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Fatty Acid Profile, Mineral Composition, and Health Implications of Consuming Dried Sago Grubs (*Rhynchophorus ferrugineus*)

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Abstract: A comprehensive evaluation of the fatty acid and mineral composition of *Rhynchophorus ferrugineus* (sago grub powder, SGP), pre-treated to meet New Zealand import biosecurity requirements, was investigated. Palmitic acid (42.5% FA), oleic acid (39.0% FA), and linoleic acid (1.02% FA) were the most abundant saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid, respectively. Lipid nutritional indices of SGP fats were ω6/ω3 (2.17), hypocholesterolemic/hypercholesterolemic acid ratio (0.88), atherogenicity index (1.01), thrombogenicity index (1.65), and health-promoting index (0.99). Although there are no established recommended values for these dietary indicators, including them in a balanced diet may be advised to reduce the risk of adverse health effects. The mineral analysis profile of the SGP found 11 essential and 29 non-essential minerals, and 4 heavy metals. SGP was rich in important minerals such as potassium (1657 mg/kg DW), magnesium (805.3 mg/kg DW), iron (23 mg/kg DW), manganese (8.8 mg/kg DW), calcium (477 mg/kg DW), and phosphorus (2950 mg/kg DW). However, the Ca:P ratio (0.16:1) of SGP samples was lower than the recommended Ca:P ratio (1.3:1) needed for optimum bone health. Heavy metals such as arsenic (0.17 mg/kg DW), cadmium (0.04 mg/kg DW), lead (0.56 mg/kg DW), and vanadium (0.01 mg/kg DW) were detectable in SGP but were below acceptable toxicity limits. These findings indicate that SGP pre-treated for export is safe for consumption and contains appreciable nutrients, especially minerals. However, the nutritional and health implications of the elevated saturated fatty acid levels, low polyunsaturated fatty acid contents, and low Ca:P ratio of SGP should be considered when choosing sago grubs as a food source.

Keywords: *Rhynchophorus ferrugineus*; fatty acid composition; lipid nutritional indices; minerals

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1. Introduction

Edible insects are increasingly being regarded as a sustainable and alternative source of nutrients and are becoming of increasing interest for commercial purposes [1]. One of the most widely eaten insects in Thailand is sago palm weevil larvae (*Rhynochophorus ferrugineus)* [2], which are either wild-harvested or farmed [3]. The larvae can be up to 35 mm long, with a brown head and a yellowish body composed of 13 segments. As adults, they develop a strong chitinised average length of about 50 mm and width of 20 mm [4]. They are reported to be nutritious [5] and can meet the nutritional needs of vulnerable groups, contributing to food security. According to published studies, the protein content for *Rhynochophorus ferrugineus* ranges from 18 to 28.5 g/100 g dry weight (DW), lipid content ranges from 52.4–60.1 g/100 g DW, and ash content ranges from 2.4–2.9 g/100 g DW, depending on the location of farming [4]. In addition to their high nutritional value, insects have a short life span, and high production capacity, are relatively easy to harvest/gather and can be consumed at any stage of their life cycle. Insect farming is gaining global traction due to its nutritional, environmental, and economic importance [3]. Sago grub larvae, for example, have a higher feed conversion, are produced without antibiotics, and have lower environmental impacts such as greenhouse emissions, and low land and water requirements when reared compared to traditional domestic farmed animals [1,3,4]. The elemental nutritional compositions of several *Rhynchophorus* species have also been established. The protein and fat contents of *R. bilineatus* (24.2 and 40.0 g/100 g DW), *R. ferrugineus* (27.9 and 59.7 g/100 g DW), *R. phoenicis* (22.1 and 66.6 g/100 g DW), and *R. palmarum* (24.4 and 15.36 g/100 g DW) have been reported in the literature, demonstrating the suitability of sago grubs as a source of protein and fat [4,6–8].

In Thailand, entomophagy is majorly concentrated in the Northern and Northeastern provinces [9]. However, *Rhynchophorus ferrugineus* larva is one of the most popular edible insects commonly farm-raised in Southern Thailand. Sago grubs are often prepared as fried, stir-fried, or as satay, and are reported to be a relatively cheap, accessible and nutritious food source [9]. According to Yhoung-Aree [10], sago grubs are eaten by either traditional consumers based in the provinces, urban consumers who have developed a taste for insects, or foreign tourists attracted by the novelty and delicacies sold across the country. Consequently, the global trade and inter-country movement of edible insect materials is likely to increase as edible insects become more popular, especially in Western countries.

However, such inter-country trade poses a potential biosecurity risk [11], and laws governing the importation of edible insect materials in various countries have been established to control such risk. For example, for importing edible insect material such as sago grub into New Zealand, there is a biosecurity pre-processing requirement involving boiling and drying of the grubs, since live grubs are not permitted to be imported (see https://www.mpi.govt.nz/biosecurity/, accessed on 29 July 2022).

Currently, there is no report assessing the impact of such pre-treatment on nutrient content. This present study aimed to evaluate the mineral and fatty acid profile of sago grub (*R. ferrugineus)* imported into New Zealand from Thailand and assess the health implications based on the fatty acid profile, lipid nutritional indices, essential minerals, and heavy metal contents.

2. Materials and Methods

2.1. Sample Acquisition and Proximate Composition

Dried sago (*R. ferrugineus*) larvae were obtained from JR Unique Foods, Udon Thani, Thailand (https://jrunique.com, accessed on 15 September 2020). The farmed and harvested *R. ferrugineus* larvae were processed following a standard protocol that meets biosecurity guidelines for importing insect material into New Zealand. The processing method involved boiling the grubs for 30 min, oven drying for 14 h at 70 °C, vacuum packing in aluminium foil, and shipping to our laboratory. Upon receipt, the dry sago grubs were pulverised (by blending), the powder sieved (425 mm cut off), and aliquoted into triplicate subsamples for use in subsequent experiments. A photo of the pre-treated sago grubs and obtained powder are shown in Figure 1. Proximate analysis of the samples found residual moisture: 4.42%, crude protein: 14.02% dry weight (DW) using a conversion factor of 5.6 [3,12], crude lipid: 58.7% DW, ash: 2.5% DW, carbohydrate: 24.7% DW, and total energy: 2833.7 kJ/100 g DW, as determined using the Association of Official Analytical Chemists methods [13]. The sago grub powder is referred to as SGP in subsequent sections.

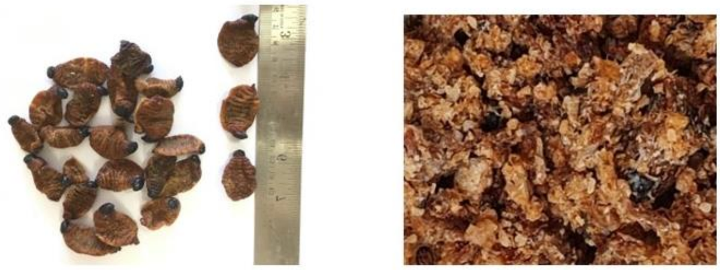

Figure 1. Representative images of sago grub and sago grub powder.

2.2. Fatty Acid Analysis

The FAMEs were analysed using a gas chromatography device outfitted with a flame ionisation detector (Agilent 6890 N, Santa Clara, CA, USA) and an autosampler (Agilent 7683, Beijing, China), as reported by Kavle, et al. [14]. In order to conduct the analysis, 1 µL of the FAME samples were injected into a GC-FID device. The ionisation detector was set at 250 °C, and the injector detector port was configured with a 10:1 inlet split ratio. The FAMEs were successfully separated on a BPX-70 silica column (Phenomenex, Torrance, CA, USA) (length: 50 m; inner diameter: 0.32 mm; film thickness: 0.25 m). The temperature program for the column oven was as follows: 40 °C for 3 min, then 10 °C/min up to 225 °C at a rate of 3 °C/min, and finally 10 °C/min to a final temperature of 250 °C. By comparing retention times with recognised FAME standards (FAMQ-005) from NuCheck Prep, Elysian, Minnesota, and Sigma, St. Louis, Missouri, it was possible to identify the FAME chromatogram peaks. The per cent peak area data from the GC data processing software were used to determine the percentage composition of each detected FAME in the sample. Using an internal standard and theoretical relative FID response corrections, the FAME values from the GC-FID were converted to free fatty acid (FFA). The values were expressed as % fatty acid, and g fatty acid/100 g dried insect SGP material of SGP.

2.3. Dietary Indicators

Lipid dietary indices, such as index of atherogenicity (IA), index of thrombogenicity (IT), hypocholesterolemic/hypercholesterolemic ratio, and health-promoting index (HPI) were calculated according to the following equations [15]:

$$\text{IA} = [12{:}0 + (4 \times 14{:}0) + 16{:}0]/\Sigma\text{UFA} \tag{1}$$

$$\text{IT} = (14{:}0 + 16{:}0 + 18{:}0)/[(0.5 \times \Sigma\text{MUFA}) + (0.5 \times \Sigma\omega6\ \text{PUFA}) + (3 \times \Sigma\omega3\ \text{PUFA}) + (\omega3/\omega6)] \tag{2}$$

$$\text{HH} = (18{:}1\ \text{c}\omega9 + \Sigma\text{PUFA})/(12{:}0 + 14{:}0 + 16{:}0) \tag{3}$$

$$\text{HPI} = \Sigma\text{UFA}/[12{:}0 + (4 \times 14{:}0) + 16{:}0] \tag{4}$$

ΣPUFA (polyunsaturated fatty acid) = 18:2 cω6 + 18:3 ω3
ΣUFA (unsaturated fatty acid) = ΣMUFA + ΣPUFA
ΣMUFA (monounsaturated fatty acid) = 16:1 cω7 + 18:1 cω9
Σ = Sum of the fatty acid components
ω = Omega, which refers to the first double bond from the methyl end of the fatty acid

2.4. Mineral Analysis

Aliquots (5 mg) of SGP were analysed in an ultraclean and metal-free Class 10 (ISO4) laboratory for minerals using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7850, Santa Clara, CA, USA; Department of Chemistry, University of Otago, New Zealand), as reported by Burrow, et al. [16]. Pre-digestion tubes were filled with approximately 0.25 g of frozen SGP. This was mixed with 1 mL of analytical reagent grade 30% peroxide, before adding 5 mL of triple quartz distilled fuming nitric acid (Labserv, Auckland, New Zealand). The fluid in the pre-digestion tube was transferred into a digestion tube (Mars X-Press, CEM Corporation, Matthews, NC, USA) after 24 h. The remainder of the contents were rinsed into the digestion tube using two 2.5 mL aliquots of nitric acid. In a MARS 6 microwave digestion device (CEM Corporation, Matthews, NC, USA), the digestion tubes were then sealed and processed as previously mentioned.

The contents of the digestion tubes were transferred back to the pre-digestion tubes after the samples had cooled for at least 2 h. The pre-digestion mixture was then diluted with deionised water to a volume of approximately 25 mL (18.2 Mcm at 23.1 °C) and placed on a heating block set to 81 °C for 24 h. Before the amount was increased to 10 mL, 0.5 mL of triple quartz distilled fuming nitric acid was added to each pre-digestion tube. To conduct an ICP-MS analysis, 0.25 mL of this solution was collected and mixed with 7.25 mL of 2% triple quartz distilled nitric acid in deionised water (18.2 Mcm at 23.1 °C).

In addition to the SGP, a reference material fish protein-certified substance (DORM-4, National Research Council of Canada (NRC-CNRC)) was used for quality control. The experimental % recovery, detection limits, and limit of quantification for SGP are also reported.

2.5. Statistical Analysis

Measurements are reported as mean ± standard deviation (SD) and calculated using Microsoft Excel.

3. Results

3.1. Fatty Acid Profile

The fatty acid (FA) profile of SGP is shown in Table 1, expressed as % of total FA and g FA per 100 g DW SGP. Among the saturated fatty acids (SFA) found in SGP, the most abundant were palmitic acid (16:0) (42.5% of total FA), followed by myristic acid (14:0) (2.39% of total FA), and stearic acid (18:0) (1.30% of total FA). A similar palmitic acid content (42.45% of total FA) has been reported for *R. bilineatus* (sago grub) [8]. A lower palmitic acid content has been reported in large nymphs of *Blaberus craniifer* (30.9% of total FA) [17], *T. molitor* (19.0% of total FA) [18], and large Huhu grubs (23.0% of total FA) [14]. Chinarak, et al. [19] reported that the diet of sago grubs can have a substantial effect on the fatty acid composition.

While the myristic acid content of *T. molitor* larvae fat (5.0% of total FA) [18] is reported to be higher than that of SGP (2.39% of total FA), that of *R. bilineatus* (0.81% of total FA) [8] and large nymph (*Blaberus craniifer*) (1.7% of total FA) [17] are lower than SGP (Table 1). Steric acid, the third most abundant SFA in SGP, was found to be at a level 10 times higher than lauric acid. This amount of stearic acid in sago grubs is lower than that found in wild-harvested Huhu grubs [14]. The WHO (World Health Organization) has recommended reduced consumption of SFA due to their association with increased incidence of heart disease and elevated low-density lipoprotein-cholesterol (LDL-C) [20]. A potential health benefit of consuming SGP was assumed due to the low levels of lauric acid and myristic acid (0.90 and 2.39% of total FA, respectively, see Table 1), which are reported to promote hypercholesterolemia when present at high levels [20].

Palmitoleic acid (16:1 cω7) (12.1% of total FA) and oleic acid (18:1 cω9) (39.0% of total FA) were the most prominent monounsaturated fatty acids (MUFA) available in SGP (Table 1). The total MUFA was higher (51.2% of total FA) than the SFA and PUFA content of SGP (Table 1), and was higher than that reported for *R. bilineatus* (47.6% of total FA) [8], *R. ferrugineus* (36.53% of total FA) [19], *Blaberus craniifer* (50.9% of total FA) [17], and *Apis mellifera* pupae (48.9% of total FA) [21]. However, the MUFA in SGP was lower than in Huhu grubs (71.5%of total FA) [14]. The FA content of the palm trunk eaten by sago grubs contains 46.8% oleic acid, and ingestion of this material likely contributes to the reported profile [8]. The MUFA content is primarily composed of *cis*-oleic acid and *cis*-palmitoleic acid. Oleic acid is commonly found in edible insect material and is reported to help reduce blood pressure and the development of immune, inflammatory, and cardiovascular diseases on consumption by humans [22]. In contrast to SGP (Table 1), *R. bilineatus* contained eicosenoic acid (20:1) and low levels of palmitoleic acid [8]. These findings could be due to species effects and differences in the analytical method or pre-processing treatment prior to the export of the samples used in our study.

Table 1. Mean fatty acid content (% of total FA and g FA/100 g dried insect material of SGP).

Fatty Acid		% Total Fatty Acid	g FA/100 g Dried Insect Material
Lauric acid	12:0	0.90 ± 0.72	0.54 ± 0.43
Myristic acid	14:0	2.39 ± 0.06	1.41 ± 0.04
Palmitic acid	16:0	42.6 ± 0.65	25.2 ± 0.38
Stearic acid	18:0	1.30 ± 0.04	0.77 ± 0.32
Arachidic acid	20:0	0.18 ± 0.01	0.11 ± 0.01
Saturated fatty acid	SFA	47.3 ± 0.23	28.1 ± 0.15
Cis-palmitoleic acid	16:1 cω7	12.2 ± 0.21	7.22 ± 0.13
Cis-oleic acid	18:1 cω9	39.0 ± 0.55	23.1 ± 0.08
Monounsaturated fatty acid	MUFA	51.2 ± 0.76	30.3 ± 0.45
Cis-linoleic acid	18:2 cω6	1.02 ± 0.13	0.61 ± 0.08
α-linolenic acid	18:3 ω3	0.50 ± 0.05	0.28 ± 0.03
Polyunsaturated fatty acid	PUFA	1.50 ± 0.18	0.89 ± 0.11
Nutritional indices			
Polyunsaturated fatty acid/saturated fatty acid ratio	PUFA: SFA	0.03 ± 0.00	-
Omega 6: Omega 3 ratio	ω-6/ω-3	2.17 ± 0.43	-
Index of atherogenicity	IA	1.01 ± 0.01	-
Index of thrombogenicity	IT	1.65 ± 0.01	-
Hypocholesterolemic/ hypercholesterolemic ratio	H/H	0.88 ± 0.01	-
Health-promoting index	HPI	0.99 ± 0.01	-

cω—cis-omega; ω—omega; All values are expressed as mean ± Standard Deviation (SD); n = 3; '-' Not determined.

In the present study, SGP was found to have low levels of polyunsaturated fatty acid (PUFA) (1.49 % of total FA), compared with *R. bilineatus* (4.57 % of total FA). SGP (Table 1) contained linoleic acid (18:2 cω-6) (1.02% of total FA), which is one of the main components of PUFA in edible insects and has been shown to confer anti-inflammatory properties on consumption [23].

SGP contained higher levels of MUFA (51.2% of total FA) than SFA (47.3% of total FA) and PUFA (1.49% of total FA). A similar trend was observed by Ekpo and Onigbinde [7] for *R. phoenicis* (MUFA, 43.4; SFA, 38.9; PUFA, 17.7% of total FA). However, SGP was found to contain high levels of SFA and MUFA and low levels of PUFA compared to the larvae of *R. phoenicis* and *R. palmarus* from Nigeria, with SFA ranging from 29–31% of total FA, MUFA from 37–40% of total FA and PUFA ranging from 29–34% of total FA [7,24]. Usually, insects contain higher unsaturated fatty acid (UFA) than SFA [25]. The results obtained for SGP in the present study agree with that reported for other edible insects in the literature. The essential fatty acid content is reported to depend on factors such as feed, gender, and developmental stage [26]. The fatty acid profiles obtained in the present study for SGP were used to calculate nutritional indices (ω-6/ω-3, AI, IT, HPI, and HH) [27] to assess the quality of the SGP lipid.

3.2. Fatty Acid Nutritional Indices

3.2.1. ω-6/ω-3

The fatty acid ω-6/ω-3 ratio for SGP fat was found to be 2.1 in the present study (Table 1) and can be considered to be within the recommended range, as the WHO has recommended the ratio of ω-6/ω-3 should be <10, as reported in Kumar, et al. [28]. The ω-6/ω-3 ratio for *T. molitor* was reported to be 21.5 [18], large Huhu grub was 16.2 [14], *B. craniifer* was 6.1 [17], *R. ferrugineus* was 5.4 [19], and *R. phoenicis* was 3.7 [7], which were higher than that observed in the present study. However, the ω-6/ω-3 reported for *R. bilineatus* of 1.9 [8] is lower than that reported for SGP (Table 1). It has been reported that the difference in the ω-6/ω-3 ratio depends on the insect diet [18]. A lower ω-6/ω-3 ratio is considered to be more beneficial for preventing chronic diseases, such as diabetes, autoimmune, and cardiovascular disease [23].

3.2.2. PUFA: SFA Ratio

The PUFA/SFA ratio is one of the key parameters currently used to assess the nutritional quality of lipids in food. It is recommended for dietary requirements that the PUFA/SFA ratio should ideally be above 0.4 [29], and a high ratio is considered to be beneficial for a positive impact on cardiovascular health [27]. The PUFA/SFA ratio for SGP was found to be 0.032, which is lower than the ratio found for *R. bilineatus* (0.09) [8], and for *R. ferrugineus* was reported to be 0.01 [19]. Interestingly, the level of UFA (MUFA+PUFA) means that sago grub fat, like plant oil, is liquid even at a storage temperature of 4 °C.

3.2.3. Index of Atherogenicity

The index of atherogenicity (IA) was developed by Ulbricht and Southgate [15] to characterise the relationship between saturated fatty acids (SFA) and unsaturated fatty acids (UFA) and the atherogenic potential of the FA content in the food. UFA is considered to be anti-atherogenic as it is reported to inhibit plaque accumulation in the vascular system and facilitate a reduction in the level of cholesterol and phospholipid, and esterified fatty acids [30,31]. The IA value for SGP was calculated to be 1.01, which is comparable to that found in animal meat (0.5 to 1.0), but higher than in cocoa butter (0.7), mealworm (0.5), death head cockroach (0.69), Huhu large larvae (0.37), *R. ferrugineus* (0.74), and eri silkworm (0.35–0.37) [14,19,27,32–34].

3.2.4. Index of Thrombogenicity

The index of thrombogenicity (IT) measures the risk of clot formation in blood vessels based on the net contribution of prothrombic Fas and anti-prothrombic Fas [15]. In the present study, the IT value for SGP (1.65) was comparable to that of the medium size Huhu grub (1.63) [14]. However, these values are higher than *Acheta domestica* (1.25) [35], locusts (1.51) [36], *R. ferrugineus* (1.35) [19], and plant oil (soybean oil, 0.4) [37]. It is considered that the lower the IT value, the better the nutritional quality and reduced risk of developing coronary heart diseases, although no specific values are recommended for IA or IT [27].

3.2.5. Hypocholesterolemic/Hypercholesterolemic Ratio

The hypocholesterolemic/hypercholesterolemic (HH) ratio is an index used to analyse the effect of FA composition on systemic cholesterol levels. In comparison to the PUFA/SFA ratio, it has been reported that H/H values are more accurate and reflect the impact of FA on the risk of cardiovascular diseases [38]. Higher HH ratios are considered to be more beneficial to human health [27]. The HH ratio for SGP was calculated to be 0.88, which is lower than that of *P. reticularis* (2.90) [14], *T. molitor* (3.83), *R. ferrugineus* (1.35) [19], and *A. domestics* larvae (2.30) [39].

3.2.6. Health-Promoting Index

The health-promoting index (HPI) was proposed by Chen, et al. [40] to estimate the nutritional value of dietary fat and a higher value of HPI is considered more beneficial to human health. The HPI (the inverse of IA) for SGP is 0.99, which is higher than dairy products (0.16 to 0.68) [40–42], but lower than those reported in another study reported for the same species (*R. ferrugineus*; 1.35) [19].

3.3. Mineral Contents

The concentrations of the 44 minerals found in SGP are reported as mg/kg DW in Table 2. Eleven essential minerals (Table 2) were found in the SGP, comprising five macrominerals and six microminerals. There were 29 non-essential minerals (see Table 3), and four heavy metals found in SGP (Table 2). Although the mineral content of SGP was expected to be affected by the processing steps applied to the sago grubs prior to export, it can be generally concluded that pre-treated sago grubs are rich in several dietary minerals.

3.3.1. Essential Macro-Minerals

Overall, the most abundant microminerals (mg/kg DW) in SGP were found to be potassium (8053), phosphorous (2950), magnesium (1657), sodium (772), and calcium (477). Potassium is an important macro-mineral required for cellular and electric signalling in the body [43]; thus, a regular intake is important to maintain the electrolyte balance. In addition, low potassium levels in the body are potentially associated with obesity and diabetes [44]. Potassium contents were within the range (5230–10,805 mg/kg DW) reported in the literature for the same species (*R. ferrugineus*) [4,12], but higher than that reported for *R. bilineatus* (4766 mg/kg DW) [8], and large Huhu grub (5936 mg/kg DW) [45].

The content of phosphorus in SGP (2950 mg/kg DW) was lower than that in mealworm (7480 mg/kg DW) [46]. Phosphorus is essential for growth and is involved in the mineralisation process that facilitates the formation of teeth and bones [47]. However, excess phosphorous can negatively affect bone mineralisation [48].

Calcium is the most abundant element in the human body, essential for bone health and other metabolic processes. Ca deficiency is associated with an increased risk of osteoporosis [49]. The calcium content in the present study (477 mg/kg DW) was within the range of the study reported by Chinarak, et al. [4] on the same insect material (380 to 530 mg/kg). Compared to other insect material, SGP contained a lower calcium content than that of Bombay locust (656 mg/kg DW), house cricket (887 mg/kg DW), and mulberry silkworm (922 mg/kg DW) [50]. In contrast, SGP was found to have a higher calcium content than the wood-dwelling Huhu grub (285 mg/kg) [45].

It is well established that bone health can be harmed by both insufficient dietary calcium and excessive dietary phosphorus [51]. Maximum bone mass and strength are mostly influenced by food choices and genetic susceptibility [52], and the ideal Ca:P range necessary for preserving bone health is 1–1.5:1.0 [53,54]. A higher Ca:P ratio in the diet is thought to prevent loss of bone mineral density and is also an important factor in calcium balance [55]. The relatively high P content (2950 mg/kg DW) in SGP resulted in a Ca:P ratio of 0.16:1, which is lower than the recommended intake ratio of 1.3:1 [55], indicating that the treated SGP might not be an ideal source of well-balanced Ca and P. Therefore, SGP must be consumed as part of a well-balanced diet, with other ingredients that can supply Ca and P, thus improving the Ca:P ratio to levels needed for optimum bone health.

Sodium is another essential mineral required for regulating body cell water levels and maintaining the osmotic pressure of body fluids [53]. Sodium content (772 mg/kg, DW) in SGP was lower than large Huhu larvae (871 mg/kg DW) [45] and higher than *R. bilineatus* (386 mg/kg, DW) [8]. The current WHO recommendation is to reduce sodium intake to 2000 mg/day [56]. However, it has been reported that reducing salt intake to 1200 mg/day would positively affect blood pressure and reduce the risk of cardiovascular disease [54].

The magnesium content of SGP was found to be 1657 mg/kg DW, which is higher than that found in large Huhu larvae (1306 mg/kg DW) [45], scarab beetle (812 mg/kg DW), and mulberry silkworm (1577 mg/kg DW) [50]. However, *Allomyrina dichotoma* larvae (2835.6 mg/kg DW), *Protaetia brevitarsis* larvae (3276 mg/kg DW), and *T. molitor* (3152 mg/kg DW) [33] contain higher magnesium concentrations than SGP. Magnesium is a co-factor of various enzymes involved in the mechanism of protein and nucleic acid synthesis. It also helps regulate phosphorus and calcium metabolism to maintain bone density [56].

3.3.2. Essential Micro-Minerals

Iron is an essential micro-mineral for bodily functions, including acting as an oxygen carrier in the blood, and being a component of some enzymes in tissues [57]. In the present study, iron content in SGP was found to be 23 mg/kg DW, indicating that consumption of 0.4 to 0.5 kg/day of SGP would meet the recommended dietary allowance (RDA) of iron of 9–15 mg per day (children), 8–18 mg per day (women), and 8 mg per day (men) [58], as a part of a balanced diet. The most vulnerable population affected by iron deficiency are infants, children, and women of childbearing age [33]. According to FAO/WHO [56], the labelling of food as being 'high in' or a 'source of' depends on the nutrient reference value (NRV). If the NRV of a food for iron is 10–19%, then the food is considered to be a good source of iron; however, if the food has a daily value of more than 20%, then it is considered to be high in iron content. Hence, potentially SGP could be considered to be a 'high in' source of iron for a diet.

The levels of manganese in SGP (8.8 mg/kg DW) were within the range reported by Chinarak, et al. [4] (6–110 mg/kg DW), and similar to *R. bilineatus* (8.2 mg/kg DW) [8]. The variations in the content of nutrients in these studies depend on several factors, such as species/variety studied, feed, location of harvesting, the reproductive cycle of the insects, and any pre-processing steps used during the post-harvest handling of the insects [1]. The nutrient quality of various foods can be altered by different food processing methods such as boiling or frying [59]. So far, the effect of the various food preparation methods on the mineral content of various insect materials has not been fully investigated [60].

Selenium was detected in SGP (0.03 mg/kg). Selenium is a trace element, and its deficiency is reported to be associated with various health implications such as suppressed biosynthesis of key selenoproteins, low lipid accumulation, high glutamine catabolism, and hypoglycaemia [61]. However, levels of selenium higher than the recommended intake of 0.45 mg/day can increase lipogenesis, cholesterol biosynthesis, and protein biosynthesis [61]. Chromium is another essential micronutrient for humans and plays an important role in regulating blood sugar levels. Consumption of 100–200 g of SGP that contains 0.25 mg/kg DW of chromium can provide the recommended daily intake for an adult (0.02–0.04 mg) [58].

The nutrient reference value (NRV) for zinc is reported to be 15–30 mg/day [56], and hence ~300 g of SGP would fulfil the daily requirement of zinc. Compared to superworm (77 mg/kg DW) [60], the zinc content was found to be relatively high in SGP (83 mg/kg DW) (Table 2), and this was similar to that found by Chinarak, et al. [4] (80–90 mg/kg DW). Zinc is an essential mineral for more than 300 enzymes involved in the metabolism of nutrients and facilities cell and organ integrity and the immune system [29]. In the present study, SGP contained 8.3 mg/kg DW copper, which is lower than those reported for *R. ferrugineus* (10–11 mg/kg) [4], but comparable to Huhu grubs (7.1 to 8.9 mg/kg). Copper plays a role in redox reactions and, together with zinc, are important co-factors of the antioxidant enzyme superoxide dismutase [62].

3.3.3. Heavy/Toxic Metals

One of the main concerns about the consumption of wild-harvested insect material is the possible presence of heavy metals that have bioaccumulated in the insect material from the environment over time. Heavy metals such as arsenic (0.17 mg/kg DW), vanadium (0.01 mg/kg DW), cadmium (0.04 mg/kg DW), and lead (0.56 mg/kg DW) were found in SGP (Table 2). Overall, the arsenic content of SGP was lower than the maximum allowable concentration of 2 mg/kg FW in foodstuffs [63]. According to the EFSA, the maximum allowed level of lead in plant foods such as cereals, legumes, and pulses is 0.20 mg/kg fresh weight, FW, and the maximum permitted level in meat is 0.10 mg/kg FW [64]. The maximum allowed level for lead content in insect materials destined for use in feed is 10 mg/kg DW [65], showing that SGP was within the maximum set limit. The lead content in some edible insects found in Thailand, such as the Bombay locust (0.009 mg/kg FW), scarab beetles (0.11 mg/kg FW), and mulberry silkworm (0.004 mg/kg FW) has been reported [50]. The lack of moisture or dry matter data for these insects makes it challenging to compare their lead contents with that found in SGP.

Table 2. Mineral content of SGP of macrominerals, micro/trace minerals, and heavy metals expressed in mg/kg, DW.

Macrominerals (mg/kg DW)						
	Sodium	Magnesium	Phosphorus	Potassium	Calcium	
SGP	772 ± 12.5	1657 ± 46.1	2950 ± 10.0	8053 ± 290.2	477 ± 24.4	
[1] DORM-4	14,052 ± 1006.0	897 ± 76.0	7113 ± 497.0	13,352 ± 962.0	2391 ± 181.0	
Recovery (%)	100	99	89	86	101	
[2] Limit of detection	4	2	10	4	8	
[3] Limit of quantification	12	6	30	12	24	
Microminerals (mg/kg DW)						
	Chromium	Copper	Iron	Zinc	Selenium	Manganese
SGP	0.25 ± 0.03	8.3 ± 0.32	22 ± 0.97	83 ± 0.55	0.03 ± 0.003	8.8 ± 0.27
[1] DORM-4	1.75 ± 0.20	15 ± 0.90	333 ± 0.90	51 ± 4.80	3.2 ± 0.500	3.04 ± 0.20
Recovery (%)	94	97	97	98	93	96
[2] Limit of detection	0.02	0.06	0.8	2	0.02	0.02
[3] Limit of quantification	0.06	0.20	2.0	6	0.06	0.06
Heavy metals (mg/kg DW)						
	Arsenic	Vanadium	Lead	Cadmium		
SGP	0.17 ± 0.003	0.01 ± 0.003	0.56 ± 0.013	0.04 ± 0.01		
[1] DORM-4	6.4 ± 0.330	1.52 ± 0.090	0.34 ± 0.040	0.30 ± 0.02		
Recovery (%)	93	97	85	100		
[2] Limit of detection	0.010	0.006	-	-		
[3] Limit of quantification	0.06	0.02	0.03	0.03		

The experiment was carried out in triplicate (n = 3). [1] DORM-4 fish protein-certified reference material (mg/g DW). This reference material is primarily intended for the calibration of procedures and developing methods for determining trace metals in materials of a similar matrix. [2] Limit of detection is the lowest amount detected using ICP-MS, with a certain confidence level (typically 99%). [3] Limit of quantitation is the smallest amount or the lowest concentration of the substance that can be determined to establish accuracy, precision, and replicability.

3.3.4. Non-Essential Minerals

Results for the 29 non-essential (trace) minerals are presented in Table 3. Elements such as boron (2.4 mg/kg DW), cobalt (0.12 mg/kg DW), nickel (0.44 mg/kg DW), rubidium (2.6 mg/kg DW), yttrium (0.03 mg/kg DW), molybdenum (0.06 mg/kg DW), and barium (13 mg/kg DW) were detected in SGP in the present study. The distribution, absorption, and biological pathways of trace elements are poorly understood [16]. For example, although an intake of 60 mg barium per kg body weight/day is reported to cause renal effects [66], more studies are needed on the long-term health risks of low concentrations of barium [67]. Cobalt was also found at a low concentration of 0.12 mg/kg DW. The recommended intake of cobalt per person per day is reported to be between 5 and 8 μg [68]. Cobalt is required for vitamin B12 metabolism, and low levels can be beneficial for the nervous system. However, a high intake (e.g., 150–500 mg/kg) can be lethal [68]. Yttrium has no known biological properties and tends to accumulate in the spleen, liver, bones, and kidney at up to 0.50 mg/kg [69]. Nickel is a trace element, and its deficiency is considered to be very rare. However, low levels of nickel in the body can lead to certain liver and kidney diseases, which can occur in juvenile small animals, such as chicks, which can lead to stunted growth, pigmentation, dermatitis, and compromised liver function [70]. The recommended dietary allowance (RDA) of nickel for different age groups is reported as ranging from 0.10 to 0.70 mg/day [70]. The nickel content in sago grub larvae was found to be 0.44 mg/kg DW, which is within the range of the RDA.

Table 3. Non-essential mineral content of SGP expressed as mg/kg DW.

	Co	B	Ni	Ga	Li	Rb	Sr	Y	Sn	Sb	Mo	Ag	Cs	Ba	La	Ce	Pr	Nd	Sm	Gd	Dy	Eu	Ho	Er	Tm	Yb	Lu	Th	U
SGP	0.12 ± 0.01	2.40 ± 0.27	0.44 ± 0.03	<0.01	<0.02	2.68 ± 0.20	1.34 ± 0.036	0.03 ± 0.003	0.01	<0.01	0.06 ± 0.006	<0.01	0.008 ± 0.001	13.30 ± 1.453	0.032 ± 0.007	0.075 ± 0.012	0.010 ± 0.001	0.040 ± 0.005	0.009 ± 0.001	0.009 ± 0.0016	0.006 ± 0.0003	0.002 ± 0.0004	0.001 ± 0.0002	0.003 ± 0.0002	<0.001	0.0024 ± 0.0002	<0.001	0.004 ± 0.002	0.03 ± 0.0005
DORM-4	0.25 ± 0.02	11	1.3± 0.099	0.38 ± 0.029	1.1 ± 0.09	5.7 ± 0.26	9.5 ± 0.65	0.17 ± 0.03	0.072 ± 0.011	<0.002	0.26 ± 0.023	0.025 ± 0.02	0.075 ± 0.01	4.8 ± 0.24	0.46 ± 0.09	0.98 ± 0.21	0.12 ± 0.025	0.47 ± 0.11	0.096 ± 0.02	0.085 ± 0.01	0.060 ± 0.01	0.019 ± 0.002	0.008 ± 0.0001	0.019 ± 0.0002	<0.002	0.012 ± 0.010	<0.002	0.16 ± 0.010	0.05 ± 0.0006
Recovery (%)	99	-	94	-	96	-	94	-	119	-	88	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	106
Limit of detection	0.004	1	0.04	0.01	0.02	0.010	0.010	0.004	0.01	0.01	0.008	0.01	0.001	0.001	0.001	0.02	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001
Limit of quantitation	0.01	3	0.1	0.03	0.06	0.03	0.03	0.01	0.03	0.03	0.02	0.03	0.003	0.03	0.003	0.006	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.006	0.003

Co—Cobalt, B—Boron, Ni—Nickel, Ga—Gallium, Li—Lithium, Rb—Rubidium, Sr—Strontium, Y—Yttrium, Sn—Tin, Sb—Antimony, Mo—Molybdenum, Ag—Silver, Cs—Cesium, Ba—Barium, La—Lanthanum, Ce—Cerium, Pr—Praseodymium, Nd—Neodymium, Sm—Samarium, Gd—Gadolinium, Dy—Dysprosium, Eu—Europium, Ho—Holmium, Er—Erbium, Tm—Thulium, Yb—Ytterbium, Lu—Lutetium, Th—Thorium, U—Uranium. BDL—Below Detectable Level. DORM-4 Fish protein-certified reference material (mg/kg DW). This reference material is primarily intended for use in the calibration of procedures and development of methods for the determination of trace metals in materials of a similar matrix. 'Limit of detection' is defined as the lowest amount that can be detected using ICP-MS, with a certain confidence level (typically 99%). Limit of quantitation is the smallest amount or the lowest concentration of the substance that can be determined to establish accuracy, precision, and replicability.

4. Conclusions

The present study provides insights into the composition and profile (proximate, fatty acid, and mineral contents) of SGP (*R. ferrugineus*) larvae obtained from Thailand. It was postulated that prior treatment (boiling and oven drying) of the grubs to meet biosecurity requirements for import into New Zealand would impact the nutrient content of the sago grub material. However, the present study has found that, despite the pre-treatment, SGP contains substantial nutrients. For example, the lipid composition shows high levels of MUFA, followed by SFA and PUFA. The relatively high abundance of SFA and the implications of this for health needs to be considered regarding SGP as a food source. The indicators of lipid nutritional quality, such as IA and ω-6/ω-3, of the sago grubs were within limits known to promote human health. However, indicators such as HPI, IT, and H/H were lower in SGP compared to another study of the same species, suggesting there may be some impact from the pre-treatment process.

ICP-MS detected 44 minerals in the sago grub material with relatively high levels of nutritionally essential minerals such as potassium, calcium, magnesium, manganese, iron, and zinc. The Ca:P ratio in the SGP samples was lower than the recommended ratio, suggesting the need to consume SGP as part of a balanced diet. Toxic minerals such as cadmium, vanadium, and lead were below the toxicity limits in SGP. Together, these findings indicate the overall safety and nutritional potential of SGP as an imported edible insect material.

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