietnam, and Myanmar are suffering from "moderate" hunger, while the Philippines, Indonesia, Cambodia, and Lao PDR with the highest GHI of 25.7, are suffering from "serious" hunger. Overall, the data indicate that the ASEAN region is ailing from moderate to serious incidence of malnutrition and child mortality.

Malnutrition is the general term used for the lack of proper nutrition caused by either insufficient or excessive food consumption, low food quality, or the inability of the human body to properly utilize the food that was consumed, exacerbated by poor health care services, water supply, hygiene and sanitation. Being a dynamic region undergoing rapid economic growth and development, the ASEAN populace is actually facing the triple burden of malnutrition, where overnutrition, undernutrition and micronutrient deficiencies co-exist.

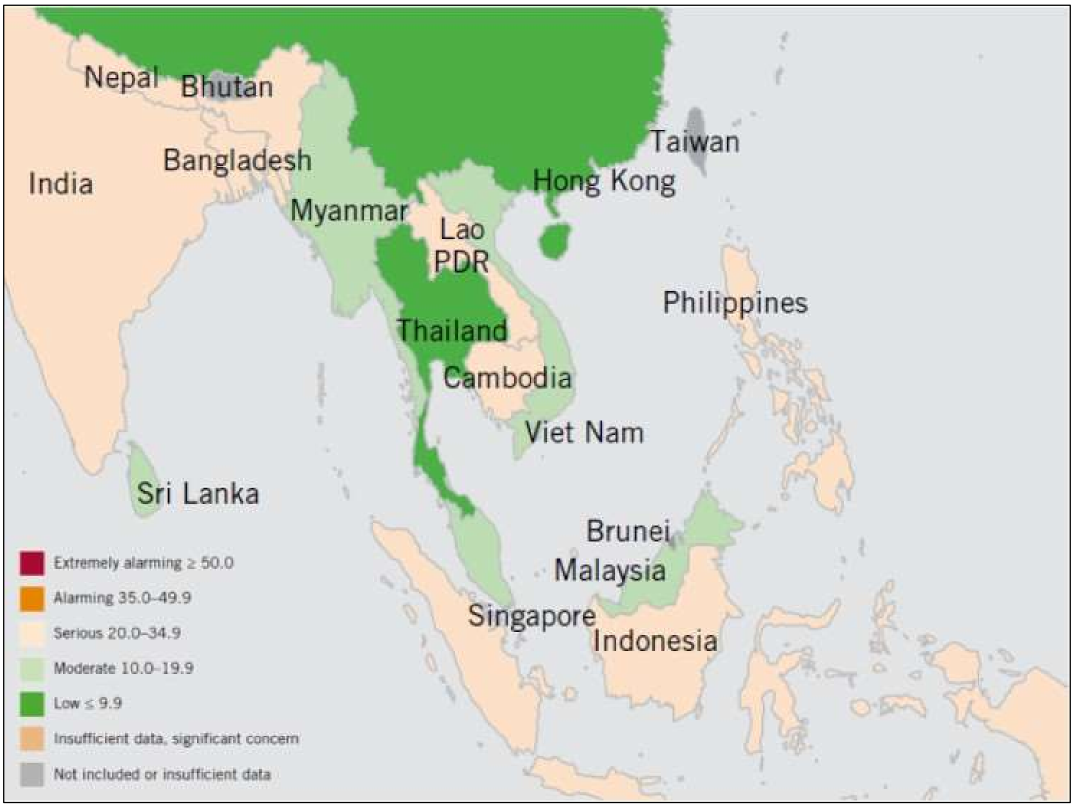

Figure 1. The map of the current Global Hunger Index according to severity in the Southeast Asian region (Source: von Grebmer et al., 2019).

Overnutrition leads to the problem of overweight and obesity. "Overweight" and "obesity" are both defined by the World Health Organization (WHO) as an abnormal or excessive fat accumulation in the body that could be detrimental to the health of an individual (WHO, 2019c). They are both major risk factors for chronic and non-communicable diseases which include diabetes, cardiovascular diseases and cancer, and their prevalence is an emerging problem in Southeast Asia (SEARO, 2019). In 2016, an estimated 41 million children below five years of age were overweight or obese (weight-for-height greater than 2 and 3 standard deviations above the WHO Child Growth Standards median, respectively), and nearly half of this number lived in Asia (WHO, 2019c). As shown in Table 1, among the ASEAN countries, Brunei Darussalam has the highest prevalence of child obesity (17.8%) – children 5 to 9 years old with body mass index (BMI) greater than 2 standard deviations above the median, while Malaysia has the highest prevalence of adult obesity (15.6%) – adults aged 18 years or more with BMI of 30 or higher (WHO, 2016). Next to Singapore, Brunei Darussalam and Malaysia are the second and third richest ASEAN countries in terms of their gross domestic products based on purchasing power parity (International Monetary Fund, 2014). Obesity has been linked to the increase in the consumption of energy-dense foods coupled with the decrease in physical activity, which is the result of transitioning economies, and consequentially, of rapid urbanization and industrialization (WHO, 2019c).

Undernutrition covers both protein energy malnutrition in the forms of wasting, stunting or underweight, as well as micronutrient deficiency. Wasting is an indication of acute undernutrition and is characterized by a low weight-for-height ratio, while stunting, an indication of chronic undernutrition, is characterized by a low height-for-age ratio (Table 1). A person who is underweight has a low weight-for-age ratio, and may be suffering from wasting, stunting, or both. In order to evaluate countries, the WHO (1997) classifies the prevalence of wasting, stunting and undernutrition based on ranges, and is also included in Table 1.

Lao PDR, as shown in Table 2, has the highest percentage of the undernourished in the population (16.5%), at the same time, having the highest percentage of stunted children under five years of age (43.85%, “very high” prevalence). Indonesia has the highest percentage of young children suffering from wasting at 13.5%, denoting “high” prevalence (FAO, 2019; UNICEF-WHO-The World Bank Group, 2018).

Table 1. Definition of undernutrition indicators and their prevalence cut-off values.

Indicator	Definition	Prevalence Cut-off Values			
		Low	Medium	High	Very High
Wasting	Weight-for-height Z-score < -2SD	< 5%	5-9%	10-14%	≥ 15%
Stunting	Height-for-age Z-score < -2SD	< 20%	20-29%	30-39%	≥ 40%
Underweight	Weight-for- age Z-score < -2SD	< 10%	10-19%	20-29%	≥ 30%

Data source: WHO, 1997. Note: SD, standard deviations of the WHO Child Growth Standards median.

Table 2. Current health and nutrition indicators in the ASEAN region.

Country	GHI Score[1]	Undernourished Population (%)[2]	Children < 5 y.o.		Obesity (%)[4]	
			Wasting (%)[3]	Stunting (%)[3]	Child (5-9 y.o.)	Adult (>18 y.o.)
Brunei Darussalam	-	3.2	2.9	19.7	17.8	14.1
Cambodia	22.8	16.4	9.6	32.4	4.5	3.9
Indonesia	20.1	8.3	13.5	36.4	8.5	6.9
Lao PDR	25.7	16.5	6.4	43.8	6.6	5.3
Malaysia	13.1	2.5	11.5	20.7	15.7	15.6
Myanmar	19.8	10.6	7.0	29.2	5.3	5.8
Philippines	20.1	13.3	7.1	33.4	6.1	6.4
Singapore	-	-	3.6	4.4	9.5	6.1
Thailand	9.7	7.8	5.4	10.5	15.0	10.0
Vietnam	15.3	9.3	6.4	24.6	4.5	2.1

Data sources: [1]von Grebmer et al., 2019; [2]FAO, 2019; [3]UNICEF-WHO-The World Bank Group, 2018; [4]WHO, 2016.
Note: GHI, Global Hunger Index; y.o., years old; - no data available; underlined data are the minimum and maximum values in each column.

In the last 30 years, as more attention has been given to micronutrient deficiency, it has gradually moved out of the collective umbrella of "undernutrition". Micronutrient deficiency is dubbed as "hidden hunger", a term coined by UNICEF and WHO (UNICEF, 1990 as cited by Maberly et al., 1994). Micronutrient deficiency is the lack of or the presence of inadequate amounts of essential vitamins and minerals in the body. A person who is suffering from overnutrition can also have micronutrient deficiencies depending on the diversity of their diet. This is an example of the double burden of malnutrition. At the household level, the double burden can be observed when the mother suffers from micronutrient deficiencies, and the breastfed baby has stunted growth. The important micronutrients mainly associated with hidden hunger are Vitamin A, Vitamin D, folic acid, iodine, iron, zinc, and selenium (Maberly et al., 1994; Biesalski, 2013). It is called "hidden hunger" because the clinical signs and symptoms are not as obvious as that of ordinary "hunger". They are often missing, and people suffer the resulting health consequences. Table 3 shows the most current data available on iron, zinc, and Vitamin A deficiencies in the ASEAN region, as well as the Hidden Hunger Index (HHI) score of each member country. The HHI was developed by Muthayya et al. (2013), and it was calculated as the average of the prevalence of stunting, anemia caused by iron deficiency (IDA), and Vitamin A deficiency (VAD) in preschool children. As recommended by the International Zinc Nutrition Consultative Group, Muthayya et al. (2013) used the data on the prevalence of stunting to represent zinc deficiency (ZnD). The HHI scores between 0 and 19.9 were considered mild, 20.0 and 34.9 as moderate, 35.0 and 44.9 as severe, and 45 and 100 as alarmingly high prevalence of micronutrient deficiencies. Among the ASEAN countries, Lao PDR has the highest HHI score with 38.7, which corresponds to having a severe case of micronutrient deficiencies.

One of the most common micronutrient deficiencies in the world is iron deficiency that most often leads to anemia. Anemia is a condition where the body has low red blood cell count or the cells' ability to carry oxygen has been compromised, unable to meet the physiological needs of the body. In its severe form, anemia is linked to problems in cognitive and motor development in children, and causes fatigue, weakness, dizziness and drowsiness, causing low productivity in adults. Pregnant women and children below five years old have the highest risk for anemia. IDA in pregnant women has been associated with higher maternal mortality rate, and poor pregnancy outcomes which include premature birth, and low birth weight babies with iron deficiency status (Chaparro et al., 2014). The prevalence of anemia presented in Table 3 is the percentage of pregnant women and children under five years of age whose hemoglobin value is less than 110 g/L at sea level (The World Bank Group, 2019). Based on the data, in 2016, Cambodia has the highest prevalence of IDA in both vulnerable groups (55.8% of pregnant women and 54.4% of children under five years old), while Brunei Darussalam has the lowest prevalence at 27.1% and 16.1%, respectively.

Another major micronutrient deficiency is VAD. Depending on the duration or severity, VAD can lead to xerophthalmia (which in turn can lead to blindness), anemia, and an increased susceptibility to infection and infectious diseases (Sommer & Davidson, 2002). Table 3 shows the percentage of pregnant women and children below five years old in the ASEAN region with serum retinol concentrations of less than 0.70 μmol/L signifying Vitamin A deficiency. The data were from 2005, the most recent year available in the WHO's global database on VAD. In the ASEAN region, Malaysia has the highest prevalence (22.2%) of Vitamin A deficiency in pregnant women, while Lao PDR has the highest prevalence (44.7%) in children below five years old (WHO, 2009).

Data on zinc deficiency is also shown in Table 3. The available data on inadequate zinc intake were based on national food balance sheets from 2005. Among the ASEAN members, Indonesia has the highest estimated prevalence of ZnD (31.2%), while Philippines has the lowest at 9.9% (Wessells & Brown, 2012). Zinc deficiency has been linked to incidence of poor pregnancy outcomes, problems in child growth and development, and child mortality from diarrhea and acute lower respiratory infections (Brown et al., 2009; Hess & King, 2009).

Table 3. Current data on micronutrient deficiencies in the ASEAN region.

Country	HHI Score[1]	Prevalence of Anemia (%)[2]		Vitamin A Deficiency (%)[3]		Zinc Deficiency (%)[4]
		Pregnant Women	Children < 5 y.o.	Pregnant Women	Children < 5 y.o.	
Brunei Darussalam	-	27.1	16.1	-	-	10.6
Cambodia	31.0	55.8	54.4	16.5	22.3	16.4
Indonesia	27.3	42.0	36.8	17.1	19.6	31.2
Lao PDR	38.7	45.7	43.8	16.6	44.7	14.8
Malaysia	11.7	37.1	30.8	22.2	3.5	11.3
Myanmar	36.3	53.8	52.9	18.0	36.7	19.5
Philippines	30.7	30.3	17.5	17.5	40.1	9.9
Singapore	-	31.8	16.9	-	-	15.4
Thailand	14.7	40.2	28.3	1.7	15.7	20.4
Vietnam	24.0	37.3	33.9	17.7	12.0	10.7

Data sources: [1]Muthayya et al., 2013; [2]The World Bank Group, 2019; [3]WHO, 2009; [4]Wessells & Brown, 2012.
Note: HHI, Hidden Hunger Index; y.o., years old; - no data available; underlined data are the minimum and maximum values in each column.

Because death is the final endpoint for many diseases and severe cases of malnutrition, mortality rates are also important statistics used to gauge the state of public health and nutrition in a country (London School of Hygiene and Tropical Medicine, 2009). As shown in Table 4, Lao PDR has the highest death rate for both children under five years of age (63 deaths per 1,000 live births) and mothers (197 deaths per 100,000 live births), while Singapore has the lowest rates at 3 deaths per 1,000 live births and 10 deaths per 100,000 live births, respectively (UN-IGME, 2018; WHO, 2015). The children under-five and maternal mortality rates are widely used indicators by the WHO, UNICEF, and other international organizations. Their reduction was targeted in the Millennium Development Goals (MDGs) and is still a target in the Sustainable Development Goals (SDGs) (UN, 2015).

Table 4. Mortality rates in the ASEAN region.

Country	Children < 5 y.o. (deaths per 1000 LB)[1]	Mothers (deaths per 100,000 LB)[2]
Brunei Darussalam	11	23
Cambodia	29	161
Indonesia	25	126
Lao PDR	63	197
Malaysia	8	40
Myanmar	49	178
Philippines	28	114
Singapore	3	10
Thailand	10	20
Vietnam	21	54

Data sources: [1]UN-IGME, 2018; [2]WHO, 2015. Note: y.o., years old; LB, live births; underlined data are the minimum and maximum values in each column.

MDG and SDG

The MDGs (Table 5) were drafted in 2000, and the signatories – members of the United Nations, gave themselves 15 years to hit all the targets. In 2015, when the MDGs expired, the SDGs were adopted to deal with the unreached MDGs, at the same time, to tackle emerging challenges in global development. Coincidentally, the ASEAN Community was established in the same year with the motto “One vision, One identity, One Community”. The vision is of an “integrated, peaceful and stable community with shared prosperity” (ASEAN Secretariat, 2019).

There are 17 SDGs (Table 5), with two of the goals directly addressing the problems of health and malnutrition. Goal 2 of the SDGs is to “end hunger, achieve food security and improved nutrition and promote sustainable agriculture”. It aims to achieve by 2025, a significant reduction in stunting and wasting in children below five years old and address the nutritional needs of other vulnerable groups in the community, like those of pregnant and lactating women, and finally, end all forms of malnutrition by 2030. Goal 3 is to “ensure healthy lives and promote well-being for all at all ages”. It targets the reduction of global maternal mortality ratio to less than 70 per 100,000 live births and end preventable deaths of newborns (at least as low as 12 per 1,000 live births) and children under five years old (at least as low as 25 per 1,000 live births) in all countries by 2030.

Table 5. The Millennium and Sustainable Development Goals.

Goal No.	Millennium[1]	Sustainable[2]
	Development Goals	
1	Eradicate extreme poverty and hunger	End poverty in all its forms everywhere
2	Achieve universal primary education	End hunger, achieve food security and improved nutrition and promote sustainable agriculture
3	Promote gender equality and empower women	Ensure healthy lives and promote well-being for all at all ages
4	Reduce child mortality	Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
5	Improve maternal health	Achieve gender equality and empower all women and girls
6	Combat HIV/AIDS, malaria and other diseases	Ensure availability and sustainable management of water and sanitation for all
7	Ensure environmental sustainability	Ensure access to affordable, reliable, sustainable and modern energy for all
8	Develop a global partnership for development	Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
9		Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation
10		Reduce income inequality within and among countries
11		Make cities and human settlements inclusive, safe, resilient, and sustainable
12		Ensure sustainable consumption and production patterns
13		Take urgent action to combat climate change and its impacts by regulating emissions and promoting developments in renewable energy
14		Conserve and sustainably use the oceans, seas and marine resources for sustainable development
15		Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss
16		Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels
17		Strengthen the means of implementation and revitalize the global partnership for sustainable development

Sources: [1]WHO, 2019b; [2]UN, 2019

Within the ASEAN Community, the member countries realized that their individual health systems should be working together to have maximum impact in solving challenging public health issues in the region (ASEAN Secretariat, 2018). In line with the previous MDGs and the present SDGs, the ASEAN adopted the Post-2015 Health Development Agenda (APHDA). The agenda focuses on 20 health priorities under four health clusters (Table 6). From 2016 to 2020, each health cluster leads the identification and implementation of regional programs, projects and other endeavors in relation to the priorities under them. Of note, under Cluster 1, Priority 7 is the "promotion of good nutrition and healthy diet", while under Cluster 3, Priority 14 is the unrealized health-related MDGs (4 - reduce children mortality, 5 - improve maternal health, and 6 - combat HIV/AIDS, malaria and other diseases).

Table 6. The clusters and priorities under the ASEAN Post-2015 Health Development Agenda.

Cluster	Priority No.	Health Priorities
Cluster 1: Promoting Healthy Lifestyle	1	Prevention and control of NCDs
	2	Reduction of tobacco consumption and harmful use of alcohol
	3	Prevention of injuries
	4	Promotion of occupational health
	5	Promotion of mental health
	6	Promotion of healthy and active ageing
	7	Promotion of good nutrition and healthy diet
Cluster 2: Responding to All Hazards and Emerging Threats	8	Prevention and control of communicable diseases, emerging infectious diseases and neglected tropical diseases
	9	Strengthening laboratory capacity
	10	Combating antimicrobial resistance
	11	Environmental health and health impact assessment
	12	Disaster health management
Cluster 3: Strengthening Health Systems and Access to Care	13	Traditional medicine
	14	Health related MDGs (4, 5, 6)
	15	Universal health coverage
	16	Migrants' health
	17	Pharmaceutical development
	18	Human resource development
	19	Health financing
Cluster 4: Ensuring Food Safety	20	Food safety

Source: ASEAN Secretariat, 2018

Guided by all the goals, targets, and priorities, each with their looming deadlines, in 2017, the ASEAN leaders finally issued a declaration, a call to action, to end all forms of malnutrition in the region (ASEAN Heads of Member States/Governments, 2017). To eradicate malnutrition, the ASEAN leaders were eager to scale up nutrition-specific and nutrition-sensitive interventions aimed towards various vulnerable groups in the region. It is now 2020, however, and from the most recent data presented in Tables 2, 3, and 4, it is evident that there is still a long way to go.

1.2 Dietary Diversification as an Intervention

There are two types of interventions that can be done to fight malnutrition. The interventions can either be nutrition-sensitive or nutrition-specific. Nutrition-sensitive interventions are those that address the underlying determinants of nutrition like poverty, food insecurity, and poor health, water and sanitation by tapping into allied sectors dealing with social services, agriculture, health, education and many more. On the other hand, nutrition-specific interventions address the immediate determinants of nutrition of specific vulnerable groups – young children, pregnant and lactating women, and others. Some examples of nutrition-specific interventions are the promotion of exclusive breastfeeding, treatment of severe cases of malnutrition, food fortification, micronutrient supplementation, and dietary diversification (Ruel & Alderman, 2013). Among the interventions, dietary diversification has been touted to be the most sustainable approach in preventing malnutrition starting from the household level (Sundaram, 2014; Bhandari & Banjara, 2015).

“Dietary diversification” basically means that a person’s diet is modified to include sufficient quantity of good quality foods containing most, if not all, the essential nutrients needed by the body. As an approach, its objective is to ensure that nutritious food is available, accessible and can be utilized the whole year round (Gibson & Hotz, 2001). Southeast Asia is rich in natural resources with hundreds of indigenous agricultural products. Considered as the food bowl of Asia (Ooraikul et al., 2008), the huge diversity of the region’s indigenous flora and fauna can be harnessed, may provide the macro- and micronutrients that people need, and be the answer to the malnutrition problem at large.

Figure 2. The typical meals in the ASEAN region.
Photo by Brunei Tourism (Brunei Darussalam), Rathna Hor (Cambodia), Euniche Ramandey (Indonesia), Pasongsin Khamchansana (Lao PDR), Meng Foong Cheon (Malaysia), Aung Ko Win (Myanmar), April Joy Alis-Talas (Philippines), Valerie Iquinia (Singapore), Kedpraveen Kate Hun (Thailand), and Trang Phan (Vietnam)

Figure 2 shows typical meals in the ASEAN region – *nasi-katok* which is rice with fried chicken in Brunei Darussalam, *chha kdov mun* which is also rice with chicken in Cambodia, rice with cucumber, water spinach (*kangkung*), and sautéed tofu in Indonesia, sticky rice and fried egg in Lao PDR, rice with fried chicken and egg in Malaysia, fried rice with roselle leaves and egg omelet in Myanmar, rice with fried fish in the Philippines, *nasi biryani* chicken in Singapore, fried rice with pork in Thailand, and rice with black beans, fried fish, vegetables (steamed, soup) in Vietnam. It is normal to see some kind of meat (chicken, pork or beef) or fish, and vegetables on the plate, together with a good portion of rice or noodles. White rice is the staple food and is usually present in every meal. It is a sad fact however that white rice, aside from being a high glycemic index (GI) food, is low in vitamins and minerals, essential amino and fatty acids, and phytochemicals, and has been paradoxically-linked to both undernutrition and micronutrient deficiencies on one end, and obesity coupled with diet-related non-communicable diseases (DR-NCDs) on the other end (Bhullar & Gruissem, 2013; Birla et al., 2017; Valarmathi et al., 2015). A healthy diet, as defined by WHO (2019a) should provide good nutrition and protect against all forms of malnutrition, as well as DR-NCDs. With dietary diversification, the region's traditional vegetables grown in home gardens or foraged from the fields and forests can be used instead of high value vegetables from the market, edible insects can be affordable substitutes to meat, and pigmented rice varieties can replace white rice.

According to Siemonsma & Piluek (1993), about a thousand plant species can be consumed as food in Southeast Asia, and about half of them are traditional cultivars. In their review of the vegetable sector in Thailand, Nath et al. (1999) were able to identify 32 indigenous and underutilized vegetable crops which included vegetables from the *Cucurbitaceae*, *Solanaceae*, *Leguminosae*, and *Zingiberaceae* families, among others. Edible insects are also abundant in the ASEAN region (Figure 3). Thailand is home to around 194 species, while Indonesia, together with Lao PDR, Malaysia, Myanmar, Philippines and Vietnam have around 150 to 200 edible insect species in total (Sirimungkararat et al., 2010). Entomophagy used to be a part of the food culture in these countries but due to Western influences and other factors, the consumption of edible insects has dwindled from one generation to the next. Similarly, only about 15% of the rice consumed worldwide is in the form of pigmented rice (Deng et al., 2013). Pigmented rice (Figure 4) comes in various shades of red, purple or black, depending on the pigmentation in the rice kernels' pericarp and bran layer (Saenjum et al., 2012; Kitisin et al., 2015). The pigmented rice is milled, just like white rice, however, the milling process is only done to remove the husk or hull, leaving the bran layer, germ and endosperm intact (GRiSP, 2013). The USDA Foreign Agricultural Service Office of Global Analysis (2019) estimated that four ASEAN members belong to the top 10 highest rice producers of the world, with Indonesia ranking 3rd, followed by Vietnam (5th), Thailand (6th), and lastly, the Philippines (8th), and although different pigmented rice varieties are available in the region, their utilization and consumption are still wanting.

Figure 3. Edible insects sold in Thailand.

There are several known factors that hinder dietary diversification. One of these factors is the lack of knowledge in the part of the consumers due to insufficient promotion and marketing of available resources. Although the foods like edible insects were traditionally eaten in the past, most people are now unfamiliar with them, and are unaware of their benefits, health- and nutrition-wise. In some instances where people are actually familiar with them, their socio-economic status dictates if they can access and afford the resources. And, if the resources are available and affordable, the cultural beliefs of the people often lead to food preferences, aversions, and/or taboos (Gibson & Hotz, 2001) that does not permit them to utilize the resources. Food taboos imposed on pregnant and postpartum women, as well as on breastfeeding mothers have been linked to babies with low birth weight, children with micronutrient deficiencies, and subsequently, to an increased risk of maternal and child mortality (Siega-Riz et al., 2009).

Figure 4. Pigmented rice in various colors.

At the regional level, the exploration of traditional vegetables – collection, characterization and promotion is being handled by the World Vegetable Center (AVRDC) East and Southeast Asia with one of their flagship programs, “healthy diets”, with the goal to “increase consumption of a diversity of vegetables among consumers, especially women and children, to improve nutrition and health” (AVRDC, 2019). On the other hand, the exploration of traditional pigmented rice varieties by the ASEAN members is being done by agencies in their respective countries i.e. the Philippine Rice Research Institute (PhilRice), the Rice Department of Thailand, and the various agriculture ministries. The International Rice Research Institute (IRRI) is also headquartered in the Philippines and was able to implement the Heirloom Rice Project under the Food Staples Sufficiency Program (FSSP) of the country’s Department of Agriculture, focusing on the varietal characterization of traditional rice varieties grown in the Cordillera Autonomous Region in Luzon and some regions in Mindanao, conserving biodiversity, optimizing agricultural production, and opening new markets for the rice products. However, currently, the agencies are more interested in rice varieties for climate change adaptation. As for the edible insects, sadly, there are no government agencies that directly handle their promotion and marketing. However, in 2008, the FAO Regional Office for Asia and the Pacific hosted a workshop in Thailand on Asia-Pacific forest resources and their potential for development as food. The workshop was able to bring together researchers on edible insects from Indonesia, Lao

PDR, Philippines, and Thailand and a few others not from the ASEAN. The bamboo borer caterpillars, house crickets, sago grubs, grasshoppers, dung beetles, cicadas, giant water bugs, silkworms, vespa wasps, weaver ants, and bee brood were identified by the participants as the most important edible insects in the Asia-Pacific region. The participants were also able to enumerate some bottlenecks to future development, and as with the traditional vegetables and the indigenous pigmented rice varieties, a major challenge for the researchers and other stakeholders in the field is really how to change people's perceptions to gain mainstream acceptance for these underutilized products.

Nutrient profiling, as defined by WHO (2011), is "the science of classifying or ranking foods according to their nutritional compositions" for health promotion and disease prevention. Some of its applications is in marketing children's foods, product labelling, declaration of health and nutrition claims, and of course, in information dissemination and education. Based on their nutritional composition, foods can be described in terms of levels such as "low in", "high in" and "source of" a particular nutrient, or in terms of the effect of the food to one's health, for example, "less healthy", and "good for you". Nutrient profiling is an important tool as it can help consumers in better understanding the nutrient composition of the foods by translating numbers and percentages into information that they can readily comprehend, and they can use this knowledge in making wholesome food choices (Wills et al., 2009; Grunert, 1997). It has been shown that health information can increase positive feelings towards a product, influence its acceptability, and enhance its purchasability (Roosen et al., 2007; Kozup et al., 2003; Roe et al., 1999). The problem, however, is that there is a scarcity of food composition data for traditional food resources that are not commercially cultivated, reared or farmed, and/or are underutilized albeit being locally available and nutritionally rich. Without these data, nutritional profiles of these underrated foods cannot be framed, and their nutritional values in the face of dietary references e.g. Recommended Safe Intake (RSI), Reference Nutrient Intake (RFI) and Adequate Intake (AI), cannot be calculated.

Currently, there is an online database that holds nutrient composition data of wild and underutilized foods to promote their conservation and sustainable use. Developed by the FAO in collaboration with the International Network of Food Data Systems (INFOODS), the FAO/INFOODS Food Composition Database for Biodiversity (BioFoodComp) had its first version in 2010 with about 2,401 foods, while the latest version 4.0 contains 11,407 food items in 12 food groups, 487 of which are of ASEAN origin (Table 7). From the 487, the most number of food items came from the food group *Cereals*, which contained 182 IR (International Rice) varieties – milled, raw, white or brown rice or just their bran, and five pigmented rice varieties (*Azucena*, *Carreon*, *Makapilay-Pusa*, *Pirurutong*, and *Tapol*) from the Philippines, five items from Thailand, and four from Malaysia. There were, however, no ASEAN contributions to the *Nuts and Seeds*, *Vegetables*, *Eggs*, *Milk*, *Herbs* and *Spices*, and *Miscellaneous* food groups (FAO, 2017).

Table 7. ASEAN's contribution in the FAO/INFOODS Food Composition Database for Biodiversity version 4.0.

Country / Region	Food Groups					
	Cereals	Starchy Roots and Tubers	Legumes	Fruits	Meat	Fish and Shellfish
Brunei Darussalam	-	-	-	-	-	-
Cambodia	-	-	-	-	-	31
Indonesia	-	-	10	3	4	-
Lao PDR	-	-	-	-	1	7
Malaysia	4	-	-	-	2	27
Myanmar	-	-	-	-	-	1
Philippines	187	-	2	-	-	-
Singapore	-	-	-	4	-	-
Thailand	5	5	21	69	20	66
Vietnam	-	-	-	1	4	-
Southeast Asia	-	-	-	13	-	-
Total	196	5	33	90	31	132

Data source: FAO (2017); Note: "-" means no contribution

All entries in the BioFoodComp database are analytical data from primary sources like scientific journals, reports or dissertations, and those coming from the INFOODS network. Researchers are encouraged to directly contribute to the database as data compilers, and it is hoped that more data, especially on micronutrients will be generated and published so they can also be added into the database (FAO, 2017).

1.3 Aims of the Dissertation

This research work was conducted to turn the spotlight towards the ASEAN region, its triple burden of malnutrition, and to dietary diversification as a sustainable way to lighten the load. It tackles one of the stumbling blocks to the acceptance of dietary diversification – food taboos, and one of the stepping stones towards its successful implementation – nutrient profiling of underutilized, indigenous foods in the region.

In order to find out the various food taboos being adhered to in the ASEAN region, a systematic literature review was conducted. Focusing on pregnant, post-partum, and lactating women, plant- (**Chapter 2**) and animal-based (**Chapter 3**) food taboos were collated, and similarities and differences among the ASEAN members were documented.

However, to truly promote dietary diversification in the region, underutilized, indigenous food products must be explored, and interests must be created or revived among the vulnerable groups, health- and nutrition-wise, in the society. Then, routes must be forged from the sources to the plates in the household while circumventing, if not, totally changing people's perspectives on food taboos. In this regard, edible insects and pigmented rice varieties were chosen in this study, and their nutrient profiles were generated to enhance their acceptability into a diversified diet. For edible insects - the Bombay locust (*Patanga succincta*), scarab beetle (*Holotrichia* sp.), house cricket (*Acheta domesticus*), and mulberry silkworm (*Bombyx mori*) from Thailand are featured in **Chapter 4**, and the sago grub (*Rhynchophorus bilineatus*) from Indonesia is in **Chapter 5**. For the pigmented rice varieties, the Camoros (red), Tinta (purple) and Malinao black rice from the Philippines are presented in **Chapter 6,** while a review of the nutritional properties of pigmented rice varieties in Thailand is presented in **Chapter 7**.

Overall, this research work aimed to contribute data and information towards (1) the promotion of dietary diversification, (2) the expansion of the BioFoodComp database for the conservation of biodiversity and sustainable use of indigenous food resources, and (3) the operations of various stakeholders who are working to reach the SDGs on health and the eradication of malnutrition in the ASEAN region.

Chapter 2

Plant-based Food Taboos in Pregnancy and the Postpartum Period in Southeast Asia – A Systematic Review of Literature

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Plant-based food taboos in pregnancy and the postpartum period in Southeast Asia – a systematic review of literature

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Abstract

Purpose – Food taboos during pregnancy and the postpartum period have been linked to increased risk of maternal and neonatal death. This paper aims to present plant-based food restrictions on Southeast Asian women during pregnancy and after giving birth and the rationale behind such cultural practices.

Design/methodology/approach – Google® Scholar, PubMed and Scopus search using the term food taboo, its synonyms and truncations, in combination with the terms pregnancy, postpartum and breastfeeding, and with the name of the Southeast Asian countries, was conducted from January to February 2017. Articles were included in the review if their full texts were accessible online, in English, published from 2005 to 2016 and if they contained primary data from either quantitative or qualitative method.

Findings – A total of 281 articles were downloaded, and 28 were included in this review. The food taboos and the reasons for avoidance were collated and grouped per their occurrence and according to the country or countries where they are practiced. In total, 14 papers generated data on food taboos during pregnancy, 16 papers on postpartum food taboos and/or 6 on breastfeeding.

Research limitations/implications – This review pools together relevant information about plant-based food taboos Southeast Asian women adhere to during pregnancy and after giving birth. However, data are absent for some of the Association of Southeast Asian Nations (ASEAN) countries, and there is a need for more research to get up-to-date information on the local women's adherence to these cultural practices.

Practical implication – The knowledge of these practices can support stakeholders who are contributing to the reduction of maternal and under-five mortality ratios in Southeast Asia.

Originality/value – This is the first review paper on food taboos covering all ASEAN members and highlighting the need for cultural sensitivity to properly address maternal and child health problems in the region.

Keywords Women, Nutrition, Pregnancy, Taboos, Asian studies, Postpartum

Paper type Literature review

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1. Introduction

Food taboo has been defined as a deliberate avoidance of certain food or food groups because of reasons other than just simple dislike (Meyer-Rochow, 2009). In most societies around the world, food taboos, restrictions or prohibitions exist and are associated with various milestones in the human life cycle, from birth to death, and every special event in between (Yusuff, 2013). Passed from one generation to another as a cultural practice, the food taboos have been imposed so people can ease through these life events successfully.

In many cultures, food taboos are commonly practiced during pregnancy and after giving birth in the belief that this can protect the mothers and their babies (Piperata, 2008). The practice, however, can be deemed as counter-productive as these periods of great vulnerability are also periods of increased energy and nutrition needs. As dietary diversification is viewed to be a sustainable approach in fighting malnutrition, the adherence to food taboos promotes the opposite. Taboos on food consumption imposed on pregnant women and lactating mothers have been linked to low-birth-weight babies, micronutrient-deficient breastfed children and subsequently to an increased risk of maternal and neonatal death (Siega-Riz *et al.*, 2009).

The Association of Southeast Asian Nations (ASEAN) is composed of Brunei Darussalam, Cambodia, Indonesia, Lao People's Democratic Republic (PDR), Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam, with roughly 600 million people or 9 per cent of the world's total population. Albeit the diverse geography, economic status and socio-cultural backgrounds of these countries, the ASEAN Community was established in 2015 to pave the way for the Association to rise as a single market much like the European Union. The ASEAN Community has three pillars: the ASEAN Political-Security Community, ASEAN Economic Community and ASEAN Socio-Cultural Community (ASCC). Under the strategic objective of the ASCC blueprint of ensuring access to adequate and affordable health care, medical services and medicine, and the promotion of healthy lifestyles for the people across the ASEAN region, improved maternal, newborn and child health is a priority area and a major challenge (ASEAN Secretariat, 2014).

In 2015, the ASEAN region had a maternal mortality ratio that ranged from 10 maternal deaths per 100,000 live births (lb) in Singapore to 197 maternal deaths in Lao PDR and an under-five mortality ratio that ranged from 3 deaths to 67 deaths per 1,000 lb in these two countries (WHO, UNICEF, UNFPA, World Bank Group and United Nations Population Division, 2015). On the other hand, the focus of most policies, programs and health interventions is on the babies, while the mothers have not received similar attention. The baby's first 1,000 days are very important, but the first 1,000 days of a woman, from conceiving to nourishing the baby up to two years, is as vital and should not be neglected.

This review aims to give an overview on the plant-based food taboos of pregnant and postpartum women and lactating mothers among the ten members of the ASEAN and the rationale behind such cultural practices. It hopes to generate information that can support various stakeholders who are working toward the reduction of the maternal and under-five mortality ratios in Southeast Asia. In addition to this, the review also aspires to contribute to the region's attainment of two of the United Nation's Sustainable Development Goals: "to ensure healthy lives and promote the well-being for all at all ages" and "to achieve gender equality and empower all women and girls" (UN, 2015).

2. Methods

Google® Scholar, PubMed and Scopus searches using the term *food taboo*, its synonyms and truncations, in combination with the terms *pregnancy, postpartum* and *breastfeeding*, and with the names of the different ASEAN-member countries, were conducted from

January to February 2017. The search results were downloaded through the University license. The articles were then screened for relevancy and were included in the review if their full texts were accessible online, in English, published from 2005 to 2016, and, most importantly, if they contained primary data on plant-based food taboos, generated by either quantitative or qualitative method. Studies undertaken within the period of 2005 to 2016 are more likely to reflect the current state of knowledge concerning food taboos in the ASEAN region; however, unpublished dissertations and masters' theses, as well as other reviews, were excluded from this review.

3. Results and discussion

Undernutrition, micronutrient deficiency and overnutrition (obesity) are collectively known as the triple burden of malnutrition. Undernutrition is basically manifested as hunger, while micronutrient deficiency is known as the hidden hunger. Plant-based foods such as cereals and root crops are staple sources of caloric energy, while fruits and vegetables are sources of micronutrients. These foods contain the four key micronutrients involved in hidden hunger: vitamin A, zinc, iron and iodine, as well as calcium and selenium, among other minerals, and vitamins B (thiamine, riboflavin, niacin and folate) and C, among other vitamins, all essential nutrients for pregnant and lactating mothers. They also contain dietary fiber that is known to reduce the risk of preeclampsia in pregnant women (Qiu *et al.*, 2008).

From the online literature searches conducted, a total of 281 articles were downloaded: 217 papers from Google® Scholar, 23 from PubMed and 41 from Scopus. From the 217 papers from Google® Scholar, only 24 were found to be relevant. From the 23 papers from PubMed, 17 were found relevant; however, 13 of these papers were already found using Google® Scholar, and only 4 were actual unique downloads. From the 41 papers from Scopus, 17 were also found relevant; however, from it, 5 papers were already found using Google® Scholar, 3 papers also found using PubMed and 9 papers found in both Google® Scholar and PubMed searches. The Scopus search did not generate any unique download. In total, 28 papers were pooled and included in this review. The plant-based food taboos and the corresponding reasons for avoidance were collated and grouped per their occurrence in the three critical periods in a woman's life – during pregnancy, the postpartum period and while lactating or breastfeeding – and according to the ASEAN-member country or countries where they are practiced.

Traditional practices in pregnancy and childbirth had kept abreast with modern times and are still strong, especially in the rural areas of Cambodia (Montesanti, 2011), but have gradually weakened in Thailand as influenced by a woman's social and economic status (Liamputtong *et al.*, 2005). In Lao PDR, food taboos during pregnancy and after childbirth are common in the rural areas (Barennes *et al.*, 2009; Alvesson *et al.*, 2013). Similarly, in Indonesia, food taboos are significantly more prevalent among rural women compared to women in the urban areas (Hartini *et al.*, 2005). On the other hand, albeit being the largest city and the national capital of Malaysia, the prevalence of food taboos among pregnant women is also high in Kuala Lumpur. In the cross-sectional study conducted in 2016, 73 out of 104 or 70.2 per cent of the pregnant respondents adhered to a food taboo (Mohamad and Yee Ling, 2016).

Postpartum food restrictions are common in the Hmong, Khmu (de Sa *et al.*, 2013), Phuthai, Yao, Akha, Lue and Phunoi ethnic groups of Lao PDR (Holmes *et al.*, 2007) and among the rural populations of Myanmar (Sein, 2013). Likewise, in a study conducted in Ho Chi Minh City in Vietnam, all the respondents ($n = 115$) adhered to restrictions on the type of food that they consumed during their postpartum period (Lundberg and Trieu, 2011).

3.1 Food taboos during pregnancy

While pregnant, a woman's eating behavior is extremely vital. Poor diet, both in quantity and quality, has been linked to overall weakness, iron-deficiency anemia, miscarriages, stillbirths or disabilities in babies and increased risk of maternal death (Klein *et al.*, 2004).

Under the Third Sustainable Development Goal, it is targeted that by 2030, the global maternal mortality ratio should be reduced to less than 70 per 100,000 live births (UN, 2015). Currently, however, five out of the ten ASEAN members are still far from achieving this goal: Lao PDR takes the top rank with 197 maternal deaths per 100,000 live births, followed by Myanmar (178), Cambodia (161), Indonesia (126) and the Philippines (114) (WHO, UNICEF, UNFPA, World Bank Group and United Nations Population Division, 2015).

The various plant-based food taboos of pregnant ASEAN women were listed from 14 research papers. Table I shows the food taboos in Cambodia (Montesanti, 2011; Wallace

Countries	Food Taboos	References
Cambodia, Indonesia, Lao PDR, Malaysia	Rice (crust from boiled black or brown rice, fermented rice, glutinous cake, porridge, sticky rice)	Alvesson *et al.* (2013), Hartini *et al.* (2005), Mohamad and Yee Ling (2016), Montesanti (2011)
Cambodia, Lao PDR, Malaysia	Coconut (fruit, milk, water)	de Sa *et al.* (2013), Mohamad and Yee Ling (2016), Montesanti (2011), Sharifah Zahhura *et al.* (2012)
Cambodia, Malaysia	Chili	Mohamad and Yee Ling (2016), Wallace *et al.* (2014)
Indonesia	Breadnut, candlenut leaves, palm sugar	Hartini *et al.* (2005)
Indonesia, Lao PDR, Malaysia	Sugarcane (branch, juice)	Ali and Howden-Chapman (2007), Hartini *et al.* (2005), Holmes *et al.* (2007), Mohamad and Yee Ling (2016), Triratnawati *et al.* (2016)
Indonesia, Lao PDR, Malaysia, Thailand	Banana (flower, fruit, fused double fruit)	Hartini *et al.* (2005), Holmes *et al.* (2007), Liamputtong *et al.* (2005), Sharifah Zahhura *et al.* (2012)
Indonesia, Malaysia	Durian, fermented cassava, ginger, jackfruit, pineapple	Ali and Howden-Chapman (2007), Hartini *et al.* (2005), Mohamad and Yee Ling (2016), Sharifah Zahhura *et al.* (2012), Triratnawati *et al.* (2016), Widyawati *et al.* (2015)
Lao PDR	Betel nut, mushrooms, vegetables from the forest	de Sa *et al.* (2013), Holmes *et al.* (2007)
Lao PDR, Malaysia	Pumpkin (fruit, leaves)	Holmes *et al.* (2007), Mohamad and Yee Ling (2016)
Lao PDR, Thailand	Medicinal plants (*Kalanchoe laciniata*, *Mirabilis jalapa*, *Phedimus* sp., *Xiphidium caeruleum*)	Lamxay *et al.* (2011), Srithi *et al.* (2012)
Malaysia	Bamboo shoot, black pepper, cabbage, cashew nuts, cucumber, flour, fermented tapioca, kale, long beans, rambutan, spinach, turmeric, watermelon	Ali and Howden-Chapman (2007), Mohamad and Yee Ling (2016), Sharifah Zahhura *et al.* (2012)
Thailand	Coffee, Northern Thai relishes, papaya salad, Thai eggplants, tea	Liamputtong *et al.* (2005)

Table I. Plant-based food taboos of pregnant women in the ASEAN region *et al.*, 2014); Indonesia (Hartini *et al.*, 2005; Triratnawati *et al.*, 2016; Widyawati *et al.*, 2015); Lao PDR (Alvesson *et al.*, 2013; de Sa *et al.*, 2013; Holmes *et al.*, 2007; Lamxay *et al.*, 2011); Malaysia (Mohamad and Yee Ling, 2016; Ali and Howden-Chapman, 2007; Sharifah Zahhura *et al.*, 2012); and Thailand (Liamputtong *et al.*, 2005; Srithi *et al.*, 2012). However, there were no available data for food taboos that are being practiced in Brunei, Myanmar, Philippines, Singapore and Vietnam at this period.

Although the staple food in the ASEAN region, several forms of rice are considered as food taboos during pregnancy in Cambodia, Indonesia, Lao PDR and Malaysia. Banana, a good source of potassium, is taboo in Indonesia, Lao PDR, Malaysia and Thailand; and sugarcane (tall grass plant that is pressed for its juice which is processed into sugar) is taboo in Indonesia, Lao PDR and Malaysia. Coconut fruit is considered as taboo in Lao PDR and Malaysia, coconut milk is taboo in Cambodia, while coconut water, also a food taboo in Malaysia, is considered as a wholesome drink in Cambodia because it is believed to give strength to a pregnant woman, smooth skin to the baby and for an easy delivery (Montesanti, 2011). Indonesia and Malaysia shared several other food taboos which included durian (spiky fruit with sweet-tasting flesh, and a distinct pungent smell), fermented cassava (a tuber better known as tapioca), ginger, jackfruit (spiky fruit with mildly-sweet, sticky, orange-yellow flesh, and sweet aroma) and pineapple. *Kalanchoe laciniata*, *Mirabilis jalapa*, *Phedimus* sp., *Xiphidium caeruleum*, or medicinal plants in general, are food taboos for pregnant women in Lao PDR and Thailand. Chili is taboo for pregnant women in Cambodia and Malaysia, while, pumpkin, rich in beta-carotene, is a food taboo in Lao PDR and Malaysia.

From the research papers reviewed, and from the corresponding reasons why these food taboos are adhered to by pregnant women, the following beliefs were extracted:

- Foods can directly, negatively affect the baby.
- Foods can negatively affect the delivery or childbirth.
- Foods can negatively affect the pregnant woman.
- Foods can negatively affect both mother and child.

Several foods are implicated in causing direct, negative effects on the baby. In Indonesia, durian is viewed as an abortifacient (Hartini *et al.*, 2005). *Kalanchoe laciniata*, *Mirabilis jalapa*, *Phedimus* sp. and *Xiphidium caeruleum* are medicinal plants from Thailand that are used to treat amenorrhea and promote the expulsion of "dead blood" after delivery; however, when taken while pregnant, these plants can cause the abortion of the baby (Srithi *et al.*, 2012). Most of the cited food taboos for pregnant women in Malaysia are imposed because of the fear of abortion as a major reason (Ali and Howden-Chapman, 2007; Mohamad and Yee Ling, 2016).

Aside from causing abortion, some foods have also been linked to deformities and other physical manifestations on the baby. In Thailand, foods believed to cause allergic reactions, and pickled foods like papaya salad, can weaken the baby, and drinking coffee or tea while pregnant is believed to make the baby unintelligent (Liamputtong *et al.*, 2005). In Malaysia, pregnant women consuming flour will deliver babies covered with sticky, white material (Sharifah Zahhura *et al.*, 2012), and in Indonesia, jackfruit has been linked to the formation of a sticky layer of thick fat around the newborn (Hartini *et al.*, 2005). Also in Indonesia, sugarcane can make babies' ears watery (Triratnawati *et al.*, 2016). Coconut milk and porridge in Cambodia (Montesanti, 2011) and banana, coconut and sugarcane in Lao PDR (Holmes *et al.*, 2007; de Sa *et al.*, 2013) are believed to make babies fat, consequently causing difficult deliveries.

Some foods, if eaten during pregnancy, are believed to affect the childbirth or the delivery of the baby negatively; hence, restrictions are imposed. In Lao PDR, glutinous rice cakes and pumpkin leaves have been linked to complications during childbirth and negative effects on the baby's health (Holmes *et al.*, 2007). In Indonesia, breadnut and candlenut leaves are believed to cause difficulties in pushing during birth (Hartini *et al.*, 2005). Similarly, in Malaysia, when jackfruit is eaten, its sticky, rubbery sap is believed to collect inside the womb, making the delivery difficult because every time the baby is pushed forward, it springs back to its original position (Sharifah Zahhura *et al.*, 2012). In Thailand, banana has been implicated in causing birth obstruction (Liamputtong *et al.*, 2005), while in Malaysia, due to its shape, eating a fused double banana is believed to cause a twin pregnancy. Aside from this, cabbage and coconut, being round, resembling the womb but with no opening, are also believed to block the delivery (Sharifah Zahhura *et al.*, 2012).

Food taboos are also implemented because some foods are viewed to have negative impacts on pregnant women. In Indonesia, eating rice crust from black or brown boiled rice can make the placenta sticky in the womb (Hartini *et al.*, 2005). Thai eggplant can cause anal pain after giving birth or during the confinement period, and Northern Thai relishes can prevent the new mother's perineum from drying out properly after giving birth (Liamputtong *et al.*, 2005).

Foods can also have negative impacts on both mother and baby, such as banana, durian, fermented cassava, ginger, jackfruit, palm sugar, pineapple, plain and sticky rice, and sugarcane, as believed in Indonesia (Hartini *et al.*, 2005). Similarly, black pepper, chili, durian, fried foods, ginger, rambutan, turmeric and vinegar are viewed as hot foods in Malaysia (Mohamad and Yee Ling, 2016). In this context, hot foods do not pertain to high temperature or the presence of spices, but to the effect that the foods have on the body of a pregnant woman (Hartini *et al.*, 2005). It is believed that these foods can cause "heat" problems in the stomach and womb, which can lead to possible abortion of the baby and hemorrhage for the mother.

3.2 Food taboos during the postpartum period

The postpartum period which follows the birth of a baby is characterized by changes in the social, mental and physical well-being of a woman as she settles into her new role as a mother. Technically, the postpartum period starts from an hour after the placenta is delivered and ends 42 days after (WHO Technical Working Group, 1999). In developing countries, it was reported that about two-thirds of all maternal deaths occur at this time (Ronsmans and Graham, 2006).

Under the Third Sustainable Development Goal, the end of preventable deaths of newborns and children under five years of age is targeted by 2030, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1,000 live births and under-five mortality to at least as low as 25 per 1,000 live births (UN, 2015). In the latest report by the UN, five out of ten ASEAN members are above the 12 neonatal death per 1,000 live births target, with Lao PDR topping the list at 29 neonatal deaths, followed by Myanmar (25), Cambodia (16), Indonesia (14) and the Philippines (13), while five out of the ten ASEAN members are still beyond the goal for under-five mortality. Ranked from highest to lowest, Lao PDR takes the top rank at 64 deaths per 1,000 live births, followed by Myanmar (51), Cambodia (31), Philippines (27) and Indonesia (26) (UN-IGME, 2017).

Different cultures in the ASEAN region adhere to different postpartum practices which constitute a period of rest, coupled with food and mobility restrictions. The Malay women (Malay, Javanese, Buginese or Minangkabaus descent) undergo a confinement period (*masa dalam pantang*) of 40-44 days (Fadzil *et al.*, 2016). Chinese women are known for "doing the month" (*zuo yuezi*), which takes 30 days (Koon *et al.*, 2005), while it takes 40 days for women of Indian descent (Ariff and Beng, 2006). Similar to Cambodians (Hoban, 2002), Thai women adhere to the traditional practice of confinement by fire or *yu fai* (Dennis *et al.*, 2007) or *yu duan* as mentioned in another study (Liamputtong *et al.*, 2005) for 30 days after giving birth. Women following the Islamic faith adhere to a 44-day confinement period (Abdul-Mumin, 2016). On the other hand, women in Lao PDR adopt *phit kam* or a restricted diet after giving birth for an average of two weeks (Boer *et al.*, 2011), while *phit kam* means "wrong condition" after childbirth in Thailand (Elter *et al.*, 2016).

In total, 16 research papers cited various plant-based food taboos for ASEAN women during their postpartum period. However, data on food taboos that are being practiced in Brunei, Cambodia, Philippines and Singapore were not available. Table II shows the food taboos in Indonesia (Hasan and Suwarni, 2012); Lao PDR (Barennes *et al.*, 2009; Srithi *et al.*, 2012; de Sa *et al.*, 2013, 2013; Holmes *et al.*, 2007; Barennes *et al.*, 2015; Boer and Lamxay, 2009; Bouphavanh *et al.*, 2009); Malaysia (Koon *et al.*, 2005; Jamaludin, 2014); Myanmar (Sein, 2013; Diamond-Smith *et al.*, 2016; Sheehy *et al.*, 2016); Thailand (Srithi *et al.*, 2012; Panyaphu *et al.*, 2011; Roesler *et al.*, 2014); and Vietnam (Lundberg and Trieu, 2011).

All sorts of vegetables, from fresh to fermented, green leafy to yellow flowering, to hairy or bitter, and strong-smelling ones, are not allowed to be eaten by postpartum women in Indonesia, Lao PDR, Malaysia, Myanmar, Thailand and Vietnam. Other vegetables considered as taboo include chili in Indonesia, Lao PDR, Myanmar and Thailand; bamboo in Lao PDR, Myanmar and Thailand; young pumpkin fruit, leaves and tops in Lao PDR, Malaysia and Thailand; water spinach, watercress, beans, sprouts and other legumes, as well as cucumber, in Malaysia and Myanmar; cabbage, lettuce and cassava tuber and its leaves in Lao PDR and Malaysia; and mushrooms in Lao PDR and Myanmar. Aside from vegetables, women's fruit consumption is also restricted. Fresh fruits, in general, are taboo in Lao PDR, Malaysia, Thailand and Vietnam. Other fruit taboos include pineapple, papaya and watermelon in Lao PDR and Malaysia and mango in Malaysia and Myanmar. Also, in Myanmar, there is a superstition that everything else, except fried fish and rice or soup, is taboo for women undergoing their postpartum period (Sheehy *et al.*, 2016). Aside from micronutrient deficiency, the absence of a high-nutrient, low-calorie diversified diet has been linked to obesity worldwide (Ruel, 2003), also a growing concern in developing countries.

The reasons why women adhere to these food taboos during the postpartum period can be grouped into two categories:

(1) adherence to the culturally prescribed confinement practices; and

(2) avoidance of postpartum sickness and overall bad health.

According to Chinese tradition, the mother is in the period of strongest *yin* (cold) during the postpartum period; hence, her diet should be composed of *yang* (hot) foods. She should avoid cold foods such as vegetables and fruits for her body to regain its balance (Koon *et al.*, 2005). During the postpartum period, it is believed that women in Lao PDR may encounter *pit duen* or postpartum sickness, which includes symptoms such as fever, body ache, numbness, tingling, weakness and bleeding that could lead to death (de Sa *et al.*, 2013), while women in Thailand may similarly encounter *phit kam*, with symptoms include dizziness, fainting, fever, headaches or death (Elter *et al.*, 2016). Food taboos are followed to avoid these conditions, with the belief that the avoidance will protect the mother and child from further negative complications such as getting leprosy, deafness, having a deformed abdomen, suffering from womb itchiness, slow wound healing, either loose bowel movement or constipation, hypertension, drowsiness and allergic reactions. On the other hand, the

Countries	Food Taboos	References
Indonesia, Malaysia	Coconut (oil, water)	Hasan and Suwarni (2012), Jamaludin (2014)
Indonesia, Lao PDR, Myanmar, Thailand	Chili	Diamond-Smith *et al.* (2016), Hasan and Suwarni (2012), Holmes *et al.* (2007), Roesler *et al.* (2014)
Indonesia, Lao PDR, Malaysia, Myanmar, Thailand, Vietnam	Vegetables (bitter, fermented, fresh, green leafy, hairy, strong-smelling, yellow flowering)	Barennes *et al.* (2009), Barennes *et al.* (2015), Bouphavanh *et al.* (2009), Boer and Lamxay (2009), de Sa *et al.* (2013), Diamond-Smith *et al.* (2016), Hasan and Suwarni (2012), Holmes *et al.* (2007), Jamaludin (2014), Koon *et al.* (2005), Lundberg and Trieu (2011), Roesler *et al.* (2014), Srithi *et al.* (2012)
Lao PDR	Banana, *bouan* and *kadom* fruits, cauliflower, coffee, eggplant, ginger, *leuang* flower, *mak nam tao*, melon, onion, spring onion, river weed, taro leaves	Bouphavanh *et al.* (2009), de Sa *et al.* (2013), Holmes *et al.* (2007)
Lao PDR, Malaysia	Cabbage, cassava (tuber, leaves), lettuce, papaya, pineapple, striped watermelon, tea	Bouphavanh *et al.* (2009), de Sa *et al.* (2013), Holmes *et al.* (2007), Jamaludin (2014), Koon *et al.* (2005)
Lao PDR, Malaysia, Thailand	Pumpkin (fruit, leaves, tops)	de Sa *et al.* (2013), Holmes *et al.* (2007), Jamaludin (2014), Panyaphu *et al.* (2011)
Lao PDR, Malaysia, Thailand, Vietnam	Fruits (fresh, 'itchy', with white sap)	Barennes *et al.* (2009), Boer and Lamxay (2009), de Sa *et al.* (2013), Holmes *et al.* (2007), Jamaludin (2014), Koon *et al.* (2005), Lundberg and Trieu (2011), Srithi *et al.* (2012)
Lao PDR, Myanmar	Mushrooms	de Sa *et al.* (2013), Sein (2013)
Lao PDR, Myanmar, Thailand	Bamboo (*dtao*, shoots)	de Sa *et al.* (2013), Panyaphu *et al.* (2011), Sein (2013)
Malaysia	*Brinjal*, *cempedak*, corn, fern shoot, *nangka*, potato, *siew pak choy*, starfruit, sugarcane, sweet potato, yam	Jamaludin (2014), Koon *et al.* (2005)
Malaysia, Myanmar	Beans, sprouts and other legumes, cucumber, *kang kung*, mango, water convolvulus or watercress	Diamond-Smith *et al.* (2016), Jamaludin (2014), Koon *et al.* (2005), Sein (2013)
Myanmar	All foods except fried fish and rice or soup, bean tendrils, bitter gourd (fruit, stem), *danjin*, pickled tea, sour food (leaves, roselle, tamarind, and snack made of plums), tomato	Diamond-Smith *et al.* (2016), Sein (2013), Sheehy *et al.* (2016)
Myanmar, Thailand	Sticky rice	Panyaphu *et al.* (2011), Sein (2013)

Table II. Plant-based food taboos of postpartum women in the ASEAN region

adherence to food taboos during the postpartum confinement is one of the cultural practices linked to postnatal depression (Yusuff, 2013). In Northern Lao PDR, the strict practice of mothers eating nothing else but water- or tea-soaked milled glutinous rice during the postpartum period has been linked to breastmilk with poor nutritional quality, leading to infantile beriberi (Barennes *et al.*, 2015).

3.3 Food taboos while breastfeeding

Postpartum breastfeeding is the natural way of providing babies with much-needed nutrients for their survival and optimal growth and development. It should be initiated within the first hour right after delivery, and exclusive breastfeeding is recommended up to six months, continuing up to two years of age or beyond, together with suitable complementary foods. Breastfeeding is not just beneficial to the child, it is also known to have good long-term effects on the health of the mother (WHO, 2018).

Only 38 per cent of infants under six months of age are exclusively breastfed in the world (UN-IGME, 2015). According to a report published in 2012, 75 per cent or more infants are exclusively breastfed in Cambodia, around 33 per cent in the Philippines, Indonesia and Malaysia, 25 per cent in Lao PDR and Myanmar and at less than 20 per cent in Thailand and Vietnam, while no data were supplied for Brunei and Singapore (OECD/WHO, 2012). Sub-optimal breastfeeding in the first six months of a baby's life was estimated to be responsible for 1.4 million deaths in children less than five years of age (WHO, 2009).

Six research papers contained information on the different plant-based food taboos in the ASEAN region for breastfeeding or lactating mothers (Table III); however, the only data available were from Cambodia (Nikles, 2008; Wallace *et al.*, 2014), Lao PDR (Holmes *et al.*, 2007; Lee *et al.*, 2013), Myanmar (Diamond-Smith *et al.*, 2016) and Singapore (Ong *et al.*, 2014). Based on the reasons for the adherence to the food taboos, three themes emerged:

(1) Foods (i.e. vegetables, bamboo shoots, chili, Chinese watercress, ginger and pumpkin and sour leaves) may cause harm to the baby.

(2) Foods (i.e. *chek nuon*, a type of banana) may cause harm to the mother.

(3) Foods (i.e. fruits and vegetables – melon, pumpkin tops, banana flower and chili) may affect the quantity of the breast milk.

As long as new mothers are given relevant information and support by their families, health-care practitioners and the community, every woman can breastfeed (WHO, 2016). Physiologically, however, breastfeeding is the most energetically demanding stage of the reproductive cycle, increasing the energy needs of the mothers by 25-30 per cent (Prentice *et al.*, 1996). On the other hand, cultural practices such as adherence to food taboos can make it more difficult for them to meet the additional energy needs (Piperata, 2008) and micronutrient demands of their recuperating bodies.

4. Conclusion

This review pools together relevant information about plant-based food taboos Southeast Asian women adhere to during pregnancy and after giving birth. However, data are absent

Countries	Food Taboos	References
Cambodia, Lao PDR	Banana (*chek nuon*, flower), vegetables	Holmes *et al.* (2007), Lee *et al.* (2013), Nikles (2008), Wallace *et al.* (2014)
Lao PDR	Bamboo shoots, fruits, melon, pumpkin (leaves, tops)	Holmes *et al.* (2007), Lee *et al.* (2013)
Lao PDR, Myanmar	Chili	Diamond-Smith *et al.* (2016), Holmes *et al.* (2007)
Myanmar	Chinese water cress, sour leaves	Diamond-Smith *et al.* (2016)
Singapore	Ginger	Ong *et al.* (2014)

Table III. Plant-based food taboos of breastfeeding women in the ASEAN region for some of the ASEAN countries, and there is a need for more research to get up-to-date information on the local women's adherence to these cultural practices.

Human behavior shaped by culture and tradition is deeply rooted in the fear of repercussions and is often hard and slow to change. Nutrition education, a key tool in maternal and child health interventions, should go hand in hand with the knowledge of various stakeholders, especially the community health workers, of these cultural practices. It is recommended that women beneficiaries should be asked if they believe and adhere to these practices and for them to be educated to allay their fears, or at the least, to be guided by prescribing them with food supplements and alternative sources of nutrients, to ensure a diversified diet and to minimize the possible negative health impacts of the food taboos that they adhere to.

Under the ASEAN Community, the divides in trade and economics are blurred; in contrast, however, to properly address maternal and child health problems in the region, cultural sensitivity when educating and empowering women among the ASEAN members should be highlighted, as beliefs such as food taboos vary from one woman, family, tribe or village to another.

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Chapter 3

Animal-based Food Taboos during Pregnancy and the Postpartum Period of Southeast Asian Women – A Review of Literature

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Review

Animal-based food taboos during pregnancy and the postpartum period of Southeast Asian women – A review of literature

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ABSTRACT

This paper gives an overview of the various taboos on animal products and the reasons behind such practices among women in the member countries of the Association of Southeast Asian Nations (ASEAN), while they are pregnant, during their postpartum period, and while breastfeeding.

Three data search engines, Google® Scholar, PubMed and Scopus, were used one after the other, to generate the papers for this review. The online searches using the term food taboo, its synonyms, and truncations, in combination with the terms pregnancy, postpartum, and breastfeeding, and with the country name, were conducted from January to February 2017. In total, 28 papers were pooled and included in this review. The taboos and the justification for avoidance were collated and grouped per their occurrence, and according to the country where they are practiced. Nine papers provided information on the food taboos during pregnancy, 16 on postpartum food taboos, and six on breastfeeding. The food taboos included various river or seafood, meat from terrestrial animals, and derived products like fish paste, blood and internal organs.

Healthcare providers should be aware of the food taboos being adhered to in the region to provide the right information and guidance to the women practicing them. There is a need to educate the public in general, and women, in particular, about the risk of malnutrition when food taboos are followed, and to support their nutritional requirements during pregnancy and the postpartum period by promoting dietary diversification.

More research could be conducted to fill in the gap in information with regards to the food taboos and their impact in some of the ASEAN members. Culture-sensitive health interventions based on timely research work can contribute to the attainment of the sustainable development goal of reducing the maternal and under-five mortality ratios in Southeast Asia.

1. Introduction

The Association of Southeast Asian Nations (ASEAN) is composed of Brunei Darussalam, Cambodia, Indonesia, Lao People's Democratic Republic (PDR), Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam. The adherence to food taboos, which is in contrast with the principles of dietary diversification where eating a variety of food is encouraged to acquire all the essential nutrients, thrives in this region, especially in the vulnerable periods of pregnancy, postpartum, and while breastfeeding (Köhler, Sae-Tan, Lambert, & Besalski, 2018). This paper gives an overview of these taboos on animal products and the reasons behind such practices among ASEAN women.

Food taboos have been previously linked to malnutrition leading to babies with low birth weight, breastfed children with micronutrient deficiencies, and subsequently, to an increased risk of maternal and child death (Siega-Riz et al., 2009). The daily energy requirements for a healthy woman of normal weight and who has a moderately active lifestyle increases during pregnancy and is dependent on the developmental stage of the fetus (Marangoni et al., 2016). According to EFSA (2013), a 12-kg mean increase in the body mass of a pregnant woman will lead to optimal maternal and fetal health outcomes. To support the increase in body mass, the average additional energy requirement for pregnancy is 320 MJ (76,530 kcal) which equates to approximately 0.29 MJ/day (70 kcal/day) in the first trimester, 1.1 MJ/day (260 kcal/day) in the second and 2.1 MJ/day (500 kcal/day) during the third trimester. For exclusively-breastfeeding mothers in the first six months postpartum, an additional 2.1 MJ/day (500 kcal/day) on top of the energy requirement before birth which includes 2.8 MJ/day (670 kcal/day) for milk production and an energy mobilization from maternal tissues of 0.72 MJ/day (170 kcal/day) is required.

The macronutrients – protein and fat, are the energy providers from animal-based foods. During pregnancy, focus should be given to protein

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in the diet as its demand in the body to support protein biosynthesis needed to supply the requirements of maternal tissues and support the growth and development of the fetus progressively increases, peaking at the last trimester (Marangoni et al., 2016). Animal products have better protein quality, providing complete essential amino acids compared to plant products (FAO, 2013). Animal products also have the highest protein digestibility, while legumes have the lowest (EFSA, 2013).

Like protein, fat is more than just an energy source during pregnancy. In this period, the quality of fat is more important than the quantity, and the focus should be on the intake of polyunsaturated fatty acids (PUFAs) like docosahexaenoic acid (DHA) rather than increasing the total fat content. DHA is essential for baby's brain and retina growth and development, and also has a positive impact on maternal health as it reduces the risk of premature delivery and postpartum depression (Mennitti et al., 2015; Sallis, Steer, Paternoster, Davey Smith, & Evans, 2014). Because of the limited ability of the human body to synthesize long-chain PUFAs, it is imperative that they be obtained in high concentrations from fatty fishes like mackerel, anchovies, and salmon (Mozaffarian & Rimm, 2006). Reaching the DHA requirement during pregnancy and when breastfeeding redounds to higher DHA content in breastmilk, and better visual acuity and cognitive development in breastfed babies (Koletzko, Cetin, & Brenna, 2007).

Animal-based foods also contain important micronutrients which include the minerals iron, iodine, calcium, and vitamins A, D and folate. Sufficient amounts of micronutrients are required during pregnancy, and inadequate intakes can negatively affect the maternal and fetal health (Marangoni et al., 2016).

2. Methodology

Three data search engines, Google® Scholar, PubMed and Scopus, were used one after the other, to generate the papers for this review. The online searches using the term *food taboo*, its synonyms, and truncations, in combination with the terms *pregnancy*, *postpartum*, and *breastfeeding*, and with the names of the ASEAN-member countries, were conducted from January to February 2017. The search results were downloaded through the University license. The articles were then screened for relevancy and were included in the review when they fulfilled four criteria: (1) the full text was accessible online, (2) written in English, (3) published from 2005 to 2016, and most importantly, (4) have primary data on food taboos, generated by either quantitative or qualitative research methods. Studies undertaken within the period specified are believed to be more likely to reflect the most recent state of knowledge concerning food taboos in the region, however, unpublished dissertations and masters' theses, as well as other reviews were excluded from this review. The taboos were then organized whether they are on plant- or animal-based foods. The plant-based food taboos were tackled in a separate paper (Köhler et al., 2018), while this paper focuses on animal-based food taboos.

During the online literature search, 281 articles were downloaded: 217 from Google® Scholar, 23 from PubMed, and 41 papers from Scopus. From the 217 papers from Google® Scholar, 26 were found to contain relevant information on animal-based food taboos. From the 23 papers from PubMed, two were found relevant and unique, not having been found during the searches conducted through Google® Scholar. From the 41 papers from Scopus, two were also found relevant and unique from the Google® Scholar search results, however, these were the same two articles found using PubMed. In total, 28 papers were pooled and included in this review. Nine papers provided information on the food taboos during pregnancy, 16 on postpartum food taboos, and six on breastfeeding.

3. Food taboos during pregnancy

Shown in Table 1 are the animal-based food taboos adhered to by pregnant women in Indonesia (Hartini, Padmawati, Lindholm, Surjono, & Winkvist, 2005; Widyawati, Utomo, van Dillen, & Janssen, 2015), Lao PDR (Holmes et al., 2007; Alvesson, Lindelow, Khanthaphat, & Laflamme, 2013; de Sa et al., 2013), Malaysia (Mohamad & Yee Ling, 2016; Sharifah Zahhura, Nilan, & Germov, 2012), Philippines (Ramos, 2012), and Thailand (Liamputtong, Yimyam, Parisunyakul, Baosoung, & Sansiriphun, 2005). No information was found for Brunei Darussalam, Cambodia, Myanmar, Singapore, and Vietnam.

The most common food taboo during pregnancy mentioned in the five countries was river or seafood. Various reasons for the avoidance were cited and ranged from the fear of abortion (Mohamad & Yee Ling, 2016), to the occurrence of odorous blood (Widyawati et al., 2015), convulsions (Sharifah Zahhura et al., 2012), sickness, vomiting, dizziness, edema, and difficulties and delay in the delivery (Mohamad & Yee Ling, 2016; Sharifah Zahhura et al., 2012). Further reasons are the fear of complications like the fetus being in the wrong position (Hartini et al., 2005), cognitive impairment of the child (Mohamad & Yee Ling, 2016), perineum not drying out properly after giving birth (Liamputtong et al., 2005), and the baby having dark skin (Mohamad & Yee Ling, 2016).

River or seafood mentioned in the reviewed articles included fish, eel, catfish, shrimp, stingray, mackerel, shellfish, and the derived product - fermented fish sauce. They are not only good protein and PUFA sources, but they are also rich in micronutrients like iodine, vitamin A and D.

In a report by Chaparro, Oot, and Sethuraman (2014), maternal malnutrition is an area of concern among the ASEAN members, particularly iodine deficiency in Myanmar, the Philippines, and Vietnam, and vitamin A deficiency in Cambodia, Lao PDR and Myanmar. Iodine is a major component of thyroid hormones and is essential for growth, formation and development of organs and tissues, in addition to the metabolism of glucose, proteins, lipids, calcium and phosphorus, and thermogenesis (EFSA, 2014). In pregnancy, iodine deficiency can increase the risk of spontaneous abortion, perinatal mortality, congenital disabilities and neurological disorders (Trumpff et al., 2015), and is considered by the WHO as the most important preventable cause of brain damage. Fish and shellfish extract the iodine from the algae that they ingest, and the algae absorbs the mineral from marine water (Marangoni et al., 2016).

Vitamin A is not just a single compound but is composed of fat-soluble retinoids, which include retinol, retinal, and retinyl esters. Vitamin A is involved in the body's immune function, vision, and cellular growth and differentiation. There are two forms of the vitamin available from the food that we consume: preformed vitamin A consisting of retinol and its esterified form, retinyl ester, and provitamin A carotenoids (Johnson & Russell, 2010). Typically found in foods from animal sources, preformed vitamin A is present in dairy products, eggs, fish, and meat, with the highest concentrations found in liver and fish oils (Ross, 2010).

Similarly, good amounts of vitamin D are also contained in fatty fish such as herring and salmon, and high amounts in cod liver oil. Vitamin D is the collective name for two molecular species that share vitamin activity: ergocalciferol (vitamin D2, derived from ergosterol, found in vegetables) and cholecalciferol (vitamin D3, derived from cholesterol and synthesized by animals). In the first stage of pregnancy, vitamin D3, the predominant form in the maternal blood, is involved in regulating cytokine metabolism and in modulating the immune system, thereby contributing to the embryo implantation and the control of several hormones (Marangoni et al., 2016). Despite the abundance of sunlight in the ASEAN region, vitamin D deficiency is prevalent, ranging from 6 to 70% (Nimitphong & Holick, 2013). The common predictors of having low vitamin D status include being young, inactive, female and urban-dweller (Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2011; Robien et al., 2013). Vitamin D deficiency is prevalent during pregnancy and is associated with an increased risk of developing pre-eclampsia and gestational diabetes mellitus. Low birth weight, impaired skeletal development, respiratory infections and

Table 1
Animal-based food taboos of ASEAN women during pregnancy.

Country	Reference	Food taboo	Justification
Indonesia	Hartini et al. (2005)	River or seafoods like eel, catfish, shrimp	Difficulties during delivery because the fetus would be upside down in the womb
		Chicken eggs	Women will perform like a chicken during delivery, they would have longer labor
		Lamb barbecue	Heat problems in the stomach and womb, cause stomachache and diarrhea, and cramping of the womb, vomiting and even abortion
	Widyawati et al. (2015)	Meat, fish, eggs	Make blood odorous
Lao PDR	Holmes et al. (2007)	Meat from wild animals, wild animals with babies, domestic animals with babies	Difficulties during delivery and the child will not be healthy and may become disabled
		Meat from animals killed by a tiger	–
	Alvesson et al. (2013)	Stingrays, monkeys, rats, snakes	Complications during childbirth
	de Sa et al. (2013)	Pork	Make the baby fat and cause a difficult birth
		Fermented fish sauce	*Amlue* or not good
Malaysia	Mohamad and Yee Ling (2016)	Meat, chicken liver, seafoods like mackerel	Fear of abortion, difficult labor, unnecessary sickness, vomiting, dizziness, edema, dark skin baby, the baby may be born with cognitive impairment
	Sharifah Zahhura et al. (2012)	Fish caught by other people besides the husband using a chain-weighted circular throwing net, caught by line fishing, caught by bubu (a type of fish trap)	Obstructed labor resulting to a difficult delivery
		Deer, mousedeer, antelope, *ikan kelah* (greater brook carp)	*Sawan* or convulsions
		Fish caught using a tuba (poison), game animals killed by blowpipe, or shotgun	Poison might harm the unborn child
		Game animals caught using a trap	Baby will be trapped in the mother's womb
		Porcupine	Baby will have blistered skin that resembles the porcupine's spines
Philippines	Ramos (2012)	Inner meat of an animal	Bad for the baby
		Shrimps, eel	Delay the delivery
Thailand	Liamputtong et al. (2005)	Shellfish	Prevent the perineum from drying out properly after giving birth

allergies in babies are often linked with the inadequate contribution of vitamin D from the mother's diet (De-Regil, Palacios, Lombardo, & Peña-Rosas, 2016).

On the other hand, meat products like lamb barbecue, pork, meat from domestic and wild animals with babies, wild animals (monkey, rat, snake, deer, mousedeer, antelope, porcupine), those caught using a trap or killed by a tiger, blowpipe or shotgun, chicken liver and inner meat (other internal organs) were not allowed to pregnant women based on similar reasoning as with the river or seafood taboos. In addition, pork is taboo as it is believed that the baby will become fat and can cause a difficult birth (de Sa et al., 2013), while eating porcupine meat will cause the baby to have blistered skin that resembles the porcupine's spines (Sharifah Zahhura et al., 2012). Aside from being a good source of protein, meat products are also an important source of iron and folates.

Iron plays a vital role in the transfer of oxygen to tissues and is also involved in various enzymatic reactions in the body. Iron deficiency causes anemia, and in 2011, 25% of children under the age of five, 21% of non-pregnant women aged 15 to 49 years, and 25% of pregnant women in the same age group in East and Southeast Asia were afflicted (Stevens et al., 2013). Meat and fish, aside from legumes and green leafy vegetables are the main dietary sources of iron. Iron can either be in the heme or non-heme form. Heme iron, derived from sources such as myoglobin and hemoglobin in meat, is better absorbed by the human body compared with non-heme iron (Marangoni et al., 2016).

Folates play a crucial role in many chemical processes in the body, like the biosynthesis of DNA and RNA, methylation of homocysteine to methionine, and amino acid metabolism. They are therefore essential for health, and inadequate dietary levels can lead to anemia, leucopenia, and thrombocytopenia (Hoey, McNulty, Duffy, Hughes, & Strain, 2013). Folates are mostly found in green leafy vegetables, fruits, cereals and in the internal organs of animals. One of the best sources of folates is chicken liver with only a portion weighing 20 to 25 g already sufficient to meet the desirable nutrient density for folates and vitamin A, at the same time (WHO/FAO, 2004). The requirement for folates undergoes a steady increase from before conception to the early stages of pregnancy, in association with the development of fetal cells and tissues (Berti et al., 2012). Folic acid supplementation is widely recommended to women of childbearing age, especially to reduce the risk of neural tube defects and congenital heart disease, as well as to support the proper development of the placenta (De-Regil, Fernández-Gaxiola, Dowswell, & Peña-Rosas, 2010).

4. Food taboos during the postpartum period

Shown in Table 2 are the animal-based food taboos adhered to by women in their postpartum period in Indonesia (Hasan & Suwarni, 2012), Lao PDR (Holmes et al., 2007; Eckermann & Deodato, 2008; Bouphavanh, Vongkhily, Xayyalath, & Ongroongruang, 2009; Barennes et al., 2009; Lamxay, de Boer, & Bjork, 2011; de Sa et al., 2013), Malaysia (Haron & Hamiz, 2014; Jamaludin, 2014; Koon, Peng, & Karim, 2005), Myanmar (Diamond-Smith, Thet, Khaing, & Sudhinaraset, 2016; Sein, 2013), Thailand (Elter, Kennedy, Chesla, & Yimyam, 2014; Panyaphu et al., 2011; Roesler, Smithers, & Moore, 2014), and Vietnam (Lundberg & Ngoc Thu, 2011). The literature search yielded no information for Brunei Darussalam, Cambodia, Philippines, and Singapore.

There are more food taboos during the postpartum period than during pregnancy. River or seafood is still taboo in the region, and include fishes like *kouan*, *khae*, *kheung*, red, *nai*, *panae*, *ahnyigyi* (a kind of fish with a strong nauseating smell) snake-head, Nile tilapia, cuttlefish (*Restrilleger kanagurta*), *hilsa* (*Hilsa ilisha*), shark and fish with no scales (*kembung*, *pari*, tuna, sardine and *bilis*), catfish (*Sa wai* fish or stripped catfish, river catfish and striped dwarf catfish), eel, ray, shellfish including crustaceans (crab, prawn), mollusks (squid, cockles, snails), turtle, frog, and derived products like fish skin, preserved salted fish, anchovy sauce, fermented fish sauce or paste (*pa-daek*, *pad-dek* or *paadek*). While a wide selection of meat was also taboo. This included beef, pork from female, in postpartum, and male pigs, mutton, poultry (white and red chicken, duck), meat from dog, deer, buffalo, tiger, animals with either white or red skin, burrowing animals (rats and snakes), wild animals (wild pig, squirrel), animals killed by a tiger, male animals, as well as derived products like blood, buffalo intestines, and dishes like *lap-mou* (pork chopped with vegetables), *sommou*

Table 2
Animal-based food taboos of ASEAN women in the postpartum period.

Country	Reference	Food taboo	Justification
Indonesia	Hasan and Suwarni (2012)	Fresh eggs, fish, salted eggs	–
Lao PDR	Holmes et al. (2007)	Meat from white buffalo, white chicken, *pa-daek* (fermented fish)	Cause diarrhea, leprosy, bleeding, other illnesses, or death
		Beef	Cause leprosy, bleeding, other illnesses or death, baby will lose consciousness
		Duck	Cause leprosy or other illnesses
		Meat from female pig	Cause headaches, dizziness, fever, fatigue, bleeding, other illnesses or death
		Deer	Cause diarrhea, bleeding, other illnesses, or death, baby will lose consciousness
		Wild pig, red chicken, fish (*kouan, khae, kheung, red, nai*)	Cause bleeding, other illnesses or death
		Dog	Cause diarrhea, bleeding, other illnesses, or death
		Pork	Umbilical cord of baby may not dry, cause diarrhea or bleeding from the uterus
		Wild animals (e.g. squirrel), buffalo meat, chicken, crab	Umbilical cord of baby may not dry
		Fried, oily foods	Cause both mother and child to get a stomachache and other diseases
		Red meat	Cause sickness, damage to uterus, and later, cough
		Animals that were killed by a tiger, blood, buffalo, intestine, roasted meat	–
		Red cow, turtle, ray, eel, frog, tiger, meat from male animals	Cause diarrhea or bleeding from the uterus
	Eckermann and Deodato (2008)	Oily meats like duck and pork	–
	Bouphavanh et al. (2009)	Meat like beef, water buffalo, pork, and fish like catfish, snake-head fish, Nile tilapia, and fish with no scales	–
	Barennes et al. (2009)	*Lap-mou* (pork meat chopped with vegetable), *sommou* (fermented pork meat with vegetable) or *pad-dek* (fermented fish sauce)	Negatively influence the health of the newborn
		White-skinned mammals	Would lead to weakness
	Lamxay et al. (2011)	Meat like chicken, pork, dogs and beef, and catfish	–
	de Sa et al. (2013)	Animals with white or red skin, deer, dog, bamboo rat, frog, cow, post-partum pig/male pig, *panae* fish, snails, *paa dek* (fermented fish paste)	*Pit duen* or post-partum sickness which includes fever, body ache, numbness, tingling, weakness, bleeding and at worst, death
Malaysia	Jamaludin (2014)	Certain kinds of fish	Digestive disorders, postpartum hemorrhage, and the womb will be swollen and watery
	Haron & Hamiz, (2014)	Egg yolk and seafood	Itchiness and not good for the wound healing process
	Koon et al., (2005)	Mutton and beef	–
		Seafood like squid, cockles, prawn, crab, and most fish (including *kembung, pari*, tuna, sardine and *bilis*)	Poison
Myanmar	Sein (2013)	Pork	Postnatal diarrhea
		Duck	Abdominal cramps
		Fish like cuttlefish (*Restrilleger kanagurta*), hilsa (*Hilsa ilisha*), shark and other seafoods	Drowsiness
		Prawn	Drowsiness, skin itchiness
		Preserved salted fish	Hypertension and drowsiness
		Anchovy sauce	Hypertension and drowsiness, loose motion
		Beef	Skin itchiness
		Burrowing animals (rats, snakes, eels, etc.)	Leprosy and edema
	Diamond-Smith et al. (2016)	Fish with no scales, fish skin, *ahnyigyi* (a kind of fish with a strong nauseating smell), manta ray, river catfish and striped dwarf catfish	Leprosy, skin will have big white and red spots
Thailand	Panyaphu et al. (2011)	Eggs	Poisonous
	Elter et al. (2014)	*Sa wai* fish or stripped catfish	*Phit kam* symptoms including dizziness, fainting, fever, headaches, or death
	Roesler et al. (2014)	Eggs from their own house, fish, meats	–
Vietnam	Lundberg and Ngoc Thu (2011)	Chicken and seafood	Cause allergy

(fermented pork with vegetables), roasted, fried, and oily meats. Eggs (i.e. fresh, salted, yolk) were taboo in Indonesia, Malaysia and Thailand.

Food taboos during the postpartum period were believed to cause: (1) diarrhea, abdominal cramps or other digestive disorders (Holmes et al., 2007; Jamaludin, 2014; Sein, 2013), (2) bleeding or postpartum hemorrhage (Holmes et al., 2007; de Sa et al., 2013; Jamaludin, 2014), (3) leprosy, skin will have big white and red spots (Diamond-Smith et al., 2016; Holmes et al., 2007; Sein, 2013), (4) other illnesses, those mentioned included fatigue, damaged uterus (swollen and watery), cough, drowsiness, itchiness of the skin, hypertension, loose motion, edema, and allergy (Holmes et al., 2007; Lundberg & Ngoc Thu, 2011; Sein, 2013), and (5) death (Holmes et al., 2007; de Sa et al., 2013; Elter et al., 2014). On the other hand, the supposed effects on the baby included (1) losing consciousness (Holmes et al., 2007), (2) umbilical cord may not dry out (Holmes et al., 2007), and in general, (3) negatively influence the health of the newborn (Barennes et al., 2009). Foods like the egg in Thailand (Panyaphu et al., 2011), and seafood like squid, cockles, prawn and crab, and most fish (including *kembung, pari*, tuna, sardine and *bilis*) in Malaysia (Koon et al., 2005) are also said to be poisonous. The fear of leprosy has been mentioned as a reason for the food taboos in Lao PDR and Myanmar. A chronic disease caused by the bacillus, *Mycobacterium leprae*, leprosy is transmitted via droplets from the nose and mouth, during close and frequent contacts with untreated cases. Leprosy is known to be endemic in Southeast Asia but the disease is now completely curable with multi-drug therapy and is considered to be one of the least infectious diseases, with > 99% of the population having adequate natural immunity against it (WHO, 2018a).

Two terms were also used that pertain to the illnesses that might spring on women during the postpartum period: *pit duen* in Lao PDR (de

Sa et al., 2013) and *phit kham* in Thailand (Elter et al., 2014). In the same line, this is *binat* in the Philippines. *Pit duen* is described as a sickness which includes fever, body ache, numbness, tingling, weakness, bleeding and at worst, death, while *phit kham* symptoms include dizziness, fainting, fever, headaches, or death. In Thailand, eating catfish may cause *phit kham*, while eating animals with white or red skin, deer, dog, bamboo rat, frog, cow, postpartum or male pigs, panae fish, snails, and fermented fish paste may cause *pit duen* in Lao PDR.

In a study by Barennes, Sengkhamyong, René, and Phimmasane (2015), mothers in northern Lao PDR with diets restricted to water- or tea-soaked, milled glutinous rice during the postpartum period produced low quality breastmilk which had been linked to infantile beriberi. On the other hand, in some instances, food taboos can have beneficial effects like in the case of fermented fish paste which contains thiaminase and also contributes to the development of maternal thiamin deficiency and infantile beriberi to breastfed babies (WHO/FAO, 2004); and in eating dog meat, which is associated with rabies. Dogs are the main cause of death from rabies, contributing up to 99% of all rabies transmissions to humans. Although contracting rabies through consumption of raw meat or animal-derived tissue has never been confirmed, the process of killing dogs pose a huge risk of being bitten and scratched and infected with the virus (WHO, 2018b).

5. Food taboos in breastfeeding

Shown in Table 3 are the animal-based food taboos adhered to by breastfeeding women in Cambodia (Wallace et al., 2014), Indonesia (Agus, Horiuchi, & Porter, 2012; Wulandari & Klinken Whelan, 2011), Lao PDR (Holmes et al., 2007; Lee, Durham, Booth, & Sychareun, 2013), and Vietnam (Lundberg & Ngoc Thu, 2012) only since there was no information as to the rest of the ASEAN members.

Seafoods like the fishes *kry*, *kahel*, *broma* and *khgoeng* have been cited to be harmful to breastfeeding women, and fishes in general, could make the breastmilk smell and taste bad (Agus et al., 2012); fishes without scales like snake fish, and crab, could reduce the milk supply (Holmes et al., 2007), while fermented fish sauce or paste (*prahok*) is deemed harmful to breastfeeding women and avoiding it could ensure sufficient breastmilk for the baby (Wallace et al., 2014). Seafood is avoided in Vietnam as it can cause allergies (Lundberg & Ngoc Thu, 2012). Tropomyosin is the major allergen present in many shellfish, especially in crustaceans (shrimp, lobster, and crab) and mollusks (squid, oyster, snail, mussels, clams, and scallops). Clinical symptoms that can be observed in allergic individuals include generalized itching, hives, swelling of the lips and tongue, pulmonary symptoms, gastrointestinal symptoms, and anaphylactic shock (Lehrer, Ayuso, & Reese, 2003). However, the evidence suggesting that a restricted diet when breastfeeding decreases the development of allergies is weak, whereas the potential for maternal malnutrition is strong, making the practice of avoiding specific foods in order to protect the babies from allergies an unnecessary health risk for the mothers (Jeong, Park, Lee, Ko, & Shin, 2017).

Meat in general is believed to make breastmilk sour, causing the baby not to want it (Wulandari & Klinken Whelan, 2011). In Lao PDR, meat from wild animals, buffalo meat, chicken, pig fat and fermented pork with vegetables are believed to cause insufficient milk supply, and when the baby is ill, beef and chicken consumption of the breastfeeding mother is believed to cause sickness, damage to the uterus, and cough (Holmes et al., 2007).

Breastfeeding mothers follow these food taboos because they are concerned with the sensory quality and quantity of the breastmilk that they produce. However, the volume of the milk produced is primarily determined by the demand of the baby, rather than the limitations of the mother, her diet - restricted or otherwise (Emmett & Rogers, 1997). As for the taste and smell of the breastmilk, infants rarely react to the food that mothers eat, and the few foods that have been observed to cause some reactions differed among infants, so there is no practical reason to recommend that all breastfeeding mothers avoid particular foods or food groups (Jeong et al., 2017).

On the other hand, the nutritional status of the mother has been observed to influence the nutritional value of the breastmilk, specifically, the amount of fat and hence the energy content, fatty acids, water-soluble vitamins, thiamin (WHO/FAO, 2004), selenium, and immunological properties of breastmilk (Emmett & Rogers, 1997). And, although a more recent review conducted by Bravi et al. (2016) found that available literature presents only varied, weak evidences and indirect causal association linking maternal diet with the composition of breastmilk, it is clear, however, that physiologically, breastfeeding is the most energetically demanding stage of the reproductive cycle, increasing the energy needs of the mothers by 25 to 30% (Prentice et al., 1996) and they have to eat a nutritious and diversified diet to meet the additional energy requirement (Piperata, 2008) and micronutrient demands. Also, where the nutrients supplied by the breastmilk are not directly derived from the diet of the mothers, they must be provided by the maternal reserves (Emmett & Rogers, 1997).

6. Conclusion

While pregnant, a woman must eat not only for herself but literally, also for another human being, tiny, albeit with big nutritional needs. Pregnant and breastfeeding women are consumers with great responsibilities to the next generation, and they must choose their food wisely. However, this choice can be driven by cultural beliefs and traditions handed down from one mother to the next, that could have either positive or negative impacts on both mother and baby.

Healthcare providers should be aware of the food taboos being adhered to in the region to provide the right information and guidance to the women practicing them. There is a need to educate the public in general, and women, in particular, to demystify the taboos using scientific information, raise awareness about the risk of malnutrition when food taboos are followed, and to support their nutritional requirements during pregnancy and the postpartum period by promoting dietary diversification.

More research could be conducted to fill in the gap in information with regards to the food taboos and their impact on some of the ASEAN

Table 3
Animal-based food taboos of ASEAN women while breastfeeding.

Country	Reference	Food Taboo	Justification
Cambodia	Wallace et al. (2014)	Fish (*kry*, *kahel*, *broma* and *khgoeng*), fermented fish paste (*prahok*)	Harmful to breastfeeding women
Indonesia	Wulandari and Klinken Whelan (2011)	Meat	Breast milk will become a bit sour and the baby will not want it
	Agus et al. (2012)	Fish	Makes the breast milk smell and taste bad
Lao PDR	Holmes et al. (2007)	Fish without scales like snake fish, wild animals, buffalo meat, chicken, pig fat, crab	Insufficient milk supply
		Beef, chicken	Cause sickness, damage to uterus, and later, cough
	Lee et al. (2013)	Fermented pork meat with vegetable or fermented fish sauce	Based on medical advice
Vietnam	Lundberg and Ngoc Thu (2012)	Seafood	Cause allergy

members. Culture-sensitive health interventions based on timely research work can contribute to the attainment of the sustainable development goal of reducing the maternal and under-five mortality ratios in Southeast Asia.

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Chapter 4

Protein, Amino Acid and Mineral Composition of Some Edible Insects from Thailand

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Protein, amino acid and mineral composition of some edible insects from Thailand

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ABSTRACT

This paper focuses on the nutritional profiles of four Thai edible insects: the Bombay locust, scarab beetle, house cricket, and mulberry silkworm. The insects were 'high' in protein ranging from 27g to 54g/100g edible portion in fresh weight basis, however, only the silkworm met the FAO/WHO requirements of 40% essential amino acids and 0.6 ratio of essential to non-essential amino acids. Tryptophan is the first limiting amino acid in the locust and cricket, lysine in the scarab beetle, and leucine in the silkworm. The locust is a 'source' of iron and is 'high' in zinc, while the scarab beetle is a 'source' of magnesium and is 'high' in iron and zinc. The cricket bought from the street is a 'source' of iron and magnesium and is 'high' in zinc, while the cricket from the supermarket is a 'source' of calcium (the only one among the insect samples) and is 'high' in iron, magnesium and zinc. And lastly, the silkworm, regardless of where it was purchased, is a 'source' of iron and is 'high' in magnesium and zinc. The arsenic, cadmium, lead and mercury content of all the insect samples were well below the maximum values and are deemed safe for consumption as either feed material or complete feed. Edible insects can contribute to people's nutrient requirements and should be sustainably utilized. Aside from direct consumption, there is a huge potential for using the insects as raw material and fortificant in food processing.

Introduction

The world is now facing the triple burden of malnutrition – obesity (overnutrition), protein energy malnutrition and micronutrient deficiency (undernutrition). With the looming deadline for the attainment of the sustainable development goal of zero hunger and malnutrition by 2030, three main strategies are available, short-term supplementation; medium-term food fortification; and long-term emphasis on balanced nutrition or dietary diversification. Of the three, dietary diversification is believed to be the most sustainable, economically-feasible, and culturally-acceptable in solving the problem.

Under the banner of dietary diversification, the Food and Agriculture Organization (FAO, 1997) had enumerated several approaches for adoption at the community and household levels. To enhance the availability, access, and utilization of foods with high nutrient content and bioavailability throughout the year, the use of underexploited traditional foods from local sources has been promoted, hand in hand with nutrition education to motivate people into eating a healthy and nutritious diet.

In Southeast Asia, where 17.9 million children under five years of age are stunted and 5.4 million are wasted (ASEAN/UNICEF/WHO, 2016), underexploited traditional foods include not only tropical fruits and green, leafy vegetables, but also edible insects. All edible insects are considered as underutilized species contributing to the nutritional indicators for biodiversity (INFOODS, 2013). More than 2000 species of insects, mostly from the forest and often classified as pests, have been collected and eaten by men in the practice known as entomophagy (Jongema, 2017). Indonesia, Lao PDR, Malaysia, Myanmar, the Philippines and Vietnam account for a total of 150 to 200 edible insect species, while Thailand alone has 194 (Sirimungkararat et al., 2010).

Though previous studies have shown that insects cannot only provide protein and amino acids, but also fat and calories, and various vitamins (A, D, thiamine, riboflavin, pantothenic acid, biotin, and folic acid) and minerals (iron, calcium, copper, magnesium, manganese, phosphorous, selenium, and zinc) (Gullan and Cranston, 2010; MacEvilly, 2000; Paoletti, 2005; Rumpold and Schlüter, 2013), in the

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Table 1
English, order, family, scientific and local names of the Thai insects.

English name	Order	Family	Scientific name	Local Thai name
Bombay locust	Orthoptera	Acrididae	*Patanga succincta* L.	*Tak ka tan*
Scarab beetle	Coleoptera	Scarabaeidae	*Holotrichia* sp.	*Malaeng ki noon*
House cricket	Orthoptera	Gryllidae	*Acheta domesticus*	*Malaeng sa ding*
Mulberry silkworm	Lepidoptera	Bombycidae	*Bombyx mori* L.	*Dak dae mai*

ASEAN (Association of Southeast Asian Nations) region, the practice of eating insects is not simply due to nutrition or poverty, but also a matter of culture and taste predilection (Jäch, 2003; Pemberton, 1999). Unfortunately, due to western influences, among other factors, the consumption of edible insects has dwindled throughout generations. In the Philippines, the consumption of insects is now seldom practiced in rural and almost never in the urban areas. In recent years however, in Thailand, Lao PDR and Cambodia, the consumption of edible insects has made a comeback as part of the local food culture and it has spread from the rural villages to the urban areas.

In the case of Thailand, entomophagy was concentrated in the Northern and Northeastern provinces but can now be seen all over the country (Hanboonsong et al., 2013). According to Yhoung-aree (2010), there are three groups of insect eaters nationwide: (1) nostalgic consumers with roots in the provinces, but maybe now residing in different parts of the country; (2) urban consumers who learned and later developed a taste for insects; and (3) foreign tourists attracted by the novelty of eating different insects prepared and sold as delicacies in the various tourist spots in the country.

Crickets, grasshoppers, beetles, ants, water bugs, bamboo caterpillars and silkworm are some of the insects commonly eaten in Thailand. These insects are either farmed or collected from the wild. In Bangkok, insects can be bought in bulk from wholesale outlets in some of the big markets like *Talaad Thai*, *Khlong Toei*, and *Si Mum Mueang*. Cooked insects are sold per piece or in small Styrofoam boxes and plastic containers in a few scattered roadside food stalls as street food, and the more sophisticated processed insects in attractive foil packs are sold in selected retail supermarkets and convenience stores, branded and advertised as nutritious ready-to-eat snacks. Most edible insects are either fried, barbecued, dried and ground, or steamed in banana leaves and curried. Spices and herbs like garlic, basil, chili, lemon grass, and soy sauce and vinegar, aside from the usual salt and pepper, are also used to enhance the flavor and sometimes mask the insects' unpleasant smell (Hanboonsong, 2010).

This paper focuses on four insects from Thailand: the Bombay locust *Patanga succincta*, scarab beetle *Holotrichia* sp., house cricket *Acheta domesticus*, and mulberry silkworm *Bombyx mori*. Compared to the huge diversity of edible insects in the world, only a small percentage has been nutritionally-profiled. In a review conducted by Rumpold and Schlüter (2013), the nutrient composition of 236 edible insects have been published. However, a problem in data utilization has risen as most data were presented in dry matter basis only, information which cannot be directly used for the assessment of human nutrition, product labelling, and dietary recommendations, as foods are consumed on a fresh weight basis and, therefore, data must be reconverted to fresh weight basis when presented in food composition references (Nowak et al., 2016). In this study, the protein content and some protein quality parameters, essential and non-essential amino acid, and mineral (essential macro and trace elements, and non-essential heavy metals) contents of the insects in focus were determined and data in fresh weight (as purchased, edible portion) basis presented herein will be submitted for inclusion in the FAO/INFOODS Food Composition Database for Biodiversity Version 4.0 where there is a need for more data especially on micronutrients, covering more insects and their diets, locations, and seasons (FAO, 2017; Nowak et al., 2016). By highlighting its nutritional benefits, this paper aims to contribute to an increased acceptance of entomophagy and the increased use of the edible insect resource as raw material in developing more acceptable products.

Materials and methods

Insect samples

Four kinds of Thai insects namely, the Bombay locust, scarab beetle, house cricket and mulberry silkworm were collected for this study. All four were purchased from a street hawker (SH) in a well-known tourist spot, while additional cricket and silkworm samples in foil packs (original flavor, each at 15 g net weight) were bought from a supermarket (SM), also in downtown Bangkok. This represents the low- and high-end insect products currently available to the public, respectively. The insects were all in their adult stage, except for the silkworms that were sold in their pupal stage. The insects' English, order, family, scientific and local names are presented in Table 1. A local entomologist was consulted for the identification of the insect samples.

The insects were obtained as ready-to-eat foods deep-fried in cooking oil except for the branded *Bombyx mori*, labeled as a non-fried snack, and was cooked using steam and hot air instead, as stated in the company website. The branded insects were better packed and exhibited a drier and crunchier texture than the other samples. The insects bought from the SH were put in resealable plastic bags, frozen and packed in a Styrofoam box with icepacks to keep them in their frozen state, while those bought from the SM were kept as is, in their original packaging. From Thailand, the insects were transferred to the Institute of Biological Chemistry and Nutrition of the University of Hohenheim, Germany. The frozen insects were then freeze-dried for 24 h (until constant weight), ground to a powder using a laboratory mill, and stored in Falcon tubes in −80 °C prior to analyses, while the insects from the SM where removed from their original packaging, and likewise, ground to a powder, and stored in Falcon tubes in −80 °C freezer.

Chemical analyses

The protein, amino acid, and mineral contents of the samples were determined at the ISO/IEC 17025:2005-accredited Core Facility of the University of Hohenheim. All the assays have a repeatability limit of < 0.25%, and the samples were analyzed in duplicate.

The protein content was analyzed according to the Kjeldahl method in the Official Journal of the European Union, Commission Regulation (EC) No. 152/2009, Annex III C, using the nitrogen conversion factor of 6.25 (European Union, 2009). The amino acid content was analyzed using Annex III F except for tryptophan which was analyzed with Annex III G.

For mineral content analysis, the samples were defatted using petroleum ether (60%, *v/v*). They then underwent microwave-heated digestion with MLS Ultraclave III. Approximately 0.3 g of each defatted sample was weighed out for the digestion, moistened with 1 mL H_2O and then 2.5 mL HNO_3 was added. After digestion, each resulting sample solution was made up to 10 mL with H_2O and was then measured against calibration curves of mineral standards, with Varian ICP-OES VistaPRO for Ca, Fe, K, Mg, Mn, Na, and P, and with PerkinElmer NexION 300X ICP-MS for Cd, Cu, Mo, Pb, and Zn.

The measurements of As and Se were carried out using Hydride Generation Atomic Absorption Spectrometer (AAS), and Hg was measured using Cold Vapor AAS (AAnalyst 400 from PerkinElmer). After a prereduction step with potassium iodide and ascorbic acid, the arsenic and selenium in each digestion solution were further reacted with sodium borohydride to form arsenic hydride and selenium hydride, respectively. The hydrides were then expelled with argon from the solutions, transferred to heated quartz glass cuvettes where they decompose to elemental arsenic or selenium, and were then measured using the AAS against their respective calibration curves.

For the measurement of Hg, the freely present Hg in each digestion solution was reduced with sodium borohydride to elemental Hg, and then transferred with an argon gas stream from the solution to the beam path of the AAS. The Hg concentrations were measured against a calibration curve.

Quantification

The results are presented as mean values with units of either g/100 g or mg/Kg of the insect samples (as purchased, edible portion in fresh weight basis). From the results of the chemical analyses, the percentages and ratio of essential and non-essential amino acids, the percentages of savory (aspartic and glutamic acids) and sweet (glycine and alanine) amino acids, the protein recovery, the amino acid score, and the most limiting amino acid were also determined, as well as whether the insect sample is a 'source' of protein and minerals (Ca, Fe, Mg, and Zn) or has 'high' content according to the definitions for food labelling in the Codex Alimentarius (FAO and WHO, 2007). A solid food product can be labeled as a source of protein, when the protein content is at least 10% of the nutrient reference value (NRV) specified in the Codex Alimentarius, and a source of vitamins and minerals, when the content is 15% of the NRV. A food product may be labeled as 'high' in a nutrient, when the nutrient value is twice the value required for a 'source'. The respective thresholds were calculated for each nutrient using the NRV and compared with the nutrient content of the insect samples.

The protein recovery (%) was calculated as the sum of amino acids divided by the amount of protein in the sample multiplied by 100. The amino acid score and the limiting amino acid were determined using the formula and suggested pattern of amino acid requirements for preschool children (2–5 years) of FAO/WHO (1991). First, the amino acid ratios (mg of an essential amino acid in 1 g of insect protein divided by the value of the same amino acid in the reference pattern) had to be calculated using the reference pattern (mg/g protein): histidine, 19; isoleucine, 28; leucine, 66; lysine, 58; methionine + cysteine, 25; phenylalanine + tyrosine, 63; threonine, 34; tryptophan, 11; and valine, 35. The lowest amino acid ratio was then taken as the amino acid score, and the corresponding amino acid as the limiting amino acid.

Results

Protein content

The protein content of the insect samples ranged from 27 g/100 g mulberry silkworm purchased from the SH to 54 g/100 g house cricket from the SM (Table 2).

Table 2
The protein content per 100 g edible portion in fresh weight basis (EP) of the insect samples compared to the limits for the labels 'source of' and 'high in'.

Insect	Source	Protein content (g/100 g EP)	'Source of' (≥5 g/100 g EP)	'High in' (≥10 g/100 g EP)
Bombay locust	SH	36.31	Y	Y
Scarab beetle		28.92	Y	Y
House cricket		33.21	Y	Y
	SM	53.90	Y	Y
Mulberry	SH	26.55	Y	Y
silkworm	SM	50.20	Y	Y

SH - Street hawker; SM - Supermarket.

Table 3
Essential amino acid content per 100 g of insect samples.

Essential amino acids (g/100 g EP)	Thai insects and source					
	Bombay locust	Scarab beetle	House cricket		Mulberry silkworm	
	SH	SH	SH	SM	SH	SM
Histidine	0.76	0.73	0.69	1.17	0.68	1.41
Isoleucine	1.48	1.28	1.09	2.08	0.95	2.02
Leucine	2.91	2.12	2.34	4.08	1.70	3.43
Lysine	1.71	1.55	1.73	3.01	1.69	3.34
Methionine	0.38	0.45	0.44	0.82	0.77	1.86
Phenylalanine	0.94	0.92	1.03	1.94	1.15	2.65
Threonine	1.21	1.07	1.20	2.14	1.10	2.19
Tryptophan	0.23	0.47	0.29	0.53	0.34	0.72
Valine	2.65	1.87	2.01	3.45	1.36	2.78
Sum	12.27	10.46	10.82	19.22	9.74	20.40

SH - Street hawker; SM - Supermarket.

Table 4
Non-essential amino acid content per 100 g of insect samples.

Non-essential amino acids (g/100 g EP)	Thai insects and source					
	Bombay locust	Scarab beetle	House cricket		Mulberry silkworm	
	SH	SH	SH	SM	SH	SM
Alanine	5.00	1.94	2.79	4.45	1.25	2.25
Aspartic acid	2.49	2.63	3.17	5.58	2.67	5.33
Arginine	2.04	1.30	2.00	3.47	1.25	2.68
Cysteine	0.19	0.35	0.28	0.49	0.32	0.61
Glutamic acid	4.09	2.90	3.84	5.11	2.51	5.25
Glycine	2.23	2.15	1.55	2.59	1.05	1.94
Proline	2.30	1.51	1.75	2.74	1.04	1.90
Serine	1.28	1.23	1.86	3.20	1.13	2.14
Tyrosine	5.04	4.05	3.29	5.16	1.88	3.93
Sum	24.66	18.06	20.53	32.79	13.10	26.03

SH - Street hawker; SM - Supermarket.

Amino acid profile

As shown in Table 3, leucine has the highest concentrations (1.70 g to 4.08 g/100 g) in all the insect samples, and except for methionine in the scarab beetle (0.45 g/100 g), tryptophan has the lowest values (0.23 g to 0.72 g/100 g) among the essential amino acids. On the other hand, the house cricket purchased from the supermarket contained the highest amount of isoleucine, leucine, and valine, while the mulberry silkworm from the same source obtained the highest amounts of histidine, lysine, methionine, phenylalanine, and tryptophan, among all the insect samples. Also, both insect samples had the highest amounts of threonine. From the total amino acid content of the samples (Table 5), the mulberry silkworm from the supermarket has the highest percentage of essential amino acids at 44% while Bombay locust has the lowest percentage at 33%. Only the mulberry silkworm, regardless of the source, meets the FAO/WHO (1973) requirements of 40% essential amino acids and 0.6 ratio of essential to non-essential amino acids.

Among all the non-essential amino acids in Table 4, tyrosine has the highest concentrations (4.05 g and 5.04 g/100 g) in the scarab beetle and Bombay locust, respectively. Glutamic acid has the highest concentration in the house cricket purchased from the street (3.84 g/100 g), while aspartic acid has the highest amount in the house cricket from the supermarket, and mulberry silkworm regardless of source (2.67 g to 5.58 g/100 g). On the other hand, cysteine has the lowest

Table 5
Parameters based on the protein and amino acid contents of the insect samples.

Parameters	Thai insects and source					
	Bombay locust	Scarab beetle	House cricket		Mulberry silkworm	
	SH	SH	SH	SM	SH	SM
Total AA content (g/100 g EP)	36.93	28.52	31.35	52.01	22.84	46.43
Essential AA (%)	33.23	36.68	34.51	36.95	42.64	43.94
Non-essential AA (%)	66.77	63.32	65.49	63.05	57.36	56.06
Ratio of essential to non-essential AA	0.49	0.58	0.53	0.59	0.74	0.78
Savory AA (%)	17.82	19.39	22.36	20.55	22.68	22.79
Sweet AA (%)	19.58	14.34	13.84	13.54	10.07	9.02
Protein recovery (%)	101.71	98.62	94.40	96.49	86.03	92.49

SH - Street hawker; SM - Supermarket.

values in all the insect samples, only ranging from 0.19 g/100 g Bombay locust to 0.61 g/100 g mulberry silkworm from the supermarket.

As shown in Table 5, the amount of savory amino acids (aspartic and glutamic acids) from the total amino acid content of each insect ranged from 18% to 23%, equivalent to 6.58 g/100 g Bombay locust and 10.58 g/100 g mulberry silkworm from the supermarket. The sweet amino acids (glycine and alanine) ranged from 9% to 20% of the total amino acids, equivalent to 4.19 g/100 g mulberry silkworm from the supermarket to 7.23 g/100 g Bombay locust. The protein recovered from each insect sample is also shown in Table 5, and it ranged from 86% (mulberry silkworm from the street) to 102% (Bombay locust).

Using the suggested pattern of amino acid requirements for preschool children (2–5 years) of FAO/WHO (1991) as reference, the amino acid scores of the insect samples ranged from 0.58 (Bombay locust) to 1.04 (mulberry silkworm from the supermarket). Tryptophan is the limiting amino acid in the Bombay locust and house cricket, lysine in the scarab beetle, and leucine in the mulberry silkworm (Table 6).

Mineral composition

Eleven essential dietary minerals were quantified in this study. Table 7 shows that among the minerals, sodium has the highest concentration in Bombay locust (516 mg/100 g), and house cricket regardless of source (999 mg to 1928 mg/100 g), while potassium was highest in scarab beetle (521 mg/100 g), and mulberry silkworm regardless of source (493 mg to 672 mg/100 g). In contrast to these, selenium has the lowest concentration in Bombay locust (9.9 μg/100 g), scarab beetle (34.3 μg/100 g), and house cricket from the street (23.2 μg/100 g), while molybdenum was lowest in the house cricket bought from the supermarket (54.2 μg/100 g) and the mulberry silkworm from both street and supermarket (15.1 μg to 51.6 μg/100 g).

The heavy metals – arsenic, cadmium, lead and mercury content of the insect samples were also measured in this study and the results are shown in Table 8. Arsenic and mercury were highest in the scarab beetle, 0.0576 mg and 0.008 mg/100 g, respectively. Lead was highest in the house cricket from the street at 0.0155 mg/100 g, while the cadmium level in all the insect samples was below 0.005 mg/100 g.

Discussion

On November 13, 2017, in the 31st ASEAN Summit held in Manila, Philippines, the ASEAN leaders issued the "Declaration on Ending All Forms of Malnutrition". In it, they noted that the most vulnerable, marginalized, poor, geographically-isolated and disadvantaged groups in the region are particularly affected by undernutrition and various types of micronutrient deficiencies, while the general population is affected by over-nutrition and obesity, leading to diet-related non-communicable diseases or DRNCDs (ASEAN Heads of Member States/ Governments, 2017).

The leaders declared several actions that they will undertake with the objective of contributing to the achievement of global goals relevant to nutrition. One of these actions was committing to increase public and multi-sectoral investments to improve nutrition and ensure healthy diets, which would hopefully mean more funding and support for health and nutrition researches on under-utilized, locally-available resources.

As recommended by Rumpold and Schlüter (2013), more studies on the composition of edible insects are necessary for a stronger claim on their value as human food and source of nutrients. For example, more

Table 6
Essential amino acid ratios calculated based on the FAO/WHO pattern for preschool children (2–5 years old), estimated amino acid score and limiting amino acid of the insect samples.

Essential amino acid ratios	Thai insects and source					
	Bombay locust	Scarab beetle	House cricket		Mulberry silkworm	
	SH	SH	SH	SM	SH	SM
Histidine	1.10	1.33	1.09	1.14	1.35	1.48
Isoleucine	1.46	1.58	1.17	1.38	1.28	1.44
Leucine	1.21	1.11	1.07	1.15	0.97	1.04
Lysine	0.81	0.92	0.90	0.96	1.10	1.15
Methionine + cysteine	0.63	1.11	0.87	0.97	1.64	1.97
Phenylalanine + tyrosine	2.61	2.73	2.06	2.09	1.81	2.08
Threonine	0.98	1.09	1.06	1.17	1.22	1.28
Tryptophan	0.58	1.48	0.79	0.89	1.16	1.30
Valine	2.09	1.85	1.73	1.83	1.46	1.58
AA score	0.58	0.92	0.79	0.89	0.97	1.04
Limiting AA	Trp	Lys	Trp	Trp	Leu	Leu

SH - Street hawker; SM - Supermarket.

Table 7
Essential mineral content per 100 g of insect samples and the limits for the food labels 'source of' and 'high in'.

Essential minerals (mg/100 g EP)	Thai insects and source						'Source of'	'High in'
	Bombay locust	Scarab beetle	House cricket		Mulberry silkworm			
	SH	SH	SH	SM	SH	SM		
Calcium	65.60	75.10	88.70	176.90	92.20	107.60	120	240
Copper	1.69	1.08	1.42	3.00	0.711	0.943		
Iron	3.45	9.10	3.11	5.81	2.83	3.18	2.1	4.2
Magnesium	39.40	81.20	63.80	108.10	157.70	174.70	45	90
Manganese	1.07	7.85	1.60	3.95	1.26	1.86		
Molybdenum	0.0232	0.0430	0.0386	0.0542	0.0151	0.0516		
Phosphorus	266.90	260.40	424.30	761.70	392.20	525.90		
Potassium	349.80	520.80	457.70	752.00	492.90	672.00		
Selenium	0.0099	0.0343	0.0232	0.0653	0.0534	0.2285		
Sodium	515.90	52.60	998.50	1028.60	128.40	362.60		
Zinc	8.22	8.80	11.60	21.80	9.80	15.40	2.25	4.5
Sum	1252.06	1017.01	2050.79	2861.98	1278.07	1864.46	–	–

SH - Street hawker; SM - Supermarket.

Table 8
Heavy metal content per 100 g of insect samples with the limits for feed material and complete feed for comparison.

Non-essential minerals (mg/100 g EP)	Feed material max. limit (mg/100 g EP)	Complete feed max. limit (mg/100 g EP)	Thai insects and source					
			Bombay locust	Scarab beetle	House cricket		Mulberry silkworm	
			SH	SH	SH	SM	SH	SM
Arsenic	0.176	0.176	< 0.005	0.0576	< 0.005	< 0.005	0.0432	0.0165
Cadmium	0.176	0.044	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Lead	0.880	0.440	0.0092	0.0117	0.0155	0.0102	0.0138	0.0044
Mercury	0.009	0.009	< 0.005	0.008	< 0.005	< 0.005	< 0.005	< 0.005

SH - Street hawker; SM - Supermarket.

information are needed on the quality of insect proteins to be able to fully assess their value in comparison to other food proteins.

Protein is needed for growth and other bodily functions and can also be utilized to provide energy which is done through the conversion of protein into carbohydrate. A pregnant woman needs an additional supply of protein for the growing fetus inside her. Likewise, a breast-feeding mother needs extra protein to produce milk. Infants require about 2.5 g of protein per kilogram of body weight in the first few months of life. This requirement decreases to about 1.5 g/kg at nine to 12 months of age. However, the protein that they consume can only be used for growth when their energy requirement is first satisfied. Because of this and the greater risks of infection, children also have a greater potential for protein deficiency. Infections lead to an increased loss of nitrogen from the body, which then must be sufficiently replaced by proteins from the diet (Latham, 1997).

Protein-energy malnutrition (PEM) is the most common and significant nutrient deficiency in the world. This occurs when protein is consumed in low amounts, and the carbohydrate and fat in the diet do not provide adequate energy, hence, the protein is used instead, resulting to a deficit in the protein needed for growth, cell replacement and other metabolic functions. In its severe manifestation, PEM exists as kwashiorkor, marasmus or its mixed form, marasmic-kwashiorkor (Latham, 1997). In PEM's less severe form, it manifests as stunting and wasting (Norgan et al., 2012). The basic treatment for PEM is through diet, by meeting the energy needs and the recommended dietary allowance (RDA) for protein, for instance, 0.8 g protein per kg body weight per day for adults, regardless of age, sex and body composition (Trumbo et al., 2002).

The nutrient reference value for protein is 50 g, and because the amount of proteins in the samples as shown in Table 2 are greater than at least 10% of the NRV or 5 g/100 g edible portion in fresh weight basis, the insects can be labeled as 'source of', and, even better, 'high in' (at least twice the value as 'source') protein based on the FAO and WHO (2007) food labelling standards which aim to protect the health of consumers and ensure fair practices in the food trade.

The results on protein content (Table 2) confirms the claims of other studies that in general, insects are good sources of protein. In the analysis of edible insects in various life cycle stages conducted since 1989, the raw protein content generally ranges from 20 to 70%, dry matter basis (Xiaoming et al., 2010), with insect protein digestibility as high as 76 to 96% (Ramos-Elorduy et al., 1997).

All the insect samples have protein contents greater than that of pork (21%), all except mulberry silkworm sold in the street contained protein higher than that of cow's milk (28%) and both house cricket and mulberry silkworm from the supermarket have protein contents higher than that of eggs (49%) (INFS, 1998). The house cricket and mulberry silkworm bought off the street have lower protein contents than those bought from the supermarket.

Protein is composed of 20 standard amino acids, and the insect samples contained 18 of them. Among the 18 amino acids, nine are deemed essential and needed to be obtained from food as they cannot be synthesized by the human body. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. According to Defoliart (1992), insect proteins tend to be low in methionine and cysteine, but high in others, especially lysine and threonine. The house cricket and mulberry silkworm from the supermarket were 1.7- and 2.1-fold of the total amount of essential amino acids (Table 3), while 1.6- and 2-fold of the total amount of non-essential amino acids (Table 4), respectively, of the same insect species sold from the street.

The difference in the protein and amino acid content could be attributed to the preparation and cooking methods (deep frying in oil vs cooking using steam and hot air) employed at the source and how the samples were prepared prior to the analysis (freezing and freeze-drying vs no pretreatment). Aside from biological factors like breeding and rearing, maturity, feed, and gut content (Nowak et al., 2016), these factors can significantly affect the nutrient and proximate composition, in terms of percentages, of the insect samples. The initial low moisture content (< 7%) of the insect samples bought from the supermarket made it possible to skip the freezing and freeze-drying process before they were milled and analyzed and may as well cause the higher estimation of their protein and amino acids content.

Despite the difference in the total amino acid contents between the house cricket and mulberry silkworm bought from the street and those bought from the supermarket, their ratios of essential to non-essential, as well as with savory and sweet amino acids were unaffected, and remained comparable with each other (Table 5). The mulberry silkworm from the supermarket contained the highest percentage of savory amino acids but the lowest percentage of sweet amino acids while the opposite is true for Bombay locust. Similarly, the study by Longvah et al. (2011) showed that the eri silkworm (*Samia ricinii*) contains 23% savory amino acids and 12% sweet amino acids.

The Bombay locust has a protein recovery of 101.71%. The protein content was measured independently from the amino acid profile, and the difference in the sensitivity of the analysis can affect the measurements and other derived values like protein recovery. On the other hand, the high recovery of crude protein in the all the insect samples (Table 5) can be interpreted to mean that the nitrogen content of the insects can be largely attributed to the amino acids, and that not > 14% of the nitrogen may have been contributed by chitin or other compounds (Finke, 2015). Fortunately, the presence of chitin in the exoskeleton of the edible insects is no longer treated as a deterrent in their consumption since bacterial chitinolytic enzyme responsible for the digestion of chitin has been discovered in the human gastrointestinal tract (Dušková et al., 2011). However, some individuals may have a very small amount of the enzyme in their body that eating insects can trigger an allergic reaction (EFSA, 2015). It has been proposed that the allergic immune response to chitin is initiated with the activation of macrophages, causing the upregulation of chitinolytic and chitin-binding proteins. The secretion of leukotriene B4 then recruits innate immune cells like eosinophils and basophils to tissues and might incite a T-helper type 2 (Th2) inflammatory response (Burton and Zaccone, 2007).

A study on four grasshopper species from the Tibetan Plateau in China found that based on the amino acid scoring pattern for chicks, their limiting amino acid is methionine + cysteine (Sun et al., 2010). In the case of Bombay locust, no other study was found to have calculated and identified its limiting amino acid. In contrast, the limiting amino acid of house crickets had already been studied, however, it was also determined based on the amino acid requirement for broiler chicks. Nakagaki et al. (1987) determined the protein quality of crickets using boiler chicks feeding trials and their data suggests that tryptophan is the limiting amino acid, which agrees with the result of this study (Table 6). For the scarab beetle, Yang et al. (2014) found that *Holotrichia parallela* Motschulsky, although coming from the same genus, has threonine as limiting amino acid, and not lysine. The result on the mulberry silkworm conforms with the previous studies of Zhou and Han (2006) on *Antheraea pernyi*, and Longvah et al. (2011) on *Samia ricinii*, that leucine is the limiting amino acid in silkworm proteins. The three studies also used the amino acid requirement for preschool children (2–5 years) of FAO/WHO (1991) in determining the amino acid scores. Some cereal- or tuber-based diets could be deficient in leucine, lysine, tryptophan and/or threonine (Bukkens, 1997; Mokrane et al., 2010; van Huis et al., 2013). The amino acid gap could be bridged by the consumption of insects as it can cover the deficient essential nutrient in the diet.

Macro and trace minerals are needed for normal growth and physiological functions of the body and should be provided in the diet adequately. Using the NRV of 800 mg for calcium, 14 mg for iron, 300 mg for magnesium, and 15 mg for zinc in 100 g edible portion, the thresholds for the food labels 'source of' or 'high in' were calculated (FAO and WHO, 2007). Based on the threshold values as shown in Table 7, the Bombay locust is a source of iron and is high in zinc, while the scarab beetle is a source of magnesium and is high in iron and zinc. The cricket from the street is a source of iron and magnesium and is high in zinc, while the cricket from the supermarket is a source of calcium (the only one among the insect samples) and is high in iron, magnesium and zinc. Because of the absence of an internal skeleton, insects are generally known to be low in calcium (Hunt et al., 2001). And lastly, the mulberry silkworm, regardless of where it was purchased, is a source of iron and is high in magnesium and zinc. For comparison, lean beef (raw fillet steak) contains only 4 mg calcium, 2.1 mg iron, 23 mg magnesium, and 2.8 mg zinc/100 g fresh weight (McCance, 2015). On the issue of mineral bioavailability, a study by Latunde-Dada et al. (2016) showed that compared to sirloin steak, grasshopper, cricket, and mealworms contain significantly higher chemically-available calcium, copper, magnesium, manganese and zinc, while buffalo worms and sirloin steak exhibited higher iron bioavailability comparable to that of $FeSO_4$. It was concluded that commonly consumed insects could be excellent sources of bioavailable minerals, especially iron, and could be used to increase people's dietary mineral intake (Latunde-Dada et al., 2016).

Just like any natural food product, there are several disadvantages of eating insects. Chitin and its derivative chitosan have anti-nutrient activities and can bind dietary lipids, fat-soluble vitamins and minerals (Koide, 1998; Ravi Kumar, 2000; Yhoung-aree, 2010). Chitosan is also linked to urinary tract stone diseases (Queiroz et al., 2014; Yhoung-aree, 2010). In addition, some insects may produce allergens and toxic bioactive compounds, host parasites and pathogenic bacteria, and be contaminated with pesticides and heavy metals from the environment (EFSA, 2015; Kouřimská and Adámková, 2016; Rumpold and Schlüter, 2013).

Due to their high level of toxicity, arsenic, cadmium, lead and mercury are among the priority metals that are of great significance to public health. These metallic elements are considered systemic toxicants that can induce multiple organ damage even at lower levels of exposure, and are also human carcinogens (Tchounwou et al., 2012). Since the standards on heavy metals in insects used as human food are not yet available, the values were compared with the maximum allowable levels for feed material and complete feed set in the Official Journal of the European Union, Commission Regulation (EU) No. 1275/2013 for arsenic, cadmium and lead (European Union, 2013), and in the Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed for mercury (The European Parliament and the Council of the European Union, 2002). The EU maximum limits were based on 88% dry matter with the unit "mg/Kg dry weight" and therefore, had to be converted to mg/100 g fresh weight basis. The heavy metal content of all the insect samples were well below the maximum values and are deemed safe for consumption as either feed material or complete feed (Table 8).

Conclusion

The insects bought from the supermarket yielded better nutrient contents compared to the insects from the street. This is evidence that different sources using different preparation and cooking methods may yield insect products with improved nutrient content. Overall, the edible insects are high in protein content and protein recovery, has the complete set of essential amino acids, are either a source of or high in calcium, iron, magnesium and zinc, and are safe from arsenic, cadmium, lead and mercury. This reinforces the fact that edible insects can contribute to people's nutrient requirements and should be sustainably utilized. Aside from direct consumption, there is a huge potential for using the insects as raw material and fortificant in food processing. The resistance towards eating insects may be overcome by modifying the insects' appearance and form, enhancing their flavors, and/or by employing strong advertising and marketing strategies to erase the stigma that insects are dirty and inedible.

Conflict of interest

The authors declare that there is no conflict of interest with regards to the submitted article.

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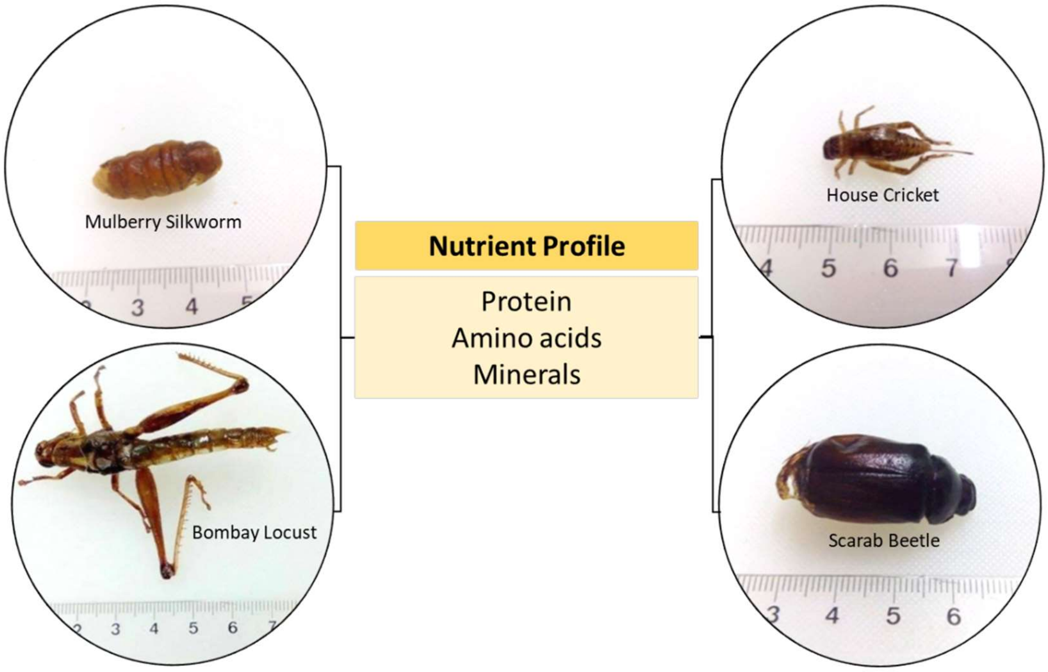

Figure 5. The graphical abstract accompanying the article published in the Journal of Asia-Pacific Entomology.

Chapter 5

Nutrient Composition of the Indonesian Sago Grub (*Rhynchophorus bilineatus*)

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ORIGINAL RESEARCH ARTICLE

Nutrient composition of the Indonesian sago grub (*Rhynchophorus bilineatus*)

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Abstract

The sago grub (*Rhynchophorus bilineatus*) [Montrouzier, Coleoptera, Dryophthoridae] is a local delicacy in the Papua Province in Indonesia. In this study, the nutrient content of the edible insect was measured using chromatographic and spectrometric methods. The results showed that it contains 10.39 g protein and 17.17 g oil/ 100 g fresh weight. The sago grub meets the FAO/ WHO requirements of 40% essential amino acids and a 0.60 ratio between essential to non-essential amino acids. Its limiting amino acid is methionine + cysteine. The major fatty acids found in the sago grub are palmitic (42%), oleic (45%), and linoleic (3%) acids. Although vitamin E is exclusively produced by photosynthetic organisms, a gram of sago grub oil contains 51 μg vitamin E, which is composed mainly of tocopherols (92%). In contrast with palm oil, the sago grub oil contains δ-tocopherol (0.12 μg/g oil), and a significantly high amount of β-tocopherol (3.85 μg/g oil). It is a source of zinc and magnesium and contains safe levels of heavy metals. Based on these nutritional properties, the Indonesian sago grub can be considered as a good source of nutrients, and its propagation and utilization should be encouraged especially in other areas of Indonesia and maybe in neighboring countries where they are also endemic, and where malnutrition is prevalent. The consumption of edible insects should be considered as a component of dietary diversification – a sustainable way of alleviating the nutritional status of the population.

Keywords Edible insects · Dietary diversification · Nutritional profiling · Micronutrients · Vitamin E

Introduction

Indonesia has the world's fourth largest population with more than 260 million people, and is the largest country in Southeast Asia with a land area of 1,904,569 km^2 (UN Department of Economic and Social Affairs, Population Division 2017). It is rich in natural resources but is plagued by high levels of acute and chronic malnutrition, together with an increasing rate of childhood obesity (UNICEF Indonesia 2018), a situation common in members of the Association of Southeast Asian Nations (ASEAN). In the fight against malnutrition, there are three possible options: fortification, supplementation and dietary diversification. Among the three, dietary diversification is viewed as most sustainable. In promoting this option, the FAO encourages people to utilize indigenous fruits and vegetables to increase the nutrients in their diet. While edible insects can also be sources of micronutrients, and most importantly, of protein, they are often overlooked because of the general negative perceptions on entomophagy (Köhler et al. 2019). Indonesia and other countries in Southeast Asia are home to around 150 to 200 edible insect species (Sirimungkararat et al. 2010).

The Papua Province is located in the easternmost part of Indonesia. The Province is divided into lowlands consisting of sago and mangrove forests, grasslands, and swamps in the coastal areas, and upland areas with primary and secondary forests, and farmed plots. Due to limited agricultural resources in the area, insects, especially grasshoppers, leaf and stick insects, cicadas and large moths and their caterpillars, remain as important protein sources in the people's daily diet. Along Papua's coastline, the most important edible insect is the *Rhynchophorus bilineatus* [Montrouzier, Coleoptera, Dryophthoridae], the sago beetle or palm weevil. The larvae, commonly known as "sago grubs" or "sago worms" are

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collected a month after the palm trees are cut down for sago starch production. One rotting sago palm trunk may yield as much as 500 to 600 sago grubs feeding on its starchy pith (Mercer 1994). Normally, they are consumed raw or roasted and is also considered as a local delicacy (Ramandey and van Mastrigt 2010). From informal talks in the community, it was gathered that the edible insects cost around 3 USD per 100 g.

There are two species of sago worm in Papua, and they are differentiated by the color in their adult stage - the *R. bilineatus* weevil is black while *R. ferrugineus* [Olivier, Coleoptera, Dryophthoridae] is brown (Ramandey and van Mastrigt 2010; Tommaseo-Ponzetta and Paoletti 2005). *R. ferrugineus* is widely distributed in Asia (Indonesia, Japan, Malaysia, Papua New Guinea, the Philippines and Thailand), while the other species, *R. palmarum* [Linnaeus, Coleoptera, Dryophthoridae] and *R. phoenicis* [Fabricius, Coleoptera, Dryophthoridae] are widely distributed and used as food in the Western Hemisphere (Central America and West Indies, Mexico and South America), and in Africa (van Huis et al. 2013), respectively. Palm weevils are often considered as pests because they attack sago palms and other important palm species like the coconut, date, oil and raffia palms (van Huis et al. 2013).

Most of the species of palm weevil have already been studied. Among the various species, the following had been profiled: African palm weevil *R. phoenicis* in Cameroon (Fogang Mba et al. 2017; Womeni et al. 2009, 2012), Nigeria (Adeyeye 2017; Edijala et al. 2009; Ekpo and Onigbinde 2005; Elemo et al. 2011; Ogbuagu et al. 2011; Okunowo et al. 2017; Omotoso and Adedire 2007) and Ghana (Atuahene et al. 2017; Laar et al. 2017); *R. palmarum* in Côte d'Ivoire (Dué et al. 2009; Gbogouri et al. 2013) and Nigeria (Adeyeye 2017), and *R. ferrugineus* in Egypt (Abdel-Moniem et al. 2017) and Malaysia (Ali et al. 2006). However, based on the review of available scientific literature, no nutritional information can be found for the species *R. bilineatus*. In general, very little research have been conducted on the edible insects in Indonesia (Lukiwati 2010).

This study was conceptualized with the objective to give an overview of the nutritional profile of the wild sago grub *Rhynchophorus bilineatus* in support to the principles of dietary diversification and the alleviation of malnutrition in Indonesia and the ASEAN region. The paper discusses the protein and amino acid content as well as the fat, fatty acid, vitamin E, and mineral content of the edible insect, the first profiling ever to be documented for the species.

Materials and methods

Sago grub samples

The grub samples (1 kg) were collected from the wild, specifically, from the sago palm forest in Sentani, the capital of the Jayapura Regency in Papua, Indonesia in December 2017. The live insects were washed, patted dry, sealed in a plastic container, and frozen at −18 °C for 24 h before the container was packed in a Styrofoam box together with icepacks, and transported to the Institute of Nutritional Science of the University of Hohenheim, Germany. The insects were freeze-dried (LyoQuest-85, Azbil Telstar Technologies S.L.U, Spain) for 24 h until constant weight was achieved, and ground for 1 min using a laboratory mill (Model A11 basic, IKA, Germany). The resulting mass was put in Falcon tubes and stored in a − 80 °C freezer until further analyses could be conducted.

Chemical analyses

The protein, amino and fatty acids, as well as the mineral content of the sago grub were analyzed in duplicate at the Core Facility of the University of Hohenheim. This is an accredited testing institute (DIN EN ISO/IEC 17025:2005) with state-of-the-art equipment coupled with well-established laboratory protocols. On the other hand, the fat and vitamin E contents were measured in triplicate at the Institute of Nutritional Science, also in the University of Hohenheim using methods developed and optimized by the researchers. All the reagents used were of the highest purity and analytical grade, and purchased from either Sigma-Aldrich Chemie (Taufkirchen, Germany) or Merck (Darmstadt, Germany), unless stated otherwise.

Protein and amino acids The protein content was determined using the Kjeldahl method, as specified in the Official Journal of the European Union (EU), Commission Regulation (EC) No. 152/2009, Annex III C, using the nitrogen conversion factor of 6.25 (European Commission 2009). The digestion unit Kjeldatherm and distillation unit VAP 50 Carousel (Gerhardt GmbH & Co. KG, Königswinter, Germany) were used in the analysis. The amino acids were determined following Annex III F except for tryptophan which was analyzed using the procedure in Annex III G. The amino acids were separated and measured by ion exchange chromatography and ninhydrin post-column derivatization using an amino acid analyzer (Biochrom 30, DKSH Management Ltd., Zürich, Switzerland).

The percentages and ratio of essential and non-essential amino acids (EAA and NEAA), the percentages of savory (aspartic and glutamic acids) and sweet (glycine and alanine) amino acids, the protein recovery, the amino acid score, and the most limiting amino acid, as well as whether the sago grub is a 'source' of protein or has 'high' content according to the rules in food labelling from the Codex Alimentarius (FAO/WHO 2007) were determined based on the results of the analysis.

The amount of protein recovered (%) was calculated as the sum of the amino acids divided by the amount of protein in the sample multiplied by 100. The amino acid score and the limiting amino acid were determined using the formula and suggested pattern of amino acid requirements for preschool children (2–5 years old) of FAO/WHO (1991). First, the amino acid ratios (amount in mg of an essential amino acid in 1.0 g of insect protein divided by the value of the same amino acid in the reference pattern) had to be calculated using the reference pattern (mg/g protein): histidine, 19; isoleucine, 28; leucine, 66; lysine, 58; methionine + cysteine, 25; phenylalanine + tyrosine, 63; threonine, 34; tryptophan, 11; and valine, 35. The lowest amino acid ratio was then taken as the amino acid score, and the corresponding amino acid as the limiting amino acid. A food product can be labelled as a protein 'source', when its protein content is at least 10% of the nutrient reference value (NRV) or may be labelled as 'high in' protein, when the measured value is twice the amount required to be a 'source'.

Crude fat and vitamin E content The fat and vitamin E (tocopherols and tocotrienols) content were extracted and quantified following the method of Grebenstein and Frank (2012) as optimized by Irías-Mata et al. (2017). This is the first time that the method was applied to an edible insect. The oil from the sago grub was extracted using 10% n-hexane (*v*/v) and stirred for 30 min at room temperature. The residue was removed using vacuum filtration, and to remove the residual n-hexane from the supernatant, a vacuum rotary evaporator (Rotavapor R-100, Büchi Labortechnik, Essen, Germany) was used at a maximum temperature of 30 °C. The extracted crude oil was transferred in a bottle wrapped in aluminum foil to prevent exposure to light, and was refrigerated at 4 °C. The vitamin E content of the sago grub oil was analyzed using high performance liquid chromatography (HPLC) together with palm oil obtained from the same Papua region, for comparison.

The daily acceptable intakes (AI) of 5 mg and 7.5 mg of α-tocopherol equivalents (α-TE) among children (1–3 years old) and adult females (19–50 years old), respectively, were used to estimate the possible vitamin E contribution of the edible insect in the diet (FAO/WHO 2001). The α-TE were calculated using the conversion factor of 1 mg α-TE = 1 mg α-tocopherol, 2 mg β-tocopherol, 10 mg γ-tocopherol, 3.3 mg α-tocotrienol, or 20 mg β-tocotrienol (FAO/WHO 2016).

Fatty acids The fatty acid profile was determined according to the method developed by the Core Facility (P23–5-008). The oil from the sago grub was dissolved in diisopropyl ether and methylated by the addition of 0.2 M trimethylsulfonium hydroxide in methanol (TMSH). The resulting solution was used for the determination of the total fatty acid pattern by capillary gas chromatography with flame ionization detection (7890A: Capillary GC with split/splitless- and PTV-injection and FI and EC detection, Agilent Technologies, USA). The standard used was Supelco 37-Component FAME Mix (Supelco, Bellefonte, Pennsylvania, USA), and the column was an Agilent J&W GC Column DB-23 (30 m × 0.25 mm × 0.25 μm).

Dietary minerals and heavy metals The sago grub was first defatted using 60% petroleum ether (*v*/v), and then digested with the ultraCLAVE III Microwave Digestor (MLS GmbH, Leutkirch im Allgäu, Germany). Approximately 300 mg of the defatted sample was weighed out for digestion, moistened with 1 mL H_2O and then mixed with 2.5 mL HNO_3. After digestion, the resulting solution was made up to 10 mL with H_2O and was then measured against the calibration curves of the different mineral standards using atom-emission spectrometers VistaPRO ICP-OES (Varian Inc., Palo Alto, USA) for Ca, Fe, K, Mg, Mn, Na, and P, and ICP-MS NexION 300X (PerkinElmer, Inc., Massachusetts, USA) for Cd, Cu, Mo, Pb, and Zn.

The measurements of As and Se were carried out using hydride generation atomic absorption spectrometer (AAS), and Hg was measured using cold vapor AAS (AAnalyst 400, PerkinElmer, Inc., Massachusetts, USA). After a pre-reduction step with potassium iodide and ascorbic acid, the As and Se in the digestion solution were further reacted with sodium borohydride to form arsenic hydride and selenium hydride, respectively. The hydrides were then expelled with argon from the solutions, transferred to heated quartz glass cuvettes where they decomposed to elemental As or Se, and were then measured using the AAS against their respective calibration curves. For the determination of Hg, the freely present Hg in the digestion solution was reduced with sodium borohydride to elemental Hg, and then transferred with an argon gas stream from the solution to the beam path of the AAS. The Hg concentration was then measured against a calibration curve.

From the results of the mineral analysis, the sago grub was evaluated if it can be labelled as a 'source of' Ca, Fe, Mg and Zn or has 'high' content according to the rules in food labelling from the Codex Alimentarius (FAO/WHO 2007). The possible contribution of these minerals (and Se) to the FAO/WHO (2001) recommended nutrient intakes (RNI) among children (1–3 years old) and adult females (19–50 years old) were also calculated.

Data handling and analysis The results generated were first in freeze-dried (FD) basis before they were converted into wet or fresh weight (FW) basis. To convert, the results in FD basis (per 100 g freeze-dried sample) were divided by a conversion factor which was calculated by dividing the fresh weight of the sample by its freeze-dried weight. The moisture loss (% of the fresh weight) due to freeze-drying was also noted. The results of the analysis are presented as mean value ± standard deviation. T-test was done to compare the fatty acid composition as well as the vitamin E content of the sago grub oil with the local palm oil using GraphPad Prism 5 (GraphPad Software, San Diego, California, USA).

Results

The calculated conversion factor in order to convert FD into FW basis was 2.33, with a moisture loss of 57%. The Indonesian sago grub contained 10.39 g protein/100 g FW, and a 92% protein recovery. The amount of protein was slightly greater than 10 g/100 g FW which means that the edible insect is not only a source of the macronutrient but can be labeled as 'high in' protein based on the Codex Alimentarius (FAO/WHO 2007).

As shown in Table 1, the sago grub contained the complete set of nine essential amino acids, from tryptophan at 0.12 g to phenylalanine at 0.79 g/100 g FW. Among the non-essential amino acids, glutamic acid was the most abundant at 1.27 g, while cysteine was the least abundant at 0.08 g/100 g FW. The total amino acid content was 9.53 g/100 g FW, comprised of 42% essential and 58% non-essential amino acids. The ratio between the essential to non-essential amino acids was 0.73. Savory amino acids (aspartic and glutamic acids) were at 23%, while the sweet amino acids (glycine and alanine) were at 14% of the total amino acid content. The amino acid score was 0.84, and the limiting amino acid was methionine + cysteine (Table 2).

The sago grub contained 17.17 g oil/100 g FW. As shown in Table 3, in 100 g fresh weight, there is 8.21 g saturated fatty acids (SFA), 8.18 g monounsaturated fatty acids (MUFA), and only 0.79 g polyunsaturated fatty acids (PUFA). In 0.5 g sago grub oil, palmitic acid has the highest concentration (42%) among the SFA, while oleic acid (45%) is highest among the MUFA, and linoleic acid (3%) among the PUFA. The same pattern was observed in the palm oil sample, although it contained 0.09% margaric acid and 0.23% eicosenoic acid, two fatty acids not detected in the sago grub oil.

Table 2 Essential amino acid ratios based on the FAO/WHO pattern for children (2–5 years old), amino acid score and limiting amino acid of the sago grub

Essential amino acid (AA)	FAO/WHO pattern for children (2–5 years old)	Ratio
Histidine	19	1.13
Isoleucine	28	1.37
Leucine	66	1.04
Lysine	58	1.08
Methionine + Cysteine	25	0.84
Phenylalanine + Tyrosine	63	1.75
Threonine	34	1.24
Tryptophan	11	1.05
Valine	35	1.49
AA Score		0.84
Limiting AA		Met + Cys

The vitamin E profile of the sago grub is shown in Table 4. Except for δ- and β-tocopherol, the sago grub oil contained significantly less tocopherols and tocotrienols compared to palm oil. The oil from the sago grub had the most α-tocopherol (43.25 μg/g oil) and the least amount of δ-tocopherol (0.12 μg/g oil) among the vitamin E congeners. δ-Tocopherol was not detected in the palm oil at all.

The sago grub contained essential minerals that ranged from <0.002 to 204.55 mg/100 g FW (Table 5). At the same time containing heavy metals - As, Cd, Pb and Hg - that ranged from <0.002 mg to 0.013 mg/100 g FW (Table 6).

Table 7 shows the sago grub's micronutrient contribution as a percentage of the AI or the RNI specified by FAO/WHO (2001) for children (1–3 years old) and adult females (19–50 years old). The sago grub can cover 10–16% of the acceptable intakes of α-tocopherol equivalents (α-TE), less than 12% of the RNI for selenium; less than 6% for iron; and less than 3% for calcium. On the other end of the spectrum, the sago grub can provide a good amount of magnesium (24–

Table 1 Essential and non-essential amino acid content (mean values in g/100 g FW ± SD; $n = 2$) of sago grub

Essential amino acid	g/100 g FW	Non-essential amino acid	g/100 g FW
Histidine	0.22 ± 0.00	Alanine	0.80 ± 0.00
Isoleucine	0.40 ± 0.00	Aspartic acid	0.88 ± 0.00
Leucine	0.71 ± 0.00	Arginine	0.46 ± 0.00
Lysine	0.65 ± 0.00	Cysteine	0.08 ± 0.00
Methionine	0.14 ± 0.00	Glutamic acid	1.27 ± 0.01
Phenylalanine	0.79 ± 0.00	Glycine	0.53 ± 0.00
Threonine	0.44 ± 0.00	Proline	0.59 ± 0.00
Tryptophan	0.12 ± 0.00	Serine	0.55 ± 0.00
Valine	0.54 ± 0.01	Tyrosine	0.36 ± 0.00
Sum	4.01 ± 0.00	Sum	5.52 ± 0.00

Table 3 Fatty acid profiles (mean values ± SD; $n = 2$) of the Indonesian sago grub and palm oil

Fatty acid (FA)		% FA in 0.5 g Oil		Sago grub (g FA/100 g FW)
		Palm	Sago grub	
C12:0***	Lauric	0.130 ± 0.000	0.030 ± 0.000	0.005 ± 0.000
C14:0***	Myristic	0.805 ± 0.007	1.075 ± 0.007	0.185 ± 0.001
C15:0*	Pentadecanoic	0.045 ± 0.007	0.085 ± 0.007	0.015 ± 0.001
C16:0**	Palmitic	34.740 ± 0.735	42.455 ± 0.035	7.290 ± 0.006
C17:0	Margaric	0.090 ± 0.014	ND	ND
C18:0^{ns}	Stearic	3.940 ± 0.438	3.670 ± 0.014	0.630 ± 0.002
C20:0^{ns}	Arachidic	0.370 ± 0.099	0.470 ± 0.014	0.081 ± 0.002
C22:0^{ns}	Behenic	0.090 ± 0.014	ND	ND
Total SFA***		40.210 ± 0.127	47.785 ± 0.035	8.205 ± 0.006
C16:1***	Palmitoleic	0.195 ± 0.035	3.015 ± 0.007	0.518 ± 0.001
C18:1n9c*	Oleic	46.810 ± 0.410	44.630 ± 0.141	7.663 ± 0.024
C20:1	Eicosenoic	0.225 ± 0.007	ND	ND
Total MUFA^{ns}		47.220 ± 0.354	47.645 ± 0.148	8.181 ± 0.025
C18:2n6c**	Linoleic	12.380 ± 0.438	3.010 ± 0.212	0.517 ± 0.036
C18:3n3c***	α-linolenic	0.215 ± 0.021	1.560 ± 0.028	0.268 ± 0.005
Total PUFA**		12.595 ± 0.460	4.570 ± 0.184	0.785 ± 0.032

Legend: $p < 0.001$, extremely significant ***; $p = 0.001$ to 0.01, very significant **; $p = 0.01$ to 0.05, significant *; $p > 0.05$, *ns* not significant; *ND* Not detected; *SFA* Saturated fatty acids; *MUFA* Monounsaturated fatty acids; *PUFA* Polyunsaturated fatty acids

88%) and zinc (29–34%) in the daily RNI of children and adult females if it is included in their diet.

Discussion

According to Payne et al. (2016), the palm weevil larva, in general, is significantly more healthful than beef and chicken based on its nutrient value score calculated from publicly available nutrient composition data. The sago grub *Rhynchophorus bilineatus* in this study has a slightly higher protein content (10.39 g protein/100 g FW) than the *Rhynchophorus ferrugineus* larva from the Southeast Sulawesi Province also in Indonesia. According to Nirmala et al. (2017), the latter contained 9.70 g protein/100 g FW. On the other hand, the sago grub has a lower protein and fat content (17.17 g oil/100 g FW) compared to the results of Abdel-Moniem et al. (2017) on their analysis of the pupae and larvae of red palm weevil, *Rhynchophorus ferrugineus* Olivier in Egypt. The Egyptian insect had a higher protein content (32%) in the pupal stage than in the larval stage

Table 4 Vitamin E profiles (mean values in μg/g ± SD; $n = 3$) of the Indonesian sago grub and palm oil

Vitamin E (μg/g Oil)	Sago grub	Palm
α-Tocotrienol***	0.80 ± 0.06	92.58 ± 8.16
β-Tocotrienol***	0.18 ± 0.09	2.30 ± 0.33
δ-Tocotrienol***	0.47 ± 0.08	23.50 ± 3.29
γ-Tocotrienol***	2.52 ± 0.12	79.45 ± 8.52
α-Tocopherol**	43.25 ± 2.69	87.47 ± 8.96
β-Tocopherol***	3.85 ± 0.15	0.19 ± 0.05
δ-Tocopherol	0.12 ± 0.01	ND
γ-Tocopherol*	0.26 ± 0.05	0.49 ± 0.12
Total T**	47.48 ± 2.67	88.14 ± 9.10
Total T3***	3.97 ± 0.29	197.82 ± 7.12
Total VE***	51.44 ± 2.45	285.97 ± 15.01

Legend: $p < 0.001$, extremely significant ***; $p = 0.001$ to 0.01, very significant **; $p = 0.01$ to 0.05, significant *; *ND* Not detected; *T* Tocopherol; *T3* Tocotrienol; *VE* Vitamin E

Table 5 Mineral content (mean values in mg/100 g FW ± SD; $n = 2$) of the sago grub and the limits for food labelling 'source of' and 'high in'

Dietary mineral (mg/100 g FW)		'Source of'	'High in'
Calcium	14.93 ± 1.00	120	240
Copper	0.38 ± 0.03		
Iron	0.77 ± 0.04	2.1	4.2
Magnesium	52.64 ± 2.56	45	90
Manganese	0.35 ± 0.02		
Molybdenum	0.003 ± 0.000		
Phosphorus	102.32 ± 4.38		
Potassium	204.55 ± 4.77		
Selenium	<0.002 ± 0.000		
Sodium	16.59 ± 0.40		
Zinc	2.87 ± 0.15	2.25	4.5

Table 6 Heavy metal content (mean values in mg/100 g FW ± SD; $n = 2$) of the sago grub with the limits for feed material and complete feed

Heavy metal (mg/100 g FW)		Feed material max. limit (mg/100 g FW)	Complete feed max. limit (mg/100 g FW)
Arsenic	<0.002 ± 0.000	0.176	0.176
Cadmium	0.013 ± 0.000	0.176	0.044
Lead	<0.004 ± 0.000	0.880	0.440
Mercury	<0.002 ± 0.000	0.009	0.009

(30%). Meanwhile, the fat content of the larvae was 22% while that of the pupae was 19%. Other palm weevil larvae like those of *Rhynchophorus palmarum* and *Rhynchophorus phoenicis* have protein contents ranging from 7 to 21 g/100 g FW (FAO 2017), and fat contents ranging from 6.24–7.48 g/ 100 g FW (Adeyeye 2017). On the other hand, the *Rhynchophorus phoenicis* larvae from Cameroon have protein and fat contents of 8 g/100 g and 21 g/100 g FW, respectively (Fogang Mba et al. 2017) while *Rhynchophorus phoenicis* larvae from Nigeria contained 24 g protein/100 g FW, and 15 g fat/100 FW (Okunowo et al. 2017). The variability in the protein and fat content or the proximate composition of the larvae, in general, could be directly attributed to the difference in measurement methods, and in the diet of the insects. Indirectly, it could also be due to the difference in body size, age during harvest, and the living environment at the geographical origin or source of the edible insects under study (Raheem et al. 2019; van Huis and Oonincx 2017). Information on the effect of these factors on the nutrient profile of edible insects is vital, as any one of them could be the deciding factor in the effective domestication and industrial production of these insects (Raheem et al. 2019).

It is predicted that the world population will grow over nine billion by 2050, and one of the biggest challenges is to increase the global food supply by at least 1.30% per year (Fischer et al. 2014; Godfray 2015; Grafton et al. 2015). Attaining food security in the future would necessitate the utilization of sustainable alternative protein and fat sources like the Indonesian sago grub, which, as a component of a diversified diet, could also contribute in attaining the Sustainable Development Goal (SDG) to end all forms of malnutrition, especially protein energy malnutrition (PEM), by 2030. PEM occurs in both developed and developing countries, and is a leading cause of death in young children all over the world (Grover and Ee 2009).

The sago grub has a high protein recovery (92%), this means that the edible insect can provide the protein in the diet of the people in the Papua Province, especially those who use sago starch, with its low protein content, as staple food. The high protein recovery can also be translated as the protein content which is mainly contributed by the amino acids, and so, only 8% of the protein may be attributed to chitin and other nitrogen-containing compounds (Finke 2015). Chitin is present in the exoskeleton of edible insects, and it could trigger an allergic reaction in some people who possess very small amount of bacterial chitinolytic enzyme in their gut (Dušková et al. 2011; EFSA 2015). On the other hand, chitin and chitosan (its most studied derivative) have also been

Table 7 Contribution (%) of the micronutrients in the sago grub to the daily acceptable and recommended nutrient intakes set by FAO/WHO (2001)

Micronutrient	Amount (mg/100 g FW)	Children (1–3 years old)		Adult Females (19–50 years old, pre-menopausal)	
Vitamin		AI (mg/day)	% AI	AI (mg/day)	% AI
α-Tocopherol	0.743 ± 0.046				
β-Tocopherol	0.066 ± 0.003				
γ-Tocopherol	0.004 ± 0.001				
α-Tocotrienol	0.014 ± 0.001				
β-Tocotrienol	0.003 ± 0.001				
α-TE	0.780 ± 0.046	5	15.60	7.5	10.40
Mineral		RNI (mg/day)	% RNI	RNI (mg/day)	%RNI
Calcium	14.93 ± 1.00	500	2.99	1000	1.49
Iron (5% bioavailability)	0.77 ± 0.04	12	6.42	59	1.31
Magnesium	52.64 ± 2.56	60	87.73	220	23.93
Selenium	<0.002 ± 0.000	0.017	<11.76	0.026	<7.69
Zinc (low bioavailability)	2.87 ± 0.15	8.4	34.17	9.8	29.29

Legend: *α-TE* α-Tocopherol equivalents; *AI* Acceptable intakes; *RNI* Recommended nutrient intake linked to adaptive immunity (Komi et al. 2018). Nagatani et al. (2012) have reported the positive effect of chitin in the control of inflammation of the intestines; Bae et al. (2013) have reported that chitin and chitosan are capable of suppressing anaphylaxis symptoms from peanut allergy; and Wiesner et al. (2015) have reported the involvement of chitin in the immunity against pulmonary cryptococcal infection.

The sago grub met the FAO/WHO (1973) requirements of 40% essential amino acids and a 0.60 ratio between essential to non-essential amino acids. In a study on mice by Romano et al. (2019), it was concluded that the ratio among EAA and NEAA is the most probable factor responsible for the health-promoting effects of proteins, and that the higher the ratio (closer to 1 or even greater than 1 if supplemented with additional EAA) in the diet, the more efficient it is to increase the lifespan of people suffering from malnutrition.

Cereals like rice and maize, staple foods in various regions around the world, are mostly low in lysine and may lack tryptophan and threonine. However, these amino acids are abundantly present in some insect species (Bukkens 2005). In the case of the Indonesian sago grub, lysine is the third most abundant essential amino acid after phenylalanine and leucine. It also contains threonine and tryptophan, although they are the least abundant among both essential and non-essential amino acids.

When compared to the FAO/WHO requirement for preschool children aged 2–5 years, the limiting amino acid in the sago grub is the sulfur amino acid – methionine + cysteine. In a study by Finke (2015), when compared to the national requirements for rats or poultry, the limiting amino acid of crickets, mealworms, superworms, and waxworms is also methionine and cystine. In contrast, the limiting amino acid of the larva of the African palm weevil, *Rhynchophorus phoenicis*, from Nigeria was tryptophan (Elemo et al. 2011).

Palmitic (42%), oleic (45%), and linoleic (3%) acids are the major fatty acids in the sago grub oil which were similarly observed by Ekpo and Onigbinde (2005) on *Rhynchophorus phoenicis,* with the larva oil containing palmitic (32%), oleic (40%) and linoleic (13%) acids. Generally, insects have more unsaturated fatty acids (UFA) compared to saturated fatty acids (SFA) (de Castro et al. 2018). The sago grub has a higher SFA (48%) and MUFA (48%), but a lower PUFA (5%) content compared to the larvae of *Rhynchophorus phoenicis* and *Rhynchophorus palmarum* from Nigeria with SFA ranging from 29 to 31%, MUFA from 37 to 40%, and PUFA from 29 to 34%. The PUFA/SFA ratio ranged from 0.92 to 1.20 (Adeyeye 2017), while the PUFA/SFA ratio of the Indonesian sago grub is only 0.096. Nevertheless, the high amount of unsaturated fatty acids (MUFA + PUFA) has kept the sago grub oil, like other plant oils, liquid even at a low storage temperature (4 °C). The oil extracted from the sago grub can not only be used in food and animal feed, it could also have possible industrial applications (e.g. cosmetics, lubrication, pharmaceutical industries).

Like vitamins A, D, and K, vitamin E is a fat-soluble vitamin. It is composed of fat-soluble compounds – four of which has the chromanol ring structure bound to a saturated 15-carbon side chain (tocopherols), and another four with three trans double bonds in the carbon side-chain (tocotrienols). In the identification of the compounds, Greek prefixes (α, β, γ, δ) are used to denote the number and position of the methyl groups substituted at the chromanol ring (Frank et al. 2012). Vitamin E is exclusively produced by photosynthetic organisms and, thus, present in plant-based foods, particularly in vegetable oils (Sen et al. 2006). On the other hand, animals can acquire their vitamin E content from the plants that they consume or by eating other animals that consumed the vitamin E-containing plants, and had stored the micronutrient in their liver, muscles and fat. It is therefore understandable that the palm oil sample yielded a significantly higher vitamin E content compared to the oil from the sago grub. A gram of palm oil contained 305 μg vitamin E, 71% of which is composed of tocotrienols, while a gram of sago grub oil only contained 51 μg vitamin E, 92% of which is composed of tocopherols. α-tocotrienol was the predominant vitamin E compound in the palm oil while α-tocopherol was the predominant compound in the sago grub oil.

The palm oil did not contain a detectable amount of δ-tocopherol, similar to the oils obtained from Costa Rican palms via screw press extraction (Irías-Mata et al. 2017). While in contrast, the sago grub oil contained δ-tocopherol (0.12 μg/g oil), and a significantly high amount of β-tocopherol (3.85 μg/g oil). The sago grub could have only obtained their vitamin E from the starchy palm pith that they consume.

The α-TE in 100 g sago grub can provide children with 16% of their daily AI level, and can cover 10% of adult females' daily AI. Both tocopherols (α, β, γ) and tocotrienols (α, β) contribute in the calculation of the α-TE. Although γ-tocopherol is the predominant class of dietary vitamin E, α-tocopherol is the most significant vitamin E form for human nutrition and physiology (Rigotti 2007; WHO/FAO 2004). On the other hand, the tocotrienols, aside from being powerful antioxidants, are known to possess great neuroprotective, anti-cancer and cholesterol-lowering properties (Sen et al. 2006).

According to the WHO, every 100 g of weevil larvae contains 4.3 mg iron, 461 mg calcium, and other vitamins and minerals (Mercer 1997). In the sago grub, *Rhynchophorus bilineatus*, potassium (204.55 mg/100 g FW), phosphorus (102. 32 mg/100 g FW), and magnesium (52.64 mg/100 g FW) were the most abundant minerals, while selenium (< 0.002 mg/100 g FE) and molybdenum (0.003 mg/100 g FW) were the least abundant. Based on the Codex Alimentarius (FAO/WHO 2007), the sago grub is not a source of iron and calcium. This is as expected since invertebrates without a mineralized skeleton can contain very little calcium (de Castro et al. 2018). On the other hand, the sago grub can be labelled as a source of zinc and magnesium. There is, however, a gap in information regardingthe bioavailability of these minerals (de Castro et al. 2018).

Zinc is an integral component of more than 300 enzymes taking part in the synthesis and metabolism of both macro and micronutrients. It also helps maintain cell and organ integrity and immunity and plays a significant role in gene expression. Likewise, magnesium is a co-factor of various enzymes that are engaged in the metabolism of energy, synthesis of protein and nucleic acids (DNA and RNA), and the regulation of the electrostatic potential of neural tissues and cellular membranes. It is also involved in the regulation of potassium and the utilization of calcium in the body (FAO/WHO 2001). The sago grub (100 g FW) can provide children (1–3 years old) with 34% and 88% of their daily RNI for zinc and magnesium, respectively, while it can provide adult females (19–50 years old) with 29% and 24% of their daily RNI for the same minerals. However, integrating the sago grub in the diet is not so easy. Aside from the sensory aspect, there are several other issues concerning entomophagy.

One of these concerns is the possibility that the edible insects are contaminated with pesticides and heavy metals from the environment they dwell in. Because of their highly toxic nature, arsenic, cadmium, lead and mercury are considered extremely dangerous to human health. However, since the standards on heavy metals in edible insects for human consumption are not yet available, the measured values in this study were compared with the maximum allowable levels (converted to mg/100 g fresh weight basis) for feed material and complete feed set in the Official Journal of the European Union, Commission Regulation (EU) No. 1275/2013 for arsenic, cadmium and lead (European Commission 2013), and in the Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed for mercury (European Parliament and Council 2002). From the results, the sago grub showed less than the maximum limits for arsenic, cadmium, lead and mercury for feed material and complete feed. The grub samples were collected from the wild, and these results can also be a confirmation that the environment where they come from is relatively safe and free of harmful pesticides and heavy metals.

Conclusion

The *R. bilineatus* is an indigenous natural product of the Papua Province in Indonesia. Based on the results of this study, it contains micronutrients (tocopherols, tocotrienols, and minerals), and can be an alternative source of fatty acids and oil, and amino acids and protein. Its propagation and utilization should be encouraged especially in areas in Indonesia, and maybe in neighboring countries where they are endemic, and where malnutrition is also prevalent. The consumption of edible insects should be considered as a component of dietary diversification – a sustainable way of alleviating the nutritional status of the population.

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Conflict of interest The authors declare that they have no conflict of interest.

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Chapter 6

Rethinking the Staple: Exploring the Nutrient Content and Morphological Characteristics of Three Heirloom Pigmented Rice Varieties from the Philippines

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Rethinking the Staple: Exploring the Nutrient Content and Morphological Characteristics of Three Heirloom Pigmented Rice Varieties from the Philippines

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Abstract

Dietary diversification has been viewed as the most sustainable way to fight undernutrition, micronutrient inadequacy, and obesity, or collectively known as the triple burden of malnutrition. In rice-eating countries like the Philippines, one way of diversifying people's diet is by shifting from polished, white rice to heirloom pigmented rice as staple food. This paper focuses on pigmented varieties locally known as Camoros, Tinta, and Malinao black rice. Their protein content, together with crude fiber, ash, amino acids, vitamins (carotenoids, riboflavin, thiamine, and vitamin E), minerals (calcium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium, and zinc), and functional components (ferulic acid and γ-oryzanol) were determined. From the results, each rice variety was then classified as a 'source' or has 'high' content of the nutrients, and the possible contribution to the recommended nutrient intakes, safe intakes, or the daily acceptable intakes for children and adult females was calculated. Based on the results of this research, it is expected that more people will be encouraged to consume these pigmented rice varieties, and that more researchers will be encouraged to explore the nutritional and functional properties of pigmented rice, as well as their cultivation and commercialization.

Keywords: Red rice; Black rice; Purple rice; Carotenoids; Vitamin E; Minerals

Introduction

Rice is one of the most important cereal crops in the world. It is the staple food for much of the Asian population and a meal in a typical household is not complete without cooked rice. Rice gruel is a popular complementary food as well, and it is the first food that most Asian mothers give to their babies. Rice comes in different varieties, but white rice is most widely eaten and only about 15% is consumed in the form of pigmented rice (Deng et al., 2013). However, it is an unfortunate and long known fact that white rice is stripped of vitamins, minerals, essential amino acids, fatty acids, and phytochemicals; it is a high glycemic index (GI) food; and has been linked to malnutrition, micronutrient deficiencies, obesity, and other diet-related non-communicable diseases (DR-NCDs) (Bhullar & Gruissem, 2013; Birla et al., 2017; Valarmathi, Raveendran, Robin, & Senthil, 2015).

The Philippines is an archipelago composed of more than 7,600 islands. It has a population of 100,981,437 as of 2015 (Philippine Statistics Office, 2019), with a Global Hunger Index of 20.2 which means a "serious case of hunger" (von Grebmer et al., 2018), and a Hidden Hunger Index of 30.7, which translates to "moderate case of micronutrient deficiencies" (Muthayya et al., 2013). It has a child obesity rate of 6.1% and adult obesity rate of 6.4% (WHO, 2016).

Diet diversification has been viewed as the most sustainable way to fight undernutrition, micronutrient deficiency, and obesity, collectively known as the triple burden of malnutrition

(Bhandari & Banjara, 2015; Sundaram, 2014). Ideally, diversifying one's diet entails that sufficient amount of a variety of nutritious foods is consumed, ensuring that the human body is supplied with the macro and micronutrients that it needs. In rice-eating countries like the Philippines, one way of diversifying people's diet is by shifting from polished, white rice to heirloom pigmented rice as staple food, while increasing or just maintaining the portions and composition of the rest of their traditional meals composed of vegetables and a main protein source (fish, pork, beef or chicken).

The Philippines has a large number of rice landraces, heirloom or local varieties. Heirloom rice are called as such because the rice seeds are considered as family treasures and are handed down from one generation to the next. These landraces come in various shades of red, purple or black, depending on the pigmentation in the rice kernels' pericarp and bran layer (Kitisin, Saewan, & Luplertlop, 2015; Saenjum, Chaiyasut, Chansakaow, Suttajit, & Sirithunyalug, 2012). The pigmented rice is milled as "brown" rice – any rice grain that has been milled to only remove the husk or hull, but with the bran layer, germ and endosperm left intact (Global Rice Science Partnership [GRiSP], 2013).

Pigmented rice is gaining popularity worldwide as it contains more dietary fiber, vitamins and minerals than polished, white rice (Trinidad, Mallillin, de Leon, & Alcantara, 2014). The pigments found in rice grains are biologically active compounds with nutraceutical functions (Chaudhary, 2003; Kitisin et al., 2015; Ryu, Park, & Ho, 1998; Yawadio, Tanimori, & Morita, 2007). Anthocyanins largely contribute to the color of black rice, while the color of red rice is attributed to oxidized proanthocyanidins, with both anthocyanins and proanthocyanidins contributing to the color of the purple rice varieties (Goufo & Trindade, 2014).

Halliwell and Gutteridge (2015) had suggested that chronic diseases develop from the attack of free radicals on the biomolecules in the body, hence, because of their strong radical scavenging activities, pigmented rice varieties can have beneficial health effects in the prevention of DR-NCDs (Rerkasem, Sangruan, & Prom-u-thai, 2015; Slavin, 2003). According to Goufo and Trindade (2014), in general, pigmented rice varieties contain more phenolic acids, flavonoids, and anthocyanins and proanthocyanidins compared with non-pigmented rice, with the black rice varieties having the highest antioxidant content and bioactivity, followed by the purple and red varieties. Pigmented rice has been used in both traditional and modern medicine in treating iron-deficiency anemia, diabetes, coronary heart disease and some forms of cancer (Chen et al., 2006; Deng et al., 2013; Guo et al., 2007; Ma, Chen, & Ling, 2000; Wang et al., 2007; Zhao, Giusti, Malik, Moyer, & Magnuson, 2004).

Although rice is the most studied cereal in the world, and various reviews have already been written on their medicinal properties (Fardet, Rock, & Rémésy, 2008; Goufo & Trindade, 2014), important nutritional properties e.g. micronutrient density, have been largely ignored. Focusing on the pigmented rice varieties of the Philippines, this paper aims to encourage people to consume them, and for researchers to further explore their nutritional value and functional properties, as well as their cultivation and commercialization, not only in the Philippines, but worldwide.

Methodology

Rice Samples

The samples were purchased from the central public market of Kalibo, Aklan in the island of Panay in the Philippines. The samples are traditional rice varieties, grown in rice fields in the upland areas of the province, mostly for the farmers' own consumption, and sold in limited amounts in the lowlands like in the capital town of Kalibo. The varieties are known locally as Camoros, Tinta, and Malinao black rice.

Morphological Characterization
The color of the rice samples (either red, purple or black) was determined visually. The 1000-grain weight of each variety was measured by weighing samples consisting of 20 rice kernels, and then multiplying the weight by 50. For the grain length, width, and thickness, a Vernier caliper was used. These measurements were done in five replications. The kernel size and shape were then classified using the scale developed by the International Rice Research Institute (IRRI). For kernel size, the following classification was followed: >7.50 mm, extra-long; 6.61 to 7-50 mm, long; 5.51 to 6.60 mm, medium; and <5.50, short, while the shape was classified based on the kernel's length-to-width aspect ratio: >3.0, slender; 2.1 to 3.0, medium; 1.1 to 2.0, bold; and <1.0, round (Frei & Becker, 2004; Juliano, 1992).

Preparation of Rice for Analysis
The rice samples in sealed polyethylene bags were taken to the University of Hohenheim, Germany for analysis. At the Institute of Nutrition Science, raw rice was first ground into a powder using a laboratory mill (Model A11 basic, IKA, Germany), and then stored at -80°C prior to analyses.

Amylose Content Determination
The amylose content was measured using an Amylose/Amylopectin test kit (Megazyme, Ireland), and following the assay procedure described in the manual, K-AMYL 06/18 (Megazyme, 2018). The method is a modified version of a lectin concanavalin A (Con A) spectrophotometric procedure developed by Yun and Matheson (1990) and requires ethanol to remove lipids in a pre-treatment step [modified from Morrison and Laignelet (1983)]. Based on the amylose content, rice can be classified as waxy (0 to 5%), very low (5 to12%), low (12 to 20%), intermediate (20 to 25%), and high (25 to 33%) (Juliano, 1992). Commercial rice can also be classified to have either low (less than 20%), medium (21 to 25%) or high (26 to 33%) amylose content according to Suwannaporn, Pitiphunpong, and Champangern (2007).

Nutrient Profiling
The protein, crude fiber, ash, amino acids, as well as the mineral contents of the rice samples were analyzed at the Core Facility, an accredited testing laboratory (DIN EN ISO/IEC 17025:2005) of the University of Hohenheim. The carotenoids, riboflavin, thiamine, vitamin E, ferulic acid, and γ-oryzanol contents were measured at the Institute of Nutrition Sciences. All the reagents used were of the highest purity and analytical grade, either from Sigma-Aldrich Chemie (Taufkirchen, Germany) or Merck (Darmstadt, Germany), unless stated otherwise.

The analyses conducted at the Core Facility, as well as the calculations of total amino acid content, percentages and ratio of essential to non-essential amino acids, savory and sweet amino acids, amino acid score and the limiting amino acid, employed the same methods described in a previous paper (Köhler, Kariuki, Lambert, & Biesalski, 2019).

The carotenoids in the rice samples were measured using the method developed by Wald, Nohr, and Biesalski (2018) with some modifications. Each rice sample (500 mg) was mixed with 1 mL 1M potassium hydroxide in methanol and 1 mL n-hexane containing the internal standard echinenone, butylated hydroxyanisole and butylated hydroxytoluene. The mixture was vortexed for 30 s and put in a rolling mixer. The extraction and saponification were done under yellow light for 3 h at room temperature. Saturated sodium chloride solution (1 mL) was added to the resulting extract, was vortexed (30 s) and then centrifuged at 16100Xg (1 min). The hexane phase (top layer) was siphoned off and stored in another Eppendorf tube. The remaining residue (bottom layer) was rinsed with 500 µL n-hexane, vortexed and centrifuged again. The top layer was siphoned once more and pooled with the first n-hexane layer. The rinsing step was done two more times. The pooled n-hexane was vaporized to dryness using a SpeedVac concentrator (Thermo Scientific Savant). The residue was then redissolved in 300 µL isopropanol. The solution was filtered (CHROMAFIL® Xtra PTFE, 0.20 µm, 13 mm) and 50 µL was injected into the HPLC system at a flowrate of 1.5 mL/min for a total runtime of 15 min. The standards, the whole HPLC system including the mobile and stationary phases and the flow gradient, as well as the method of quantification employed were as described by Wald et al. (2018). The retinol activity equivalents (RAE) were calculated using the conversion factor of 1 µg RAE = 1 µg all-trans-retinol, 12 µg β-carotene, and 24 µg other provitamin A carotenoids (Institute of Medicine, 2000).

Thiamine (vitamin B_1) and riboflavin (vitamin B_2) were determined using the protocol DIN EN 14152:2014 and DIN EN 14122:2014 respectively, published by the Deutsches Institut für Normung, 2014a, 2014b). The extraction and analysis of the two vitamins from the samples were done simultaneously. Each rice sample (50 mg) was mixed with 2 mL 0.1M hydrochloric acid and was put in a water bath (95°C) for 1 h. After the solution has cooled down at room temperature, 500 µL 2.5M sodium acetate buffer and 250 µL enzyme cocktail (phosphatase, amylase, and papain protease) were then added. The resulting mixture was vortexed (15 s), incubated in a water bath (37°C) for 18 h, and then centrifuged at 6000 rpm (5 min). For the riboflavin analysis, 100 µL of the supernatant was collected, and temporarily stored in an Eppendorf tube in a -80°C freezer while 100 µL of a fluorescent reagent cocktail (potassium ferricyanide and sodium hydroxide) was added to the rest of the mixture. The mixture was then vortexed (1 min) and left to settle down in the dark for 10 min. The 0.5M potassium dihydrogen phosphate buffer (800 µL) was then added to adjust the pH to 9, and the mixture was centrifuged (5 min) at 16100Xg. Of the resulting supernatant, 300 µL was then transferred into an HPLC vial for the thiamine analysis. The sample injection volume was 20 µl at a flowrate of 1.0 ml/min. The HPLC system consisted of the stationary phase – ReproSil Pur C18 AQ column (5 µm, 250 × 4.6 mm; Dr. Maisch, Tübingen, Germany), and the mobile phase with 75 mM K_2HPO_4/KH_2PO_4 buffer as Eluent A, and a 1:1 mixture of Eluent A with methanol as Eluent B for the thiamine analysis, while only one eluent consisting of 67% 70 mM KH2PO4 and 33% methanol was used for the riboflavin analysis.

The total run time for the thiamine analysis was 10 minutes, with a fluorometer set for excitation at 366 nm and emission at 435 nm. For quantification, six standard solutions with the concentrations of 5, 20, 40, 60, 80, and 100 ng/ml were measured together with the samples. The total run time for the riboflavin analysis was 15 minutes, with a fluorometer set for excitation at 468 nm and emission at 520 nm. For quantification, seven standard solutions with the concentrations of 5, 10, 50, 100, 200, 300, and 400 ng/ml were also measured with the samples. Peak integration, and the calculation of the amount of thiamine and riboflavin against their respective calibration curves were done using the chromatography software LabSolutions (version 5.71 SP1; Shimadzu Corporation, Kyoto, Japan).

Rice oil was extracted and the vitamin E (tocopherols and tocotrienols) content was measured using a modified version of the method by Grebenstein and Frank (2012) previously used on palm fruit by Irías-Mata et al. (2017) and on Indonesian sago grub by Köhler, Irias-Mata, Ramandey, Purwestri, and Biesalski (2020). The rice sample (50 mg) was weighed into a test tube, and 2 mL of 1% ascorbic acid in ethanol (w/v), 900 µL of deionized water, and 600 µL of saturated potassium hydroxide were added. The mixture was vortexed (15 s), incubated in a shaking water bath (70°C) for 30 min, and then quickly cooled down by putting it into an ice bath. Ethanolic butylated hydroxytoluene (25 µL, 1 mg of butylated hydroxytoluene/mL of ethanol), 1 mL of deionized water, 600 µL of glacial acetic acid, and 2 mL of n-hexane were added, and the test tube was closed and the sample was mixed for 1 min by mechanical inversion (Bio RS-24 mini-rotator) and centrifuged (3 min, 4°C) at 188Xg. From the supernatant, 1.5 mL was collected into a fresh test tube, and the second and third extractions were done with 2 mL of n-hexane each, collecting an additional 3.5 mL of the supernatant. The combined supernatants (5 mL) was then dried completely using a SpeedVac concentrator (RVC 2-25 CD Plus, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany). The residue was then dissolved in 250 µL of methanol, vortexed, transferred to an HPLC vial, and injected (15 µL) into the HPLC. Except for a shorter run time of 45 min, and the isocratic flow of the methanol/water (80:20, v/v) eluent, the HPLC system used and the method of quantification of the vitamin E content in the sample are as described by Irías-Mata et al. (2017). As in a previous paper (Köhler et al., 2019b), the α-tocopherol equivalents (α-TE) were calculated using the conversion factor of 1 mg α-TE = 1 mg α-tocopherol, 2 mg β-tocopherol, 10 mg γ-tocopherol, 3.3 mg α-tocotrienol, or 20 mg β-tocotrienol (FAO/WHO, 2016).

The method developed by Calvo-Castro et al. (2019) for the extraction and quantification of ferulic acid and γ-oryzanol (cycloartenyl ferulate, 24-methylenecycloartanyl ferulate + campesteryl ferulate, and β-sitosteryl ferulate) from rice bran extract was optimized for pigmented rice and was used for the analysis. Each rice sample (250 mg) was added with 1 mL ethyl acetate, sonicated for 10 min (Sonorex Digitec, Bandelin Electronic, Berlin, Germany), mixed using a vertical rotator for 20 min, centrifuged (1 min, 4°C) at 13000 rpm, and 600 µL of the supernatant was then collected. The samples went through the extraction procedure seven more times using 1 mL 80:20 (v/v) ethyl acetate: ethanol, mixed for 5 min, and centrifuged (1 min, 4°C) at 13000 rpm each time. After every extraction, the supernatant was collected and pooled together. The pooled supernatants amounting to around 7.4 mL was evaporated to dryness using a centrifugal evaporator. The resulting residue was then dissolved in 100 µL of methanol, vortexed (15 s), incubated in the dark for 10 min, vortexed again (15 s), and finally, centrifuged (1 min, 4°C) at 13000 rpm before a sample volume of 90 µL was taken, and then injected into the HPLC system. The HPLC system, peak integration using the software ChromNAV (version 1.19.1; JASCO, Pfungstadt, Germany), and the quantification by peak area comparison against the calibration curves of the ferulic acid and γ-oryzanol external standards were as described by Calvo-Castro et al. (2019).

From the results of the protein, riboflavin and thiamine, and mineral analysis, each rice variety was further evaluated to be labelled as either a 'source' or has 'high' content of the nutrient according to the rules in food labelling from the Codex Alimentarius (FAO/WHO, 2007). The possible contribution of the minerals (calcium, iodine, iron, magnesium, selenium and zinc), thiamine and riboflavin to the FAO/WHO (2001) Recommended Nutrient Intakes (RNI) for children (1-3 years old) and adult females (19–50 years old) were also calculated. While the possible contributions of the RE and the α-TE to the Recommended Safe Intakes (RSI) and the daily Acceptable Intakes (AI) of the same vulnerable groups (FAO/WHO, 2001), respectively, were also determined

Statistical Analysis

The results are presented as mean value ± standard deviation. The descriptive statistics, and One-way Analysis of Variance (ANOVA) followed with Tukey's Multiple Comparison Test (significant at p-value < 0.05) was done using GraphPad Prism 5 (GraphPad Software Inc., San Diego, California, USA).

Results and Discussion

The heirloom rice varieties under study are usually planted in the summer season (from March to beginning of April), and are harvested in the middle of the rainy season (end of August to October). These rice varieties are part of the culture and traditions in the locality and are not only consumed in ordinary meals but are also made into special dishes that are served during festivals and other occasions.

Table 1 shows the morphological characteristics of the rice samples as well as their amylose content. Jasmine rice, a popular Asian white rice variety was used as control. Camoros is a red pigmented rice while Tinta is purple, and Malinao black rice, is black rice originally from the town of Malinao. Jasmine and Tinta rice were not significantly different from each other in terms of their 1000-grain weight. Jasmine and Camoros rice were not significantly different in terms of thickness, while Jasmine rice was not significantly different from the rest of the rice samples in terms of amylose content.

Jasmine rice has the longest kernel (7.60 mm), while the Camoros and Tinta rice samples have the shortest kernels (6.04 to 6.92 mm). In the same line, Jasmine rice is significantly narrower (2.00 mm) compared to the rest of the samples, while Tinta and Malinao black rice has the widest kernel (2.60 to 2.71 mm). As for their aspect ratio (length-to-width), Jasmine rice (3.80) is significantly different compared to the rest of the samples and has a less round shape (3.12). Based on the scale of kernel size and shape developed by IRRI, Jasmine rice is classified as "extra-long" and "slender", Malinao as "long" and "medium", and Camoros and Tinta as "medium" in both size and shape.

According to Frei and Becker (2004), the farmers in the area had reported that the light and small grain varieties are preferred by the consumers and can fetch good market prices, and this could very well explain why Camoros, Malinao black and Tinta were able to enter the lowland market dominated by commercial white rice varieties.

Based on the classification proposed by Juliano (1992), Jasmine, Camoros and Malinao black rice have "low", while Tinta has "very low" amylose content, while based on the commercial classification, all the rice samples have "low" amylose contents. Rice with low amylose content is preferred in the ASEAN region because of its soft and sticky texture when cooked (Jungtheerapanich, Tananuwong, & Anuntagool, 2016). The amylose content was found to strongly influence the eating quality of cooked rice, having positive correlation with hardness, and negative correlation with stickiness (Juliano & Pascual, 1980; Singh, Sodhi, Kaur, & Saxena, 2003; Windham et al., 1997). In contrast, in a study by Suwannaporn et al. al. (2007) using discriminant analysis, they observed that only the pasting property of cooked rice was correlated with amylose content, while texture was more influenced by factors like protein content.

Rice protein consists mainly of glutelin and oryzenin which can form a complex with starch, and in rice with high protein content, this could lower the stickiness, and yield a harder texture (Chrastil, 1990; Suwannaporn et al., 2007).

In general, rice is known for having a better protein quality compared to wheat and other cereals (Juliano, 1985). Tinta rice had the lowest protein content (7.34 g/100 g) compared to Jasmine (7.73 g/100 g), Camoros (8.05 g/100 g), and Malinao black (9.00 g/100 g) rice; at the same time, the protein content of the Camoros and Malinao black rice were significantly higher than of the Jasmine rice (Table 2). Based on the labelling guidelines from the Codex Alimentarius (FAO/WHO, 2007), since all the rice samples have more than 5 g protein in every 100 g, they can be considered as a "source of" the macronutrient. Although the protein content of rice, at 7% in general, is low in comparison to other cereal grains (Shih, 2004), in areas with limited animal-based food products, rice can actually be a significant protein source. The protein contents of the rice samples are well within the range of 6 to 10.5% protein content of commercial rice varieties in the Philippines (Juliano & Villareal, 1993). Protein is highest in the outer layers of the rice grain (Resurreccion, Juliano, & Tanaka, 1979), hence, leaving the outer layers intact can boost the protein content of the rice.

The crude fiber content was not significantly different across samples, while the ash content was highest in Malinao black (1.19 g/100 g) and lowest in Jasmine rice (0.40 g/100 g). The significant differences in the ash content of the rice samples can be attributed to the variability in the mineral content of the soil where they were planted (Juliano & Bechtel, 1985). Similarly, Frei and Becker (2004) found that the crude ash content of rice from the upland areas of Balete and Libacao, also in the province of Aklan, ranged from 0.4 to 1.8%.

Table 3 has the amino acid profiles, while Table 4 shows the essential amino acid ratios based on the FAO/WHO pattern for children (2-5 years old), estimated amino acid scores and the limiting amino acid in the rice samples. The Malinao black rice had the highest amount of both essential (3.34 g/100 g) and non-essential (5.43 g/100 g) amino acids, while Tinta had the lowest amount of both essential (2.64 g/100 g) and non-essential (4.33 g/100 g) amino acids. The sum of all the amino acids per sample ranged from 6.96 (Tinta) to 8.76 g/100 g (Malinao black). The amount of essential amino acids (EAA) was always less than the non-essential amino acids (NEAA), and the ratio between the two is either 0.60 or 0.61. The rice samples did not meet the FAO/WHO (1973) requirement of having at least 40% essential amino acids, but they did meet the 0.6 ratio of essential to non-essential amino acids. The ratio between EAA and NEAA is believed to be responsible for the health-promoting properties of proteins from the diet (Romano et al., 2019). The savory amino acids (aspartic and glutamic acids) were more than 2-fold the amount of sweet amino acids (glycine and alanine) in all the rice samples, although, rice has a predominantly bland taste which makes it a very good staple food that can go along with any dish in a meal. The amino acid score of Jasmine rice is 0.60, and those of the samples ranged from 0.58 (Tinta) to 0.72 (Malinao black), with lysine as the limiting amino acid. Aside from lysine, other cereals, tubers and staple foods also have leucine, tryptophan and/or threonine as limiting amino acids (Kazuo, 1995).

Based on the 2013 Philippines National Nutrition Survey, there is a high prevalence of inadequate intake among adult Filipinos for the vitamins – riboflavin (86-91% of the adults), thiamine (73-89%) and vitamin A (54-66%), and the minerals – iron (97-99%) and calcium (95-98%) (Angeles-Agdeppa et al., 2019). The rice samples' micronutrient contents are shown in the succeeding tables. The carotenoids (including provitamin A carotenoids: β-carotene, α-carotene and β-cryptoxanthin), riboflavin and thiamine, tocopherols and tocotrienols, and ferulic acid and γ-oryzanol contents are presented in Table 5.

Jasmine rice contained the least amount of carotenoids (7.93 mg/100 g) and Malinao black rice contained the highest amount (33.69 mg/100 g). Among the colored rice varieties, Malinao black rice had the highest lutein, β-carotene, 9-cis-carotene, and followed by Camoros (red) and Tinta (purple) rice. Zeaxanthin and α-carotene were highest in red rice, followed by black and purple rice. β-cryptoxanthin was highest in red rice followed by purple and black rice. On the other hand, Jasmine rice only contained β-cryptoxanthin and β-carotene. In their study, Frei and Becker (2004) found that the β-carotene content was highest in the black and purple rice, followed by red and brown rice varieties. Carotenoids have antioxidant activity that has been linked to the reduction of the risk of cancer and cardiovascular diseases (Fiedor & Burda, 2014; Olson, op. 1999); and some of them have the well-known function as dietary source of vitamin A (Institute of Medicine, 2000).

The term vitamin A denotes either preformed vitamin A found in animal-based foods, or provitamin A carotenoid precursors of retinol, that are found in plant-based foods. Of the provitamin A carotenoids present in nature, the most important are α-carotene, β-carotene, and β-cryptoxanthin. Their values are converted into retinol activity equivalents (RAE) and represent vitamin A in the determination of food composition and dietary requirements (Institute of Medicine, 2002). The recommended safe intake (RSI) for vitamin A is 400 µg/day for children (1-3 years old) and 500 µg/day for female adults (19-50 years old). Camoros had the highest RAE (710 µg/100 g) as shown in Table 7b and can cover 177% and 142% of the respective RSIs. On the other hand, Jasmine rice can cover 98% and 78% of the RSIs with the lowest RAE among the samples at 392 µg/100 g, mostly contributed by β-cryptoxanthin. Vitamin A plays an important role in maintaining healthy eyesight, gene expression, reproduction and immunity (Institute of Medicine, 2002).

Thiamine (vitamin B_1) was highest in Camoros and Tinta (2.01-2.07 mg/100 g), and lowest in Jasmine (0.28 mg/100 g) rice, while riboflavin (vitamin B_2) was highest in Malinao black (0.60 mg/100 g) and lowest in Jasmine (0.16 mg/100 g) rice. Based on the Codex Alimentarius (FAO/WHO, 2007), given that the values are greater than 0.24 mg/100 g, all the pigmented rice varieties can be considered as "source of" riboflavin, with Malinao black rice classified as "high in" riboflavin due to its value greater than 0.48 mg/100 g. On the other hand, Jasmine ice is a "source of" thiamine (>0.21 mg/100 g), and all the pigmented rice samples are "high in" thiamine with values not lower than the calculated reference value of 0.42 mg/100 g.

Thiamine is not uniformly distributed in the rice grain. It is mostly concentrated in the layer between the germ and the endosperm called the scutellum (Carpenter, 2000). Thiamine deficiency or beriberi in the Southeast Asian region is attributed to the high consumption of polished, white rice (Khush, 1997); with parboiling being hardly practiced as a method for rice preparation (Soukaloun et al., 2003); and low dietary diversity (National Institute of Statistics, Directorate General for Health, & ICF Macro, 2011). Infantile beriberi also exists and has been linked to low thiamine level in breastmilk coming from mothers having insufficient thiamine intake (Allen, 2012). Riboflavin deficiency, on the other hand, is prevalent in under-developed countries where people have low intakes of meat and dairy products (Whitfield et al., 2015).

Several studies have been conducted to look into the thiamine and riboflavin status of vulnerable groups in the region, specifically in Thailand (Doung-ngern, Kesornsukhon, Kanlayanaphotporn, Wanadurongwan, & Songchitsomboon, 2007), Lao PDR (Khounnorath et al., 2011), and Cambodia (Whitfield et al., 2015; Whitfield et al., 2017). In the Philippines, there is a high prevalence of thiamine and riboflavin deficiency in adults (Angeles-Agdeppa et al., 2019) which could lead to increased risks toward anemia, cardiovascular diseases, cancers, and neurological problems (Gibson et al., 2016; Powers, 2003).

The recommended nutrient intake (RNI) for children under 3 years old is 0.50 mg/day for both thiamine and riboflavin, and 1.10 mg/day for female adults (19-50 years old). Camoros rice can cover 414% and 188% of the RNIs for children, and female adults, respectively. Likewise, the Malinao black rice can cover 120% and 54% of the RNIs. Jasmine rice, on the other hand, can only cover 55% and 25% of the thiamine requirement; and 32% and 14% of the riboflavin requirement for children and adult females, respectively.

The bran layer, specifically, the bran oil, in rice kernels is also known to be a good source of bioactive compounds like γ-oryzanols, ferulic acid and vitamin E, and these components have various health benefits which range from lowering blood cholesterol levels, slowing the onset of diabetes and Alzheimer's disease to inhibiting tumors, and preventing cancers (Gul, Yousuf, Singh, Singh, & Wani, 2015). Malinao black rice had the highest ferulic acid (0.42 ng/mg) and γ-oryzanol (350 ng/mg, pooling together cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate) content among the rice samples, while Jasmine rice had the lowest ferulic acid (0.02 ng/mg) and γ-oryzanol (9.85 ng/mg) content.

Vitamin E is a fat-soluble vitamin composed of phenolic compounds – tocopherols and tocotrienols. The tocols are considered as one of the major antioxidants in food (Shahidi & de Camargo, 2016). As shown in Table 5, in all the pigmented rice samples, the total tocopherol content (6.05 to 7.82 µg/g) was less than the total tocotrienol content (9.29 to 17.90 µg/g). The total tocotrienols was highest in Malinao black rice at 17.90 µg/g, with γ-tocotrienol as the main contributor (10.60 µg/g). The β-tocotrienol and α-tocopherol were only present in Malinao black rice (0.18 and 0.39 µg/g, respectively), and γ-tocopherol was absent in Jasmine rice. The total vitamin E content was highest in Camoros and Malinao black rice, which ranged from 21.47 to 23.96 µg/g. In the study of Shammugasamy, Ramakrishnan, Ghazali, and Muhammad (2014) on Malaysian rice, the red-pigmented varieties had a total vitamin E content that ranged from 19.36 to 37.00 µg/g, while the black-pigmented varieties had a total vitamin E content that ranged from 28.54 to 51.33 µg/g. Similarly, they also found that there are more tocotrienols than tocopherols, and that γ-tocotrienol was the major congener, contributing almost half of the vitamin E contents in the rice samples.

The vitamin E was calculated as α-tocopherol equivalents (α-TE) for all the rice samples and their contribution towards the acceptable intake (AI) for children (5 mg/day) and adult females (7.5 mg/day) are shown in Tables 7a-d. Camoros rice can cover 16%, the highest among the rice samples, of the AI for children and 11% for female adults. In comparison, Jasmine rice can only cover 13% and 8% of the AI for children and female adults, respectively.

The mineral content of the rice samples is shown in Table 6. Calcium (75.95 mg/kg), manganese (18.88 mg/kg), and zinc (24.01 mg/kg) were highest in Tinta rice. Copper (5.88 mg/kg), magnesium (1050.24 mg/kg), potassium (2256.59 mg/kg), and sodium (9.61 mg/kg) were highest in Malinao black rice. Iron (9.14 mg/kg) and molybdenum (0.80 mg/kg) were highest in Camoros rice. Iodine was less than 0.50 mg/kg in all the samples, while selenium was less than 0.05 mg/kg in all the rice samples except in Camoros rice (0.06 mg/kg).

Among the rice samples, Malinao black rice had the highest phosphorus content (2808 mg/kg), while Jasmine rice had the lowest content at 656 mg/kg. In cereal grains, 65% to 85% of the total phosphorus is stored as phytic acid (Raboy, 2000), and this is also true in the case of rice (Resurreccion et al., 1979). Unfortunately, phytic acid is an anti-nutrient, as it can chelate positively charged minerals like calcium, magnesium, potassium, iron, and zinc (Raboy, 2003). The resulting salts (phytate) are very insoluble and hinder the absorption of micronutrients in the gastrointestinal tract (Mitchikpe et al., 2008). Although phytic acid can be reduced in white rice (Liu, Zheng, Wang, & Chen, 2019), vegetables and legumes (Fabbri & Crosby, 2016, 2016) by cooking and other household processes like washing and soaking, these methods can also have negative effects on the vitamins and minerals present. Hence, approaches in reducing phytic acid while preserving the micronutrients should be explored. Plant breeding and modern techniques in molecular biology could be the answer to this problem (Perera, Seneweera, & Hirotsu, 2018), on the other hand, degree of milling and milling time has been proven to significantly influence the mineral content of rice, as the milling process can decrease the amount of the minerals highly concentrated in the outer layer of the grain as fractions are removed, going from one layer to the next (Wang et al., 2011).

Based on the Codex Alimentarius (FAO/WHO, 2007), the pigmented rice samples could be considered as "high in" iodine, although this should be verified to get the specific values. Camoros and Malinao black rice are "high in" magnesium, while Tinta is a "source of" magnesium and zinc. Furthermore, as shown in Tables 7a-d, based on the RNI for children and adult females, respectively, Camoros rice can provide the highest amount of selenium (38% and 25% RNI); Malinao black rice can provide the highest amount of iron (8% and 2% RNI) and magnesium (175% and 48% RNI); and Tinta rice can provide the highest amount of calcium (albeit, only 2% and 1% RNI) and zinc (29% and 25% RNI). In the study of Jati, Nohr, and Biesalski (2014) on pigmented rice from Indonesia, the black (n790 and jowo) and red (mandel) rice varieties showed significantly higher iron and zinc contents compared to white rice (ir64).

Among the essential minerals, iodine, iron and zinc are associated with hidden hunger because their deficiencies exhibit invisible symptoms (Biesalski, 2013). Iodine is an important component of thyroxine and triiodothyronine in the thyroid gland. These hormones regulate many essential biochemical reactions in the body like the synthesis of protein and enzyme activities. Iodine deficiency disorders, resulting from insufficient hormone production due to inadequate iodine, include mental retardation, goiter, cretinism. Iron is a component of several protein classes – heme proteins, iron-sulfur enzymes, iron storage and transport proteins, and other iron-containing or activated enzymes. Iron deficiency results in reduced physical and mental performance, anemia and adverse pregnancy outcomes (Institute of Medicine, 2002). Zinc is a component of various enzymes that are involved in the maintenance of proteins' structural integrity, and the regulation of gene expression (Cousins, 1996). Zinc deficiency is often exhibited in non-specific symptoms like growth retardation, alopecia, diarrhea, impotence, eye and skin lesions, and decrease in appetite (Institute of Medicine, 2002).

Conclusion

Camoros, Malinao black and Tinta rice contain essential nutrients that make them very good substitutes for white rice in the diet – as staple food for adults and as complementary food ingredient for children. Based on the results of this study, the pigmented rice exhibited higher content of the most of the nutrients compared to the white rice, so the rice variety can even be tailored to one's dietary requirement e.g. consumption of Camoros for its vitamins A and E; Malinao black rice for its essential amino acids, riboflavin, ferulic acid and γ-oryzanol; and Tinta for its magnesium and zinc. There is really a huge potential for pigmented rice, and future investigations should be focused on elevating these pigmented rice varieties as functional food and finding ways of increasing the bioavailability of the micronutrients that they contain while reducing the anti-nutrient components.

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Table 1. Morphological characteristics and amylose content of the rice samples (mean±SD; n=5)

Characteristics	Jasmine	Pigmented Rice		
		Camoros	Malinao	Tinta
Color	White	Red	Black	Purple
1000-grain weight (g)	18.68±0.38[a]	15.50±0.09[b]	22.18±0.99[c]	18.37±0.79[a]
Length (mm)	7.60±0.19[a]	5.79±0.26[b]	6.92±0.16[c]	6.04±0.62[b]
Width (mm)	2.00±0.00[a]	2.34±0.09[b]	2.60±0.15[bc]	2.71±0.29[c]
Thickness (mm)	1.73±0.04[a]	1.62±0.08[a]	1.89±0.07[b]	1.93±0.10[b]
Aspect ratio	3.80±0.09[a]	2.48±0.14[b]	2.67±0.18[b]	2.26±0.40[b]
Shape	3.12±0.10[a]	2.50±0.05[b]	2.57±0.07[b]	2.26±0.18[c]
Amylose (%)	17.79±6.33[a]	18.01±4.25[a]	17.34±2.43[a]	7.58±3.74[a]

Values in a row sharing the same letter superscripts are not significantly different at $p<0.05$.

Table 2. Protein, crude fiber and ash content (mean±SD; n=2) of the rice samples

Component (g/100g)	Jasmine	Pigmented Rice			'Source of'	'High in'
		Camoros	Malinao	Tinta		
Protein	7.73±0.01[a]	8.05±0.03[b]	9.00±0.04[c]	7.34±0.11[d]	5	10
Crude fiber	<0.90±0.00[a]	<0.90±0.00[a]	<0.90±0.00[a]	<0.90±0.00[a]		
Ash	0.40±0.06[a]	1.10±0.06[b]	1.19±0.01[c]	0.73±0.03[d]		

Values in a row sharing the same letter superscripts are not significantly different at $p<0.05$.

Table 3. Amino acid profiles (mean±SD; n=2) of the rice samples

Amino Acid Profile		Jasmine	Pigmented Rice		
			Camoros	Malinao	Tinta
EAA (g/100g)	Histidine	0.18±0.01[a]	0.18±0.01[a]	0.21±0.01[b]	0.18±0.01[a]
	Isoleucine	0.31±0.01[a]	0.31±0.01[a]	0.37±0.04[b]	0.29±0.02[a]
	Leucine	0.62±0.00[a]	0.63±0.01[a]	0.72±0.03[b]	0.58±0.01[c]
	Lysine	0.27±0.00[a]	0.29±0.01[b]	0.33±0.01[c]	0.26±0.00[a]
	Methionine	0.18±0.00[a]	0.19±0.00[ab]	0.31±0.01[c]	0.21±0.01[b]
	Phenylalanine	0.41±0.01[a]	0.41±0.00[a]	0.46±0.01[b]	0.37±0.01[c]
	Threonine	0.26±0.00[a]	0.27±0.00[a]	0.31±0.01[b]	0.26±0.01[a]
	Tryptophan	0.09±0.01[a]	0.10±0.01[a]	0.11±0.01[a]	0.09±0.01[a]
	Valine	0.44±0.01[a]	0.45±0.01[a]	0.52±0.04[b]	0.41±0.01[a]
	Sub-total	2.74±0.01[ab]	2.82±0.04[a]	3.34±0.06[c]	2.64±0.06[b]
NEAA (g/100g)	Alanine	0.42±0.01[a]	0.44±0.00[b]	0.52±0.01[c]	0.40±0.00[d]
	Aspartic acid	0.68±0.01[a]	0.69±0.03[a]	0.79±0.03[b]	0.63±0.01[c]
	Arginine	0.63±0.00[a]	0.64±0.01[a]	0.70±0.00[b]	0.59±0.01[c]
	Cysteine	0.16±0.01[a]	0.18±0.00[b]	0.23±0.00[c]	0.18±0.01[b]
	Glutamic acid	1.32±0.02[a]	1.35±0.00[b]	1.54±0.01[c]	1.25±0.01[d]
	Glycine	0.33±0.01[a]	0.35±0.00[b]	0.41±0.01[c]	0.33±0.00[a]
	Proline	0.36±0.01[a]	0.38±0.01[b]	0.42±0.01[c]	0.33±0.00[d]
	Serine	0.38±0.01[a]	0.38±0.00[a]	0.46±0.02[b]	0.35±0.02[c]
	Tyrosine	0.34±0.00[a]	0.33±0.00[a]	0.37±0.00[b]	0.28±0.00[c]
	Sub-total	4.60±0.01[a]	4.73±0.02[b]	5.43±0.08[c]	4.33±0.02[d]
Total AA content (g/100g)		7.33±0.00[a]	7.55±0.06[b]	8.76±0.13[c]	6.96±0.04[d]
EAA (%)		37.31±0.07[a]	37.31±0.19[a]	38.07±0.06[a]	37.86±0.58[a]
NEAA (%)		62.69±0.07[a]	62.69±0.19[a]	61.93±0.06[a]	62.14±0.58[a]
Ratio of EAA to NEAA		0.60±0.01[a]	0.60±0.01[a]	0.61±0.01[a]	0.61±0.02[a]
Savory AA (%)		27.22±0.07[a]	27.04±0.07[ab]	26.60±0.05[c]	26.94±0.08[b]
Sweet AA (%)		10.10±0.14[a]	10.47±0.08[b]	10.50±0.04[b]	10.49±0.06[b]

AA, amino acids; EAA, essential amino acids; NEAA, non-essential amino acids; Values in a row sharing the same letter superscripts are not significantly different at $p<0.05$.

Table 4. Essential amino acid ratios based on the FAO/WHO pattern for children (2-5 years old), estimated amino acid score, and limiting amino acid in the rice samples

Essential Amino Acid	FAO/WHO Pattern for Children	Jasmine	Pigmented Rice		
			Camoros	Malinao	Tinta
Histidine	19	1.23	1.23	1.43	1.23
Isoleucine	28	1.41	1.43	1.71	1.32
Leucine	66	1.22	1.23	1.41	1.13
Lysine	58	0.60	0.64	0.72	0.58
Methionine + Cysteine	25	1.76	1.91	2.79	2.02
Phenylalanine + Tyrosine	63	1.54	1.52	1.70	1.33
Threonine	34	0.99	1.03	1.18	0.97
Tryptophan	11	1.06	1.18	1.29	1.06
Valine	35	1.61	1.64	1.92	1.52
AA score		0.60	0.64	0.72	0.58
Limiting amino acid		Lys	Lys	Lys	Lys

Table 5. Vitamin content (mean±SD; n=3) of the rice samples

Vitamin		Jasmine	Pigmented Rice		
			Camoros	Malinao	Tinta
Carotenoid (mg/100g)	Lutein	0.00±0.00^{a}	9.67±0.16^{b}	19.00±0.23^{c}	9.59±0.16^{b}
	Zeaxanthin	0.00±0.00^{a}	2.21±0.01^{b}	2.02±0.00^{c}	1.99±0.01^{d}
	β-Cryptoxanthin	6.47±0.34^{a}	5.85±0.08^{b}	5.03±0.12^{c}	5.35±0.10^{c}
	α-Carotene	0.00±0.00^{a}	1.47±0.09^{b}	1.31±0.01^{b}	1.29±0.22^{b}
	β-Carotene	1.47±0.04^{a}	4.86±0.12^{b}	5.11±0.11^{c}	3.86±0.09^{d}
	9-cis-Carotene	0.00±0.00^{a}	1.05±0.03^{b}	1.22±0.02^{c}	0.90±0.02^{d}
	Total	7.93±0.32^{a}	25.12±0.15^{b}	33.69±0.39^{c}	22.99±0.20^{d}
Riboflavin (mg/100g)		0.16±0.02^{a}	0.28±0.03^{b}	0.60±0.04^{c}	0.35±0.04^{b}
Thiamine (mg/100g)		0.28±0.02^{a}	2.07±0.03^{b}	1.59±0.06^{c}	2.01±0.01^{b}
Ferulic acid (ng/mg)		0.02±0.00^{a}	0.17±0.01^{b}	0.42±0.09c	0.07±0.01^{ab}
γ-Oryzanol (ng/mg)		9.85±1.48^{a}	237.79±6.63^{b}	350.34±22.92^{c}	92.67±10.72^{d}
Vitamin E (μg/g)	α-Tocotrienol	3.29±0.39^{a}	4.68±0.92^{a}	5.33±1.37^{a}	4.40±0.28^{a}
	β-Tocotrienol	0.00±0.00^{a}	0.00±0.00^{a}	0.18±0.04^{b}	0.00±0.00^{a}
	δ-Tocotrienol	0.20±0.01^{a}	1.84±0.10^{b}	1.84±0.17^{b}	0.52±0.06^{c}
	γ-Tocotrienol	1.67±0.17^{a}	7.13±1.04^{b}	10.56±1.94^{c}	4.37±0.96^{ab}
	α-Tocopherol	0.00±0.00^{a}	0.00±0.00^{a}	0.39±0.10^{b}	0.00±0.00^{a}
	β-Tocopherol	5.95±0.08^{a}	6.29±1.07^{a}	3.51±0.00^{b}	4.74±0.63^{ab}
	δ-Tocopherol	0.96±0.06^{a}	1.08±0.02^{a}	1.23±0.18^{ab}	1.47±0.13^{b}
	γ-Tocopherol	0.00±0.00^{a}	0.45±0.11^{b}	0.93±0.27^{c}	0.99±0.13^{c}
	Total T	6.91±0.10^{a}	7.82±1.19^{a}	6.05±0.42^{a}	7.19±0.76^{a}
	Total T3	5.16±0.56^{a}	13.65±0.67^{b}	17.90±1.90^{c}	9.29±1.16^{d}
	Total VE	12.07±0.61^{a}	21.47±1.54^{b}	23.96±1.88^{b}	16.49±1.84^{c}

T, tocopherol; T3, tocotrienol; VE, vitamin E; Values in a row sharing the same letter superscripts are not significantly different at $p<0.05$.

Table 6. Mineral content (mean values in mg/kg±SD; n=2) of the rice samples and the limits for food labelling 'source of' and 'high in'

Mineral (mg/kg)	Jasmine	Pigmented Rice			'Source of'	'High in'
		Camoros	Malinao	Tinta		
Ca	32.86±0.05[a]	57.63±2.09[b]	53.85±1.09[c]	75.95±1.59[d]	1200	2400
Cu	1.49±0.03[a]	3.19±0.01[ab]	5.88±2.07[c]	3.60±0.01[b]		
I	<0.500±0.00[a]	<0.500±0.00[a]	<0.500±0.00[a]	<0.500±0.00[a]	0.225	0.450
Fe	1.32±0.05[a]	9.14±1.49[b]	9.43±0.02[b]	6.50±0.03[c]	21	42
Mg	112.70±1.81[a]	961.29±16.50[b]	1050.24±20.19[c]	529.43±2.20[d]	450	900
Mn	7.17±0.03[a]	17.46±0.23[b]	16.36±0.31[c]	18.88±0.15[d]		
Mo	0.45±0.00[a]	0.80±0.01[b]	0.37±0.01[c]	0.12±0.00d		
P	656.42±5.97[a]	2604.06±19.77[b]	2808.00±38.85[c]	1391.96±14.32[d]		
K	645.16±1.01[a]	2058.45±1.71[b]	2256.59±47.82[c]	1408.25±9.40[d]		
Se	<0.050±0.00[a]	0.06±0.01[b]	<0.050±0.00[a]	<0.050±0.00[a]		
Na	2.81±0.04[a]	3.20±0.06[b]	9.61±0.16[c]	4.53±0.08[d]		
Zn	16.13±0.06[a]	16.46±0.41[ab]	16.82±0.25[b]	24.01±0.28[c]	22.50	45.00

Values in a row sharing the same letter superscripts are not significantly different at $p<0.05$.

Table 7a. Jasmine rice's contribution (%) to the micronutrient requirements of children (1-3 years old) and adult females (19-50 years old, pre-menopausal)

Micronutrient	Amount	Children		Adult Females	
		RSI (µg/day)	%	RSI (µg/day)	%
RAE (µg/100g)	391.73±12.65	400	97.93	500	78.35
B vitamin (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Thiamine	0.28±0.02	0.50	55.28	1.10	25.13
Riboflavin	0.16±0.02	0.50	31.66	1.10	14.39
		AI (mg/day)	%	AI (mg/day)	%
α-TE (mg/100g)	0.63±0.04	5.0	12.53	7.5	8.36
Mineral (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Calcium	3.29±0.00	500	0.66	1000	0.33
Iodine	<0.05±0.00	0.075	<66.67	0.110	<45.45
Iron†	0.13±0.01	12	1.10	59	0.22
Magnesium	11.27±0.18	60	18.78	220	5.12
Selenium	<0.01±0.00	0.017	<29.41	0.026	<19.23
Zinc‡	1.61±0.01	8.40	19.21	9.80	16.46

RSI, recommended safe intake; RAE, retinol activity equivalents; AI, acceptable intake; TE, tocopherol equivalents; RNI, recommended nutrient intake; †at 5% bioavailability; ‡at low bioavailability

Table 7b. Camoros rice's contribution (%) to the micronutrient requirements of children (1-3 years old) and adult females (19-50 years old, pre-menopausal)

Micronutrient	Amount	Children		Adult Females	
		RSI (µg/day)	%	RSI (µg/day)	%
RAE (µg/100g)	709.93±6.38	400	177.48	500	141.99
B vitamin (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Thiamine	2.07±0.03	0.50	413.80	1.10	188.09
Riboflavin	0.28±0.03	0.50	55.41	1.10	25.19
		AI (mg/day)	%	AI (mg/day)	%
α-TE (mg/100g)	0.79±0.04	5.0	15.74	7.5	10.50
Mineral (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Calcium	5.76±0.21	500	1.15	1000	0.58
Iodine	<0.05±0.00	0.075	<66.67	0.110	<45.45
Iron†	0.91±0.15	12	7.62	59	1.55
Magnesium	96.13±1.65	60	160.21	220	43.69
Selenium	0.01±0.00	0.017	37.67	0.026	24.63
Zinc‡	1.65±0.04	8.40	19.60	9.80	16.80

RSI, recommended safe intake; RAE, retinol activity equivalents; AI, acceptable intake; TE, tocopherol equivalents; RNI, recommended nutrient intake; †at 5% bioavailability; ‡at low bioavailability

Table 7c. Malinao black rice's contribution (%) to the micronutrient requirements of children (1-3 years old) and adult females (19-50 years old, pre-menopausal)

Micronutrient	Amount	Children		Adult Females	
		RSI (µg/day)	%	RSI (µg/day)	%
RAE (µg/100g)	689.97±12.46	400	172.49	500	137.99
B vitamin (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Thiamine	1.59±0.06	0.50	317.44	1.10	144.29
Riboflavin	0.60±0.04	0.50	119.64	1.10	54.38
		AI (mg/day)	%	AI (mg/day)	%
α-TE (mg/100g)	0.75±0.16	5.0	14.97	7.5	9.98
Mineral (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Calcium	5.38±0.11	500	1.08	1000	0.54
Iodine	<0.05±0.00	0.075	<66.67	0.110	<45.45
Iron†	0.94±0.00	12	7.85	59	1.60
Magnesium	105.02±2.02	60	175.04	220	47.74
Selenium	<0.01±0.00	0.017	<29.41	0.026	<19.23
Zinc‡	1.68±0.02	8.40	20.03	9.80	17.17

RSI, recommended safe intake; RAE, retinol activity equivalents; AI, acceptable intake; TE, tocopherol equivalents; RNI, recommended nutrient intake; †at 5% bioavailability; ‡at low bioavailability

Table 7d. Tinta rice's contribution (%) to the micronutrient requirements of children (1-3 years old) and adult females (19-50 years old, pre-menopausal)

Micronutrient	**Amount**	**Children**		**Adult Females**	
		RSI (μg/day)	%	RSI (μg/day)	%
RAE (μg/100g)	598.67±3.01	400	149.67	500	119.73
B vitamin (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Thiamine	2.01±0.01	0.50	401.59	1.10	182.54
Riboflavin	0.35±0.04	0.50	70.17	1.10	31.89
		AI (mg/day)	%	AI (mg/day)	%
α-TE (mg/100g)	0.69±0.06	5.0	13.73	7.5	9.16
Mineral (mg/100g)		RNI (mg/day)	%	RNI (mg/day)	%
Calcium	7.59±0.16	500	1.52	1000	0.76
Iodine	<0.05±0.00	0.075	<66.67	0.110	<45.45
Iron†	0.65±0.00	12	5.42	59	1.10
Magnesium	52.94±0.22	60	88.24	220	24.06
Selenium	<0.01±0.00	0.017	<29.41	0.026	<19.23
Zinc‡	2.40±0.03	8.40	28.58	9.80	24.50

RSI, recommended safe intake; RAE, retinol activity equivalents; AI, acceptable intake; TE, tocopherol equivalents; RNI, recommended nutrient intake; †at 5% bioavailability; ‡at low bioavailability

Chapter 7

Thailand’s Pigmented Rice Varieties against the Triple Burden of Malnutrition

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Thailand's Pigmented Rice Varieties against the Triple Burden of Malnutrition

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Abstract

Thailand, considered as the world`s "rice basket", has more than 5,000 rice varieties. In this paper, we look into Thai pigmented rice and their micronutrient content, in view of dietary diversification. This review includes consolidated data on the glycemic index, proximate composition, and micronutrient contents, to highlight these rice varieties' potential as safe and inexpensive sources of nutrients and as viable staple food substitute for white rice. The review aims to encourage the consumption of pigmented rice, not only in Thailand but globally. There is still a lot of nutritionally-unprofiled pigmented rice varieties worldwide, and the search for the best white rice replacement – the perfect combination of low glycemic index, and high protein, crude fiber, vitamin and mineral contents – should be pursued.

Keywords

Red, purple, and black rice, glycemic index, proximate composition, micronutrient content

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Introduction

Malnutrition, as defined by the World Health Organization [1], is either deficiencies, excesses, or imbalances in a person's energy and/or dietary intake. The term malnutrition includes three broad cases, often referred to as the "triple burden" of malnutrition: (1) undernutrition, manifested as wasting (low weight-for-height), stunting (low height-for-age) and underweight (low weight-for-age); (2) micronutrient-related malnutrition, also called "hidden hunger", which includes micronutrient deficiencies or excess; and (3) overweight, obesity that leads to diet-related noncommunicable diseases (DR-NCDs like heart disease, stroke, diabetes and some forms of cancer).

Rice is the staple food in most Asian households, and a meal is not complete without it. It comes in different forms and varieties, but white rice is most popular, and only about 15% of what is eaten is in the form of pigmented rice [2]. It is very unfortunate, however, that white rice is known as a high glycemic index (GI) food, and through milling and polishing, is stripped of vitamins and minerals, essential amino and fatty acids, and bioactive components, and has been implicated in all the burdens of malnutrition [3–5].

Thailand is one of the largest producers and the largest exporter of rice in the world [6]. It is the world's "rice basket" and more than 5,000 rice varieties exist in more than 10 million hectares of riceland in the country [7]. Thai rice, especially the aromatic Jasmine rice (*Oryza sativa* cultivar Kao Dok Mali 105), is exported to more than 130 countries and territories [8], and with the continued rise in demand in both the Asian and African regions, Thai rice is currently being consumed by more people internationally than the whole population of Thailand [9].

Due to economic progress and effective nutrition programs, Thailand, a high-middle income country as of 2011 [10], is now enjoying a much-improved nutrition status. From the report on the food security status of Thailand from 2005 to 2011, published in 2013, the country had already achieved both the Millennium Development Goal (MDG) in the reduction of the proportion of undernourished, and the World Food Summit (WFS) target of 50% reduction in the number of undernourished individuals, way ahead of the 2015 deadline [11]. Although malnutrition has not been fully eradicated, with some dregs of undernutrition, like stunting, and iron and iodine deficiencies in the recesses of urban areas and some far-flung villages, like in the refugee camps along the Thailand-Myanmar border, a new health threat has emerged. Like China and other countries enjoying a rapid increase in wealth together with urbanization, globalization and technological advancement [12], Thailand is dealing with nutrition transition, and the shift in the dietary patterns and physical activities of the population has led to a problem of obesity and DR-NCDs amongst women and children [13]. Thailand has the highest incidence of death from DR-NCDs in the ASEAN region, and the DR-NCDs are deemed as priority public health problems in the country [14]. Currently, Thailand has a Global Hunger Index of 9.7 which means a "low case of hunger" [15], and a Hidden Hunger Index of 14.7% which translates to a "mild case of micronutrient deficiencies" [16]. However, it has a child obesity rate of 15% and adult obesity rate of 10%, 3rd highest among the ASEAN (Association of Southeast Asian Nations) members [17].

Among the possible solutions to the problem of malnutrition, diet diversification has been deemed as the most sustainable [18, 19]. And in rice-eating countries, it is high time to focus on finding a better staple food and shifting the diet towards pigmented rice. Pigmented rice come in red color due to oxidized proanthocyanidins, in purple due to anthocyanins and proanthocyanidins, or in black due to anthocyanins, in the pericarp and bran layer [20–22]. The pigmented rice is milled to only remove the husk or hull, but with the bran layer, germ and endosperm left intact [6]. Thailand has a large number of pigmented rice landraces or local varieties which are still grown by indigenous people and small farmers in rural areas of the country [23].

Several reviews on pigmented rice from Indonesia [24], Thailand [25], South India [26], and from other parts of the world have been done over the years, focusing on the bioactive components of the rice [2, 21], and their medicinal benefits [27, 28]. The most recent review by Zhao et al. [29] presented information on pigmented rice with proanthocyanidins and anthocyanins accumulation, how various rice nutrients are synthesized and metabolized, and the status of their biofortification. So far, no review has been done on the myriad Thai pigmented rice varieties and their micronutrient content, linking them to dietary diversification.

This review paper aims to remedy this, and showcase consolidated data on the glycemic index, proximate composition, and micronutrient contents to highlight Thai rice varieties' potential as an inexpensive source of nutrients and as a viable staple food replacement for white rice. Furthermore, this review aims to encourage the consumption of pigmented rice, not only in Thailand but world-wide.

Glycemic Index

Consumers are now more conscious about the impact of food on their health, and there is an increasing interest in foods with low GI. Table 1 shows the glycemic index of seven pigmented Thai rice varieties, classified as low (≤55), medium (56-69) and high (≥70) GI [30].

The glycemic index is a tool to quantify the change in the glucose level in the blood as a response to an ingested carbohydrate in comparison with the response to an ingested reference standard like glucose or white bread [31]. A food's GI is positively correlated to the blood glucose level in the body, and this has been linked to the occurrence of various DR-NCDs [32, 33]. Due to the high GI of polished white rice, its consumption as a staple food has been strongly implicated in increasing the risk of type 2 diabetes in Asian populations [34], in contrast with brown rice which has been lauded to be very effective in controlling hyperglycemia [35].

Pongjanta et al. [36] estimated the GI of red and purple Thai rice varieties using the methods of Goñi et al. [37] and Patindol et al. [38], while Inpun [39] and Owolabi et al. [40] determined the GI of Sangyod Phatthalung, and Khao Niaw Dam Peuak Dam, respectively, using another version of the method of Goñi et al. [41]. The GI ranged from 62.00±0.57 to 82.79±2.23, with Homlanna and Sangyod Phatthalung having "medium" glycemic indices. The rest of the pigmented rice varieties have higher GI values than brown Jasmine rice with a GI of 70.30 [42], but lower than the GIs of milled Jasmine rice grown in the US and Thailand which ranged from 96 to 116 [30].

Aside from the milling process, the inherent starch characteristics, postharvest, and consumer processing are main factors that could explain the differences in the glycemic responses to rice [43]. The GI of pigmented rice is inversely correlated to their pasting temperature, final viscosity, and total phenolics compounds, and positively correlated with its resistant starch and amylose contents [36]. On the other hand, the study of Inpun [39] and Owolabi et al. [40] showed that soaking and germination can significantly reduce the GI of pigmented rice.

Proximate Composition

Table 2 shows the proximate composition of pigmented Thai rice varieties with the proximate compositions of the brown and white Jasmine rice for comparison.

Yodmanee et al. [7], Inpun [39], Laokuldilok et al. [44], Fasahat et al. [45], Payakapol [46], Sangpimpa and Utama-ang [47], and Settapramote et al. [48] mainly used AOAC methods [49, 50] to elucidate the proximate composition of the pigmented Thai rice varieties. The moisture content was determined by oven-drying at 105°C until constant weight was achieved, Soxhlet extraction was used to determine the crude fat content, and protein content was evaluated using the Kjeldahl method, with 5.95 as conversion factor, except for Yodmanee et al. [7] who used a conversion factor of 5.85. Ash content was determined using a muffle furnace at 550°C, and the carbohydrate content was estimated by difference from the sum of the moisture, fat, protein, and ash contents of the rice samples.

On the other hand, some studies employed methods developed by other associations. Inpun [39] analyzed the total dietary fiber using the method of the American Association for Clinical Chemistry (AACC) [51]. Melini and Acquistucci [52] determined the moisture and protein contents using the standard methods (No. 110/1 and No. 105/2, respectively) of the International Association for Cereal Science and Technology (ICC) [53]. Sompong et al. [54] also determined the crude protein content using the ICC [55] method, while the total lipids was analyzed using the method of Matissek et al. [56], and the total dietary fiber content was measured using the Megazyme kit (K-TDFR).

The data from the ASEAN Food Composition Database were compiled from six national databases, with independently determined components from different samples and in different laboratories. The total carbohydrate contents of THA19, THA24 and the white Jasmine rice had to be recalculated to exclude the value of dietary fiber so that the data can be compared with the carbohydrate contents of the other varieties.

Presented as percentages (%), the moisture content of the pigmented Thai rice varieties ranged from 5.96±0.06 (Chormaiphai) to 13.30±0.00 (Jasmine Red Rice), the protein content from 6.15±0.21 (black Kum Doi Saket) to 13.11±0.34 (purple KumDoiSaket), fat content from 0.39±0.01 (Hom-Nin) to 3.72±0.06 (Niaw Dam Pleuak Khao), crude fiber content from 0.16±0.05 (Black Waxy Rice-96044) to 4.90 (THA24), and the ash content from 1.08-1.63 (Riceberry) to 2.15 (Sangyod). The carbohydrate content of the pigmented Thai rice varieties ranged from 71.99±0.08 (Niaw Dam Pleuak Dam) to 85.27±0.14 (black Kum Doi Saket).

In comparison with the proximate composition of the brown Jasmine rice, 14 pigmented rice varieties had higher moisture contents, nine with higher protein, and five with higher fat contents, 14 with higher amounts of crude fiber, 13 with higher ash and eight with higher carbohydrate contents. For white Jasmine rice, six pigmented rice varieties had higher moisture contents, all varieties had higher amounts of protein and ash, all except one had higher fat content, 14 varieties had higher crude fiber content, and three varieties with higher carbohydrate content.

The proximate composition of rice, in general, is influenced by its variety, environmental conditions, and the soil and the fertilizers provided to it [57]. The difference in the ash content is attributed to the minerals in the rice, taken up from the soil [58]. In the study by Settapramote et al. [48], it was established that Riceberry grown in different provinces in Thailand showed significantly different macronutrient composition.

The ideal moisture content of the rice seeds is between 13% and 14% to prevent them from cracking and breaking during the milling process. In storage, however, the rice kernels should have a moisture content of less than 14% to avoid mold growth, discoloration, and insect damage [59]. As for the rest of the rice components like protein, fat and crude fiber, they are concentrated in the outer layers of the rice grain [60], and leaving the bran fraction intact as in the case of pigmented rice, can boost the macronutrient value of the rice.

For food to be labeled as a "source of" protein, it must have more than 5 g protein in every 100 g [61]. All the pigmented rice varieties documented in Table 2 has protein content greater than 5% or 5 g per 100 g rice, and in this regard, especially in places where people have limited access to animal-based proteins, pigmented rice can be an important protein source. The diversity in the proximate composition of pigmented Thai rice varieties shows that they are better sources of macronutrients when compared to the staple white rice and can provide a better option to consumers with different nutritional needs.

Micronutrients

The micronutrient content of pigmented Thai rice varieties is presented in Tables 3 and 4. The mineral and vitamin contents of brown and white Jasmine rice were also included for comparison.

Rerkasem et al. [9] used the method of Zarcinas et al. [62] to determine the iron and zinc content of the purple rice varieties Bieisu, Kam Doi Saket, Kam Hom Morchor and Luem Pua. Yodmanee et al. [7] measured the iron contents using an Atomic Absorption Spectrophotometer (AAS) after wet-acid digestion and solubilisation of the dehusked rice samples [63]. Inpun [39] analyzed the iron, zinc, sodium, calcium, magnesium, potassium and phosphorus content of the red rice Sangyod Phatthalung by Inductively Coupled Serum Atomic Emission Spectrometer (ICP-AES). Htwe et al. [64] measured the β-carotene content of Hom Mali and Hom Nil by HPLC analysis with a photodiode array detector [65, 66], while Fasahat et al. [45] and Norkaew et al. [67] measured the amount of tocopherol and tocotrienol isomers, the sum of which was the total Vitamin E content of the rice samples, using the HPLC methods with fluorescence detection of Adam et al. [68], and Huang and Ng [69], respectively.The micronutrient data from Puwastein et al. [70] and the ASEAN Food Composition Database [71] were generated using various methods (AAS, colorimetric, titrimetric or gravimetric method for the minerals and HPLC, fluorometric, microbiological, titrimetric or colorimetric method for the vitamins) from different participating laboratories.

Iron and zinc are two of the essential minerals associated with "hidden hunger" because unlike undernutrition, they do not exhibit visible symptoms [72]. As shown in Table 3, the purple rice Kam Hom Morchor had the highest iron content at 4.00 mg/100 g edible portion, and red rice THA19 with the least amount of iron at 0.80 mg/100 g edible portion. Rice varieties, Kam Hom Morchor and THA24, with iron contents greater than 2.1 mg/100 g can be considered as "source of" iron based on the rules in food labelling from the Codex Alimentarius [61]. Kam Hom Morchor can provide 33% and 7% of the recommended nutrient intake (RNI) for children and adult females, respectively. The RNI data for minerals were taken from FAO/WHO [73].

Yodmanee et al. [7] and Meng et al. [74] observed that purple rice varieties have higher iron content than red rice varieties, which can be attributed to environmental factors and genetics. Zinc content ranged from 2.26 ± 0.01 mg/100 g Sangyod Phatthalung to 5.50 mg/100 g Luem Pua. Sangyod Phatthalung, Bieisu, Kam Doi Saket and Kam Hom Morchor have zinc contents greater than 2.25 mg/100 g and can be considered as "source of" zinc, while Luem Pua is "high in" zinc for having more than 4.5 mg of the mineral in 100 g rice. Luem Pua can cover 65% and 56% of the RNI for children and adult females, respectively.

Calcium content ranged from 14.00 mg/100 g THA19 to 48.26 mg/100 g Sangyod Phatthalung. Sangyod Phatthalung can only cover 10% and 5% of the RNI for children and adult females, respectively. Meanwhile, it is the only Thai pigmented rice variety with data on its magnesium content (98.58 ± 0.87 mg/100 g edible portion). The rice variety is "high in" magnesium (greater than 90 mg/100g) and can cover 164% and 45% of the RNI for children and adult females, respectively.

The amount of sodium ranged from 2.00 to 16.30 ± 0.23 mg/100 g rice, while the potassium content ranged from 82.00 to 211.81 ± 1.70 mg/100 g, with THA24 always having less than Sangyod Phatthalung. The phosphorus content ranged from 134.00 mg/100 g THA19 to 215.00 mg/100 g THA24. Unfortunately, in rice, phosphorus is stored as phytic acid – an anti-nutrient that can chelate other minerals hindering their absorption in the body [60, 75, 76].

In comparison, brown Jasmine rice had higher iron and zinc content than white Jasmine rice, however, only two pigmented varieties had lower iron contents than the brown Jasmine rice and none with lower zinc content. According to Saenchai et al. [77], the polishing step in making regular white rice reduces the concentration of iron by 46% and zinc by 31%. In purple rice varieties, iron can be found in the surface layer of the rice kernel while zinc is concentrated in the endosperm [9]. No data was available for the sodium, calcium, magnesium, potassium, and phosphorus content of brown Jasmine rice. White Jasmine rice had a higher sodium content compared to Sangyod and THA24, but lower calcium and phosphorus contents.

The vitamin content of six pigmented Thai rice varieties is shown in Table 4. Khao Mali Dang or Red Hom Mali is a well-known nutritious pigmented rice variety in Thailand but it was observed that it has no detectable β-carotene content. On the other hand, Khao Hom Nil, a black aromatic rice had a β-carotene content of 83.63 ± 1.01 μg per 100 g edible portion. β-carotene, together with α-carotene and β-cryptoxanthin are precursors of Vitamin A, and their values are taken as retinol activity equivalents (RAE) and this, in turn, is considered as the vitamin A content in food [78]. The RAE can be calculated using the conversion factor of 1 μg RAE = 1 μg all-trans-retinol, 12 μg β-carotene, or 24 μg other provitamin A carotenoids [79]. The recommended safe intake (RSI) for vitamin A is 400 ug/day for children (1-3 years old) and 500 μg/day for female adults (19-50 years old) [73]. Khao Hom Nil has an RAE of 7 μg per 100 g and can cover only 1.75% and 1.40% of the RSI for children and female adults, respectively.

As for the B-complex vitamins, THA19 and THA24 have a mean thiamine, riboflavin and niacin contents of 0.37 mg, 0.075 mg, and 2.70 mg/100 g, respectively. According to FAO/WHO [73], The RNI for children under 3 years old is 0.50 mg/day for both thiamine and riboflavin and less than 6 mg niacin equivalents (NEs)/day for niacin, and 1.10 mg thiamine or riboflavin/day and 14 mg NEs/day for female adults (19-50 years old). Niacin equivalents can be calculated using the conversion factor 1 mg NE = 1 mg preformed niacin, or 60 mg tryptophan [73]. Based on the mean values, the pigmented rice can cover 74% of the recommended thiamine intake for children, and 34% for adult females, 15% and 7% of the RNI for riboflavin, 45% and 19% of the RNI for niacin, for children and adult females, respectively. Brown Jasmine rice had higher thiamine content than the white Jasmine rice, while the two pigmented rice varieties THA19 and THA24 have higher thiamine, riboflavin, and niacin contents than white Jasmine rice.

The vitamin E content of Thailand Red Rice and Luem Pua is 0.06 ± 0.00 mg [45] and 2.58 ± 0.05 mg [67] per 100 g edible portion, respectively. To determine the possible contribution of the pigmented rice towards the acceptable intake (AI) of vitamin E for children (5 mg/day) and adult females (7.5 mg/day), the α-tocopherol equivalents (α-TE) were first determined using the conversion factor of 1 mg α-TE = 1 mg α-tocopherol, 2 mg β-tocopherol, 10 mg γ-tocopherol, 3.3 mg α-tocotrienol, or 20 mg β-tocotrienol [80]. Thailand Red Rice has an α-TE of 0.002 mg/100 g and can contribute 0.04% and 0.03% towards the AI for children and adult females, respectively. On the hand, Luem Pua has an α-TE of 1.01 mg/100 g and can cover

20% and 13% of the AI for children and female adults, respectively. Vitamin E in grains, with its tocopherol and tocotrienol components, prevents the oxidative damage of unsaturated fatty acids in the cellular membranes [81].

Concluding Remarks

This review gives a summary of the various Thai rice varieties that had already been studied and puts together the available data to make the information more accessible. However, in the course of this review, some problems were encountered. The first problem was in color classification, especially for purple and black rice varieties. For example, in the study of Sangpimpa and Utama-ang [47], Kum Doi Saket was classified as purple rice while Laokuldilok et al. [44] classified it as black rice in their study. The same is true for Luem Pua which was classified as purple rice by Rerkasem et al. [9] and as black rice by Norkaew et al. [67]. On the other hand, several research papers were excluded from this review because they failed to specify the color of the pigmented Thai rice varieties that were being studied.

Another problem that was encountered was the non-uniformity and ambiguity in the rice variety names. Some examples are KumDoiSaket and Kum Doi Saket, and Sangyod, Sang Yod, Sungyod and Sung Yod with their slight variations in spelling, spacing, and capitalization. Rerkasem [9] has also noted the same problem, with different varieties possibly sharing the same names or the same varieties, but having different names depending on the region, or maybe the group cultivating the landrace. There also exists some ambiguity in rice varieties named Black Waxy Rice-96025 and Black Waxy Rice-96044 that were then described as having dark purple color in the study of Yodmanee et al. [7].

The development of pigmented rice identification tools such as color charts, and the creation of an online database that will aid in the proper identification of these pigmented rice varieties, leading to accurate classification and unified naming would be the permanent solution. Meanwhile, researchers should endeavor to use the verified local names together with the cultivar/variety/landrace numbers (if available) to avoid further confusion and misinformation.

The last problem encountered was the use of different methods in measuring the nutrient composition of the pigmented rice varieties. The given values from various studies cannot be satisfactorily compared with each other, and formulating conclusions is problematic. Of course, the development and use of different analytical methods are unavoidable, and necessary in the progressive field of research. In this regard, it is perhaps timely to select pigmented rice varieties that may be used as reference rice. Institutions like the International Rice Research Institute (IRRI) or perhaps national agencies like the Rice Department of Thailand may take this challenge. The selected reference rice may be nutritionally-profiled using known standard methods, and the grains be made available to researchers so that they can be included in experiments as control to test the precision of the new methods being used, and to be able to compare the nutrient values of other rice varieties in relation to the reference rice. Using the relative nutrient values, different rice varieties can be then easily compared to each other.

In view of the number of studies on the phytochemical compounds in pigmented Thai rice varieties, there has only been a handful of researches made on identifying the varieties' glycemic index or determining their micronutrient content. More researches should be conducted in this line, and micronutrient profiles should be coupled with the quantification of antinutrients like phytic acid to make the data more relevant. The anti-obesity and anti-diabetic properties of pigmented Thai rice varieties, as well as their sensory attributes and consumer acceptability, should also be explored.

In line with the concept of "medical rice" [27], the data gathered on the Thai pigmented rice varieties can be used to tailor-fit their inclusion into people's diet based on health needs. Sangyod Phatthalung having the lowest GI (62, classified as "medium") can be recommended for people who are controlling their blood sugar levels, while Hom Nil can be included in the diet of people with vitamin A deficiency. Also, Luem Pua can be recommended for its vitamin E and zinc, and THA24 for its iron content. On the other hand, there is still a lot of nutritionally-unprofiled pigmented rice varieties in Thailand and worldwide, and the best white rice substitute – the perfect combination of low/medium glycemic index, intermediate amylose content, high protein, crude fiber, vitamin and mineral contents, but low in anti-nutrients, and high in antioxidant activity – is still out there.

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Table 1 Glycemic index of pigmented Thai rice varieties

Bran Color	Thai Rice	Glycemic Index	Classification
Red	Dangmaejang[a]	69.64 ± 0.28	High
	Dayuneanae[a]	80.40 ± 4.02	High
	Khowhoaw[a]	79.46 ± 3.42	High
	Sangyod Phatthalung[b]	62.00 ± 0.57	Medium
Purple	Homlanna[a]	68.5 ± 0.284	Medium
	Khaokum[a]	75.20 ± 3.62	High
	Khao Niaw Dam Peuak Dam[c]	72.64 ± 0.75	High
	URCN-2001[a]	73.83 ± 1.92	High

a, Pongjanta et al. (2016); b, Inpun (2014); c, Owolabi et al. (2020)

Table 2 Proximate composition of pigmented Thai rice varieties and Jasmine rice

Bran Color	Thai Rice	Component (%)					
		Moisture	Protein	Fat	Crude Fiber	Ash	Carbohydrate
Red	Bahng Gawk[a]	11.55 ± 0.04	9.21 ± 0.13	2.86 ± 0.02	3.63 ± 1.30	1.33 ± 0.01	75.04 ± 0.15
	Haek Yah[a]	12.38 ± 0.03	7.40 ± 0.11	2.91 ± 0.05	4.18 ± 0.14	1.40 ± 0.01	75.92 ± 0.11
	Homkradunga[b]	6.76 ± 0.26	6.96 ± 0.10	1.47 ± 0.09	0.28 ± 0.00	1.44 ± 0.10	-
	Jasmine Red Rice[h]	13.30 ± 0.00	9.80 ± 0.00	-	-	1.40 ± 0.04	-
	Kamyan[b]	8.65 ± 0.14	6.63 ± 0.11	2.17 ± 0.04	0.35 ± 0.05	1.64 ± 0.13	-
	Kramrad[b]	7.60 ± 0.21	8.44 ± 0.03	1.93 ± 0.39	0.26 ± 0.01	1.52 ± 0.12	-
	Niaw Dawk Yong[a]	12.01 ± 0.01	9.62 ± 0.16	3.19 ± 0.06	3.75 ± 0.79	1.45 ± 0.06	73.73 ± 0.10
	Niaw Lan Tan[a]	13.12 ± 0.16	7.35 ± 0.06	3.08 ± 0.08	3.27 ± 1.20	1.26 ± 0.05	75.20 ± 0.16
	Niaw Look Pueng[a]	11.45 ± 0.03	7.16 ± 0.01	2.37 ± 0.06	3.17 ± 0.84	1.50 ± 0.07	77.53 ± 0.01
	Red Waxy Rice-96060[b]	8.19 ± 0.30	8.18 ± 0.12	1.58 ± 0.04	0.35 ± 0.05	1.78 ± 0.35	-
	Sangyod[b]	7.18 ± 0.22	8.06 ± 0.03	1.65 ± 0.56	0.26 ± 0.01	2.15 ± 0.05	-
	Sangyod Phatthalung[d]	11.56 ± 0.38	8.22 ± 0.05	3.18 ± 0.03	-	1.36 ± 0.01	-
	Sung Yod Phatthalung[a]	9.28 ± 0.06	10.36 ± 0.04	2.67 ± 0.06	4.51 ± 1.60	1.42 ± 0.12	76.27 ± 0.13
	THA19[e]	11.20	6.80	2.70	4.00	1.10	74.20 (78.20)
	Thailand Red Rice[f]	11.80 ± 0.10	8.18 ± 0.00	2.05 ± 0.00	-	-	-
Purple	Chormaiphai[b]	5.96 ± 0.06	8.23 ± 0.17	1.67 ± 0.09	0.29 ± 0.00	1.35 ± 0.07	-
	KumDoiSaket[i]	12.92 ± 0.10	13.11 ± 0.34	2.59 ± 0.08	1.39 ± 0.08	1.88 ± 0.02	81.03 ± 0.45
	Riceberry[j]	9.69 - 11.59	7.84 - 9.25	2.46 - 3.35	1.82 - 2.40	1.08 - 1.63	73.45 - 76.50
Black	Black Waxy Rice-96025[b]	6.83 ± 0.26	7.69 ± 0.09	1.44 ± 0.11	0.28 ± 0.01	1.38 ± 0.10	-
	Black Waxy Rice-96044[b]	6.08 ± 0.23	8.46 ± 0.17	1.50 ± 0.04	0.16 ± 0.05	1.58 ± 0.20	-
	Hom-Nin[i]	12.28 ± 0.12	11.52 ± 0.23	0.39 ± 0.01	1.44 ± 0.10	1.87 ± 0.08	84.78 ± 0.40
	Khao Nim[h]	12.3 ± 0.10	9.60 ± 0.20	-	-	1.58 ± 0.03	-
	Kum Doi Saket[c]	-	6.15 ± 0.21	1.83 ± 0.02	2.82 ± 0.14	1.75 ± 0.04	85.27 ± 0.14
	Niaw Dam Pleuak Dam[a]	12.03 ± 0.13	10.85 ± 0.09	3.65 ± 0.05	3.41 ± 0.24	1.48 ± 0.02	71.99 ± 0.08
	Niaw Dam Pleuak Khao[a]	12.59 ± 0.16	8.17 ± 0.41	3.72 ± 0.06	4.01 ± 0.58	1.42 ± 0.01	74.09 ± 0.48
	THA24[e]	12.70	8.50	2.60	4.90	1.40	69.90 (74.80)
Jasmine	Brown Rice[g]	11.44 ± 0.06	8.87 ± 0.06	2.92 ± 0.03	1.12 ± 0.04	1.42 ± 0.08	74.23 ± 0.02
	White Rice[e]	11.90	6.10	0.70	0.80	0.30	80.20 (81.00)

"-" not specified; recalculated value in parenthesis; a, Sompong et al. (2011); b, Yodmanee et al. (2011); c, Laokuldilok et al. (2013); d, Inpun (2014); e, Institute of Nutrition, Mahidol University (2014); f, Fasahat et al. (2012); g, Payakapol et al. (2011); h, Melini and Acquistucci (2017); i, Sangpimpa and Utama-ang (2018); j, Settapramote et al. (2018)

Table 3 Mineral content of pigmented Thai rice varieties and Jasmine rice

Bran Color	Thai Rice	Mineral (mg/100g edible portion, dry weight basis)						
		Fe	Zn	Na	Ca	Mg	K	P
Red	Homkradunga[b]	1.16 ± 0.03	-	-	-	-	-	-
	Kamyan[b]	1.26 ± 0.03	-	-	-	-	-	-
	Kramrad[b]	1.48 ± 0.04	-	-	-	-	-	-
	Red Waxy Rice-96060[b]	0.91 ± 0.04	-	-	-	-	-	-
	Sangyod[b]	1.21 ± 0.04						
	Sangyod Phatthalung[c]	1.21 ± 0.01	2.26 ± 0.01	16.30 ± 0.23	48.26 ± 0.44	98.58 ± 0.87	211.81 ± 1.70	210.46 ± 2.64
	THA19[d]	0.80	-	-	14.00	-	-	134.00
Purple	Bieisu[a]	1.40	3.40	-	-	-	-	-
	Chormaiphai[b]	1.66 ± 0.03	-	-	-	-	-	-
	Kam Doi Saket[a]	1.80	3.50	-	-	-	-	-
	Kam Hom Morchor[a]	4.00	3.80	-	-	-	-	-
	Luem Pua[a]	1.90	5.50	-	-	-	-	-
Black	Black Waxy Rice-96025[b]	1.46 ± 0.03	-	-	-	-	-	-
	Black Waxy Rice-96044[b]	1.31 ± 0.03	-	-	-	-	-	-
	THA24[d]	3.50	-	2.00	21.00	-	82.00	215.00
Jasmine	Brown Rice[e]	1.14 ± 0.12	1.75 ± 0.16	-	-	-	-	-
	White Rice[d]	0.90	0.10	34.00	5.00	-	113.00	65.00

"-" not specified; a, converted from Rerkasem et al. (2015); b, Yodmanee et al. (2011); c, converted from Inpun (2014); d, Institute of Nutrition, Mahidol University (2014); e, Puwastein et al. (2009)

Table 4 Vitamin content of pigmented Thai rice varieties and Jasmine rice

Bran Color	Thai Rice	Component (per 100g edible portion, dry weight basis)						
		β-carotene (µg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Tocopherols (mg)	Tocotrienols (mg)	Vitamin E (mg)
Red	Hom Mali[a]	ND	-	-	-	-	-	-
	THA19[b]	-	0.35	0.05	3.40	-	-	-
	Thailand Red Rice[c]	-	-	-	-	(α) 0.00 ± 0.00 (γ) 0.02 ± 0.00	(α) 0.00±0.00 (γ) 0.04±0.00 (δ) 0.00±0.00	0.06 ± 0.00
Black	Hom Nil[a]	83.63 ± 1.01	-	-	-	-	-	-
	Luem Pua*[e]	-	-	-	-	(α) 0.77 ± 0.01 (β) 0.02 ± 0.00 (γ) 0.35 ± 0.01	(α) 0.65 ± 0.01 (γ) 0.71 ± 0.02 (δ) 0.09 ± 0.00	2.58 ± 0.05
	THA24[b]	36.00	0.39	0.10	2.00	-	-	-
Jasmine	Brown Rice[d]	-	0.37 ± 0.05	-	-	-	-	-
	White Rice[b]	-	0.12	0.02	1.50	-	-	-

"-" not specified; *with unit conversion; ND, not detected; a, Htwe et al. (2010); b, Institute of Nutrition, Mahidol University (2014); c, Fasahat et al. (2012); d, Puwastein et al. (2009); e, Norkaew et al. (2017)

Chapter 8 Concluding Discussion

This dissertation posits that food taboos adhered to by pregnant, post-partum, and lactating Southeast Asian women can hinder dietary diversification in the most vulnerable and crucial moment of the first 1,000 days of life. In the first 1,000 days of life (from conception up to two years of age), the focus of most health interventions is naturally on the baby, however, pregnant women and new mothers in the post-partum period, and those breastfeeding should also be given proper attention. The health and well-being of these women can greatly influence the health and well-being of their babies, and hence, the growth and development of a whole new generation. Motherhood demands higher energy and micronutrient requirements than normal as the body also provides for the energy and micronutrient requirements of a growing fetus before birth, and for a growing baby after birth through the production of breastmilk.

In Chapter 2, the plant-based food taboos (Köhler et al., 2018) are presented, and Chapter 3 is on animal-based food taboos (Köhler et al., 2019b). These review papers were the first to showcase researches on food taboos covering the region. They highlight the need for culture-sensitive health interventions to address maternal and child health problems that could lead to the attainment of the sustainable development goals of reducing the maternal and under-five mortality ratios and empowering women in Southeast Asia, as well as the priority health goals of the ASEAN.

The systematic method of reviewing literature and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist was used in keeping track of the collected data. Overall, 281 articles were downloaded from the search engines Google Scholar, PubMed and Scopus, and using the screening parameters, the number was narrowed down to 28. The papers were then segregated whether they contained information on plant- or animal-based food taboos to prepare two manuscripts for publication. This step was done because putting all the data in one paper was not possible, as most journals have limits to the number of pages, tables and references that can be included in an article.

Plant-based food taboos included various types of cereals, legumes, vegetables, and fruits (fresh and processed e.g. cooked, fermented). There were more papers that mentioned postpartum (16) food taboos compared to those that included food taboos during pregnancy (14), and while breastfeeding (6). On the other hand, animal-based food taboos included meat from terrestrial and aquatic animals, and eggs and other derived products (e.g. fish paste and sauce). Similarly, there were more papers (16) on postpartum animal-based food taboos compared to those on pregnancy (9) and while breastfeeding (6).

Lack of knowledge and information may be blamed, but fear of the unknown is the underlying reason why food taboos still exist today. While pregnant, even educated women may still fear that specific foods can harm the baby and/or themselves and can negatively affect the delivery. They will accept beliefs and follow traditional practices handed down from one generation to the next, from someone who has personal experience and indigenous knowledge, someone who they trust, like their mothers and grandmothers. After giving birth, the women may fear that specific foods can make them ill. This post-partum sickness is called *pit duen* in Lao PDR, *phit kham* in Thailand, and *binat* in the Philippines. The symptoms include fever, dizziness, fainting, headache, body ache, numbness, tingling, and weakness, bleeding, and at worst, death. Also, women have to adhere to the food taboos because they are part of culturally-prescribed confinement practices. These confinement practices, often administered on the women by their elders (mother, aunt or grandmother), constitute being restricted at home to rest, with food and activity limitations. In the ASEAN region, women of various descents (Malay, Chinese, Indian, etc.) as well as those of various faiths (Islamic, etc.) follow different confinement periods. When breastfeeding, women also follow food taboos to avoid causing harm to themselves and the baby, as well as having to deal with bad sensorial quality and low milk production.

Despite many of them being unfounded, and with various negative health outcomes linked to food taboos, the review revealed that there are also some positive points to it. The case of medicinal plants that are abortifacient; of fermented fish paste that contains thiaminase that can cause thiamine deficiency and beriberi, and of dog meat, which is associated with rabies, are three good examples. It was also uncovered during the review that while there are food taboos while breastfeeding, women were also encouraged to eat certain foods – indigenous plants that are considered as galactagogues that can increase the milk production. More researches should be conducted in this area, as galactagogues could be beneficial in alleviating the doubts and fears in breastfeeding mothers, and maybe prolong the breastfeeding period at least up to the age of two.

It is also recommended that more studies be conducted on food taboos and diet diversity in the ASEAN region, since there were no available data on food taboos that are being practiced during pregnancy in Brunei Darussalam, Myanmar, Singapore, and Vietnam; during the post-partum period in Brunei Darussalam, Cambodia, Philippines, and Singapore, and while breastfeeding in Brunei Darussalam, Malaysia, Philippines, and Thailand. The information generated from such studies can be used in nutrition education campaigns in the promotion of dietary diversification and in providing proper nutritional support (using the right food supplements and alternative sources of nutrients) to minimize the impact of the food taboos on women and babies.

The underutilized indigenous resources in the ASEAN region have the potential to be valuable components of a diversified diet. To prove this statement and to further promote dietary diversification, the dissertation tackles the nutrient profiling of several edible insects and pigmented rice varieties. Chapter 4 focuses on the Bombay locust (*Patanga succincta*), scarab beetle (*Holotrichia* sp.), house cricket (*Acheta domesticus*), and mulberry silkworm (*Bombyx mori*) from Thailand (Köhler et al., 2019a), and Chapter 5 focuses on the sago grub (*Rhynchophorus bilineatus*) from Indonesia (Köhler et al., 2020). On the other hand, Chapter 6 focuses on Camoros (red), Tinta (purple) and Malinao black rice from the Philippines, and Chapter 7 gives a literature review of the pigmented rice varieties in Thailand.

In the ASEAN region, edible insects are not considered as food taboo by pregnant women, as well as those in their post-partum period and breastfeeding. Unfortunately, rice (crust from black or brown boiled rice, fermented rice, glutinous rice cake, porridge, and sticky rice) is taboo for pregnant women in Cambodia, Indonesia, Lao PDR, and Malaysia, and sticky rice is taboo for women in the post-partum period in Myanmar and Thailand.

The nutrient profiles of the edible insects from Thailand consisted of their protein, amino acid, and mineral contents, while the nutrient profile of the sago grub from Indonesia included its protein and amino acids (AA), fat and fatty acids, vitamin E, and mineral content. On the other hand, the nutrient profiles of pigmented rice varieties from the Philippines included their protein, amylose, crude fiber, ash, amino acids, vitamins (carotenoids, riboflavin, thiamine, and vitamin E), minerals, and functional components (ferulic acid and γ-oryzanol). The rice varieties' morphological characteristics (color, 1000-grain weight, grain length, width, and thickness) were also documented.

All the samples were prepared (freeze-drying, grinding) and stored (frozen at -80°C) at the Institute of Nutritional Science, University of Hohenheim, Stuttgart, Germany, prior to the analyses. The protein, crude fiber, ash, amino and fatty acids, as well as the mineral contents were analyzed at the Core Facility (with DIN EN ISO/IEC 17025:2005 accreditation) of the University of Hohenheim, while the amylose, fat, vitamins (carotenoids, riboflavin, thiamine, and vitamin E), ferulic acid, and γ-oryzanol contents were analyzed at laboratories of the Institute of Nutritional Science. The protein content was determined using the Kjeldahl method, while the amino acids were measured using ion exchange chromatography. The amylose content was determined using a commercial test kit. The crude fat was extracted using n-hexane, and the crude fiber and ash were determined by ashing at different temperatures using a muffle furnace. The fatty acids were determined using gas chromatography (GC) with flame ionization detection (FID). The carotenoids, riboflavin, thiamine, vitamin E, ferulic acid and γ-oryzanol contents were determined using high-performance liquid chromatography (HPLC), while the minerals were determined using spectrometry – inductively coupled plasma - optical emission spectrometry (ICP-OES), inductively coupled plasma - mass spectrometry (ICP-MS), and atomic absorption spectrometry (AAS).

The results showed that the five edible insects can be labelled as “high in” protein. They all contain the complete set of essential amino acids, however, only the silkworm pupa and the sago grub met the FAO/WHO requirements of having 40% essential amino acids, and a 0.60 ratio between essential to non-essential AA. All the edible insects contained more savory than sweet amino acids, except for the locust. The limiting AA were tryptophan for the locust and cricket, lysine for the beetle, leucine for the silkworm pupa, and methionine + cysteine for the sago grub. The protein recovery were equal to 86% or more. Based on the Codex Alimentarius on food labelling, only the cricket bought from the supermarket can be labelled as a “source of” calcium, while all the edible insects except for the sago grub can be a “source of” iron, with beetle and cricket from the supermarket even containing “high” amounts. Except for the locust, all can be a “source of” magnesium, and the cricket from the supermarket and the silkworm pupa can even be labelled as “high in” magnesium. These edible insects (for every 100g) can cover more than 88% of the RNI of the mineral for children (1-3 years old), and more than 24% of the RNI for adult females (19-50 years old). The sago grub is a “source of” zinc, while the rest are “high in” zinc. This means that all the edible insects can cover more than 34% and 30% of the RNIs for children and adult females, respectively. The heavy metal content of the edible insects was all well below the maximum values, and are therefore, deemed safe for consumption as either feed material or as a complete feed. Unfortunately, the standards on heavy metals in edible insects for human consumption is not yet available.

In the sago grub oil, the major saturated fatty acid (FA) was palmitic acid, while oleic and linoleic acids were the major mono- and polyunsaturated FAs, respectively. It contained more unsaturated than saturated FA. A gram of the oil has 51 μg of vitamin E, 92% of which is tocopherols. The α-tocopherol was the predominant compound. The α-TE in 100 g sago grub can provide 16% and 10% of the AIs for children and adult females, respectively.

Aside from edible insects, the dissertation also showcases pigmented rice varieties from the ASEAN region. The nutrient profiling of three pigmented rice varieties from the Philippines showed that they can be considered as a “source of” protein based on Codex Alimentarius on food labelling. Camoros and Malinao black rice have “low”, while Tinta has “very low” amylose content. The crude fiber content was less than 0.90 g/100g, while the ash content was highest in Malinao black (1.19 g/100 g) and lowest in Tinta rice (0.73 g/100 g). The total amino acids in the pigmented rice varieties ranged from 6.96 (Tinta) to 8.76 g/100 g (Malinao black). Lysine was the limiting amino acid.

The Malinao black rice contained the highest amount of carotenoids. It had the highest amount of lutein, β-carotene, and 9-cis-carotene, while zeaxanthin, α-carotene, and β-cryptoxanthin were highest in Camoros. The Camoros rice has the highest RAE, and can cover 177% and 142% of the respective RSIs for children and adult females. Camoros and Tinta rice can be considered as “source of” riboflavin, while Malinao black rice is “high in” riboflavin. The Malinao black rice can cover 120% and 54% of riboflavin RNIs for children, and female adults, respectively. All the pigmented rice samples are “high in” thiamine, and Camoros rice can cover 414% and 188% of the thiamine RNIs. The total vitamin E content is highest in Camoros and Malinao black rice, with more tocotrienols than tocopherols, and with γ-tocotrienol as the major congener. Based on the calculated α-TE, Camoros rice can cover 16%, the highest among the rice samples, of the AI for children and 11% for female adults.

Camoros and Malinao black rice are "high in" magnesium, while Tinta is a "source of" magnesium and zinc. Based on the RNIs for children and adult females, respectively, Camoros rice can provide the highest amount of selenium (38% and 25% RNI); Malinao black rice can provide the highest amount of iron (8% and 2% RNI) and magnesium (175% and 48% RNI); and Tinta rice can provide the highest amount of calcium (albeit, only 2% and 1% RNI) and zinc (29% and 25% RNI). Malinao black rice has the highest phosphorus content (2808 mg/kg), unfortunately, in rice, phosphorus is stored as phytic acid, an antinutrient. Malinao black rice also has the highest ferulic acid (0.42 ng/mg) and γ-oryzanol (350 ng/mg) content among the rice samples.

Aside from the pigmented rice from the Philippines, pigmented rice varieties from Thailand were also explored thru a literature review. Information on 33 pigmented Thai rice varieties were pooled together, and the review included data on the glycemic index, proximate composition, and micronutrient contents, to highlight these rice varieties' potential as safe and inexpensive sources of nutrients and as viable staple food substitute for white rice.

Homlanna (purple) and Sangyod Phatthalung (red) have "medium" glycemic indices, while KumDoiSaket (purple/black) can be considered as "high in" protein. Kam Hom Morchor (purple) and THA24 (black) can be "sources of" iron and can cover more than 33% and 7% of the RNIs for children and adult females, respectively. Sangyod Phatthalung, Bieisu (purple), Kam Doi Saket and Kam Hom Morchor have zinc contents greater than 2.25 mg/100 g and can be considered as "source of" zinc, while Luem Pua (purple/black) is "high in" zinc, and can cover 65% and 56% of the RNI for children and adult females, respectively. Sangyod Phatthalung can cover 10% and 5% of the calcium RNI for children and adult females, respectively. The rice variety is also "high in" magnesium and can cover 164% and 45% of the RNIs.

From the available information on vitamin content, Khao Hom Nil (black) has an RAE of 7 µg per 100 g and can cover only 1.75% and 1.40% of the RSIs for children and female adults, respectively. On the other hand, the average values between THA19 (red) and THA24 can cover 74% of the recommended thiamine intake for children, and 34% for adult females, 15% and 7% of the RNI for riboflavin, and 45% and 19% of the

RNI for niacin. Luem Pua has an α-TE of 1.01 mg/100 g and can cover 20% and 13% of the AI for children and female adults, respectively.

Overall, data showed that the pigmented rice varieties from the Philippines and the pigmented Thai rice varieties have higher mineral and vitamin contents in comparison with white Jasmine rice.

The importance of energy-giving macronutrients and various essential micronutrients in the human body, especially in children and women in the reproductive age, those who are pregnant, or in their postpartum period and breastfeeding their babies, is well established. The findings in this dissertation have shown that edible insects and pigmented rice varieties can be added to diversify and improve the nutritional quality of people's diets and to fight malnutrition from the household level. However, key issues have also been identified that warrant further investigation: food safety issues concerning edible insects, and the issue of phytic acid in pigmented rice varieties. In view of the Coronavirus disease 2019 (COVID-19) global pandemic, it is also recommended to study the possibility of including commodities like edible insects and pigmented rice as survival food of the future.

The novel research into indigenous food resources contributes to the advancement of knowledge in the field of entomology and biodiversity conservation, and of course, in food science and nutrition. Most importantly, the dissertation's contribution to the promotion of dietary diversification in the hope of attaining improved human health and nutrition will benefit the whole ASEAN region.

Summary

The Association of Southeast Asian Nations (ASEAN) is composed of Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam. The ASEAN region is ailing from moderate to serious incidence of malnutrition. Among the member countries, Brunei Darussalam has the highest prevalence of child obesity (17.8%), while Malaysia has the highest prevalence of adult obesity (15.6%). Indonesia has the highest percentage of young children suffering from wasting at 13.5%. Lao PDR has the highest percentage of the undernourished in the population (16.5%), at the same time, having the highest percentage of stunted children under five years of age (43.85%). It also has the highest HHI score with 38.7, which corresponds to having a severe case of micronutrient deficiencies, and the highest death rates for both children under five years of age (63 deaths per 1,000 live births) and mothers (197 deaths per 100,000 live births).

To fight malnutrition, nutrition-specific interventions address the immediate determinants of nutrition of specific vulnerable groups – young children, pregnant and lactating women, and others. Dietary diversification is an example of a nutrition-specific intervention. This dissertation was conducted to turn the spotlight towards the ASEAN region, its triple burden of malnutrition, and to dietary diversification as a sustainable way to lighten the load. It tackled one of the stumbling blocks to the acceptance of dietary diversification – food taboos, and one of the stepping stones towards its successful implementation – nutrient profiling of underutilized, indigenous resources in the region.

This dissertation postulated that plant- and animal-based food taboos adhered to by pregnant, post-partum, and lactating Southeast Asian women can hinder dietary diversification in the most vulnerable and crucial moment of the first 1,000 days of life. The two review papers generated were the first to consolidate and showcase researches on food taboos covering the region. They highlighted the need for culture-sensitive health interventions to address maternal and child health problems that could lead to the attainment of the sustainable development goals of reducing the maternal and under-five mortality ratios and empowering women in Southeast Asia, as well as the priority health goals of the ASEAN.

The underutilized, indigenous resources in the ASEAN region have the potential to be valuable components of a diversified diet. To prove this statement and to further promote dietary diversification, the dissertation tackled the nutrient profiling of the edible insects – Bombay locust (*Patanga succincta*), scarab beetle (*Holotrichia* sp.), house cricket (*Acheta domesticus*), and mulberry silkworm (*Bombyx mori*) from Thailand, and the sago grub (*Rhynchophorus bilineatus*) from Indonesia. For the pigmented rice varieties, the Camoros (red), Tinta (purple) and Malinao black rice from the Philippines were analyzed, while a review of pigmented rice varieties from Thailand was also conducted.

The results of the analysis showed, and based on the Codex Alimentarius on food labelling, that the edible insects are "high in" protein and can be "sources of" or "high in" minerals. Also, data showed that the pigmented rice varieties from the Philippines and the pigmented Thai rice varieties have higher mineral and vitamin contents in comparison with white Jasmine rice. The findings in this dissertation have shown that edible insects and pigmented rice varieties can be added to diversify and improve the nutritional quality of people's diets and to fight malnutrition from the household level.

The novel research into indigenous food resources contributes to the advancement of knowledge in the field of entomology and biodiversity conservation, and of course, in food science and nutrition. Most importantly, the dissertation's contribution to the promotion of dietary diversification in the hope of attaining improved human health and nutrition will benefit the whole ASEAN region.

Zusammenfassung

Dem Verband Südostasiatischer Nationen (ASEAN) gehören Brunei Darussalam, Kambodscha, Indonesien, die Demokratische Volksrepublik Laos, Malaysia, Myanmar, die Philippinen, Singapur, Thailand und Vietnam an. Die ASEAN-Region leidet an mäßiger bis schwerer Unterernährung. Unter den Mitgliedsländern hat Brunei Darussalam die höchste Prävalenz von Fettleibigkeit bei Kindern (17,8%), während Malaysia die höchste Prävalenz von Fettleibigkeit bei Erwachsenen (15,6%) aufweist. Indonesien hat mit 13,5% den höchsten Prozentsatz an Kleinkindern, die an Wasting leiden. Die Demokratische Volksrepublik Laos hat den höchsten Prozentsatz an Unterernährten in der Bevölkerung (16,5%) und gleichzeitig den höchsten Prozentsatz an Kindern unter fünf Jahren mit Stunting (43,85%). Sie hat auch den höchsten HHI-Wert mit 38,7, was einem schwerwiegenden Mikronährstoffmangel entspricht, und die höchsten Sterblichkeitsraten sowohl bei Kindern unter fünf Jahren (63 Todesfälle pro 1.000 Lebendgeburten) als auch bei Müttern (197 Todesfälle pro 100.000 Lebendgeburten).

Zur Bekämpfung der Mangelernährung befassen sich ernährungsspezifische Interventionen mit den unmittelbaren Determinanten der Ernährung bestimmter gefährdeter Gruppen - Kleinkinder, schwangere und stillende Frauen und andere. Die Diversifizierung der Ernährung ist ein Beispiel für eine ernährungsspezifische Intervention. Diese Dissertation wurde durchgeführt, um das Augenmerk auf die ASEAN-Region, ihre dreifache Last der Unterernährung, und auf die Diversifizierung der Ernährung als nachhaltige Möglichkeit zur Milderung dieser Last zu lenken. Sie befasste sich mit einem der Stolpersteine auf dem Weg zur Akzeptanz der Diversifizierung der Ernährung - den Nahrungstabus - und mit einem der Sprungbretter auf dem Weg zu ihrer erfolgreichen Umsetzung - der Erstellung von Nährwertprofilen zu wenig genutzter, einheimischer Ressourcen in der Region.

In dieser Dissertation wurde angenommen, dass pflanzliche und tierische Nahrungstabus, an die sich schwangere, postpartale und stillende südostasiatische Frauen halten, die Diversifizierung der Ernährung im verletzlichsten und wichtigsten Moment der ersten 1000 Lebenstage behindern können. Die beiden erstellten Übersichtspapiere waren die ersten, die die Forschungen über Nahrungstabus in der Region zusammenfassten und vorstellten. Sie betonten die Notwendigkeit

kultursensibler Gesundheitsinterventionen zur Bewältigung der Gesundheitsprobleme von Müttern und Kindern, die zur Erreichung der Ziele einer nachhaltigen Entwicklung, nämlich der Senkung der Sterblichkeitsrate von Müttern und Kindern unter fünf Jahren und der Stärkung der Rolle der Frauen in Südostasien, sowie der vorrangigen Gesundheitsziele der ASEAN führen könnten.

Die wenig genutzten, einheimischen Ressourcen in der ASEAN-Region haben das Potenzial, wertvolle Bestandteile einer diversifizierten Ernährung zu sein. Um diese Aussage zu belegen und die Diversifizierung der Ernährung weiter zu fördern, befasste sich die Dissertation mit der Nährstoffzusammensetzung der essbaren Insekten - der Bombay-Heuschrecke (*Patanga succincta*), dem Skarabäuskäfer (*Holotrichia* sp.), der Heimchengrille (*Acheta domesticus*) und dem Maulbeerseidenspinner (Bombyx mori) aus Thailand sowie der Sago-Raupe (*Rhynchophorus bilineatus*) aus Indonesien. Bei den Sorten von pigmentiertem Reis wurden Camoros (rot), Tinta (violett) und Malinao schwarzer Reis von den Philippinen analysiert, ebenso wie eine Überprüfung der Sorten von pigmentiertem Reis aus Thailand.

Die Ergebnisse der Analyse zeigten, und basierend auf dem Codex Alimentarius über die Lebensmitteletikettierung, dass die essbaren Insekten "proteinreich" sind und "Quellen von" oder "reich an" Mineralien sein können. Die Daten zeigten auch, dass die pigmentierten Reissorten aus den Philippinen und die pigmentierten thailändischen Reissorten im Vergleich zu weissem Jasminreis höhere Mineralien- und Vitamingehalte aufweisen. Die Ergebnisse dieser Dissertation haben ergeben, dass essbare Insekten und pigmentierte Reissorten hinzugefügt werden können, um die Ernährung der Menschen zu diversifizieren und die Ernährungsqualität zu verbessern und die Unterernährung auf Haushaltsebene zu bekämpfen.

Die Forschung über einheimische Nahrungsressourcen trägt dazu bei, das Wissen auf dem Gebiet der Entomologie und der Erhaltung der biologischen Vielfalt und natürlich auch der Lebensmittelwissenschaft und Ernährung zu erweitern. Am wichtigsten ist, dass der Beitrag der Dissertation zur Förderung der Diversifizierung der Ernährung in der Hoffnung auf eine verbesserte menschliche Gesundheit und Ernährung der gesamten ASEAN-Region zugute kommen wird.

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Affidavit

Annex 3 to the University of Hohenheim doctoral degree regulations for Dr. rer. nat.

Declaration in lieu of an oath on independent work.

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic:

Promoting Dietary Diversification in the ASEAN Region: Exposing Food Taboos, and Exploring the Nutrient Profiles of Underutilized, Indigenous Food Resources

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Contributions to Publications

The following are the doctoral candidate's contributions in all the published works included in this dissertation:

- Formulation of the research questions;
- Literature review;
- Optimization of laboratory techniques;
- Collection of samples;
- Sample preparation and storage;
- Submission of samples for analysis at the University's Core Facility;
- Laboratory analysis;
- Data analysis;
- Preparation and subsequent revisions of the manuscripts;
- Collaboration with co-authors to review the manuscripts;
- Online submission of the manuscripts to journals;
- Communication with the journal editors and reviewers; and
- Revision and approval of the proofs before publication.

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TGIFF: Thank God It's Finally Finished!