



The nutritional profile of the yellow mealworm larvae (*Tenebrio molitor*) reared on four different substrates

Keandra Langston^a, Letlhogonolo Selaledi^{a,b}, Chrysantus Tanga^c, Abdullahi Yusuf^{a,*}

^a Department of Zoology and Entomology, University of Pretoria, Private bag X20, Hatfield 0028, South Africa

^b Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

^c International Centre of Insect Physiology and Ecology, Kasarani, P. O. Box 30772-00100, Nairobi, Kenya

ARTICLE INFO

Keywords:

Edible insects
Food and feed
Insect rearing diets
Nutritional quality
Sustainable Development Goals (SDGs)

ABSTRACT

The utilisation of *Tenebrio molitor* L. (the yellow mealworm) as a cheaper, alternative and readily available ingredient for food and feed is gaining interest globally. However, there has been limited research on locally and readily available substrates for mealworm mass rearing in South Africa. This study evaluated the impact of four substrates; wheat bran (control diet), wheat flour, maize flour, and Lucerne (pellets) on the rearing of mealworm larvae under controlled conditions over two generations and analysed the nutrient composition using standard nutritional analysis techniques. The results revealed that the crude protein contents of *T. molitor* larvae ranged between 28 – 36 % when raised on different substrates and varied significantly in the order Lucerne > wheat bran > wheat flour > maize flour. The major minerals found in the larvae included sodium, magnesium, phosphorus, potassium, copper, and zinc. The larvae were also rich in saturated, mono and polyunsaturated Fatty Acids (FAs) with oleic, linoleic, and palmitic as the main FAs. The nutritional profiles of first- and second-generation larvae raised on the same substrates remained the same. Wheat flour was found to be the best alternative to wheat bran. The finding demonstrates the suitability of local, inexpensive substrates for commercial production of yellow mealworm without compromising their nutritional quality and utilisation for food and feed.

1. Introduction

There are over 2000 edible insect species that are used by humans as food, feed for their animals and for therapeutic purposes (Meyer-Rochow, 2005; van Huis, 2020; Devi et al., 2023; Tanga and Ekesi 2024). Edible insects provide protein and essential nutrients and contribute to the daily food requirements of people in countries where insects form part of their traditional diets (Anankware et al., 2015; Banjo et al., 2006; Bukkens, 1997; DeFoliart, 1999; Elemo et al., 2011; Ladrón de Guevara et al., 1995; Ramaswamy, 2015; Ramos-Elorduy, 1997). More recently, the use of edible insects as food and feed is being addressed with more urgency due to changing lifestyles, rising cost of food sources and the quest for sustainable and alternative protein sources. Some of most reared and researched insects used by humans around the world include domestic crickets, palm weevils, giant water bugs (van Huis et al., 2013; Melo-Ruíz et al., 2016; Fernandez-Cassi et al., 2019; Anankware et al., 2019), black soldier flies, locusts, silkworms, honeybees, termites, cicadas and mealworms (Usman and Yusuf, 2021; Grau et al. 2017; Tanga

and Ekesi 2024).

Insects as food or feed have been found to be beneficial to humans, the environment, livelihoods, and economies and can aid in attaining most of the United Nations Sustainable Development Goals (SDGs) (Tanga and Ekesi, 2024; Oonincx et al., 2015; van Huis et al., 2013; Ramos-Elorduy, 1997; Nakagaki and Defoliart, 1991). Malnutrition and land use remain a global concern, and the use of edible insects could represent an inexpensive and environmentally sustainable solution to food security (Tanga and Ekesi, 2024) and the land crisis. Furthermore, establishing a strong foundation for the novel edible insect market, requires research on their nutritional value and their use as a source of food.

Tenebrio molitor L. (Coleoptera) larvae commonly known as the yellow mealworm (here after referred to TM) is an insect that is easy to rear with modest requirements for space, water, resources, and maintenance (Langston et al., 2023 and references therein). Mealworms have a relatively short life cycle (4 – 6 months) and a high reproductive rate. *Tenebrio molitor* is currently used as food by humans or feed for animals

* Corresponding author.

E-mail address: abdullahi.yusuf@up.ac.za (A. Yusuf).

<https://doi.org/10.1016/j.fufo.2024.100388>

Received 1 August 2023; Received in revised form 17 April 2024; Accepted 26 May 2024

Available online 27 May 2024

2666-8335/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

in Africa, Asia, Americas, and Australia (Alves et al., 2016), and has recently been approved for use as food by the European Union (Alves et al., 2016; Petrescu-Mag et al., 2022).

The larvae of TM are generally reared on wheat bran (Mancini et al., 2020). However, in South Africa a kilogram of wheat bran is sold for US\$ 1.03 compared to Lucerne pellets, maize and wheat flours which are sold at US\$ 0.46, 0.53 and 0.73 respectively. Thus, making wheat bran expensive and out of reach for many (Langston et al., 2023). This coupled with other multi-competitive uses of wheat bran by humans, makes it an unsustainable substrate in the long run. Hence the need to explore other locally and cheaper alternatives that are readily available and accessible. Previously, we evaluated the suitability and affordability of different substrates for mass rearing of TM larvae (Langston et al., 2023). Here we evaluated the effect of four promising substrates [wheat bran (control diet), wheat flour, maize flour, and Lucerne pellets] on the nutritional composition of TM larvae. TM larvae were reared for two generations (F1 and F2) on the same substrate type to ensure that substrate quality mirrored the nutrients of the larvae without compromising its nutrient quality and biomass over time for use as food and feed.

2. Materials and methods

2.1. *Tenebrio molitor* adult rearing

Tenebrio molitor adults (60 mating pairs = 120 individuals) were obtained from the colony at the Department of Zoology and Entomology, University of Pretoria, and maintained in plastic boxes containers (68.4 × 38.4 × 20.9 cm), placed in a climate-controlled chamber, kept at a constant temperature of 26 ± 1 °C, 50 % relative humidity, and a 0-h light: 24-h dark photoperiod on a standard wheat bran substrate (Langston et al., 2023).

2.1.1. Substrates and their sources

White self-raising wheat flour (Supreme Foods Limited), Lucerne pellets (Midfeeds Limited), maize flour (IWISA® Premier Foods), and wheat bran (Standard) were obtained locally in South Africa and used as substrates to rear TM larvae. Two (2) kg of each substrate was evenly distributed into clean black plastic rearing containers (68.4 × 38.4 × 20.9 cm) previously sterilised using 75 % ethanol (v/v). A total of 48 rearing containers were used with each treatment group replicated thrice. This was also repeated for the two generations (F1 and F2).

2.1.2. Rearing of *T. molitor*

Ten pairs of adult *T. molitor* (10 males and 10 females) were placed in each container together with the rearing substrate for three weeks and then removed. This was to allow them enough time to mate and lay eggs. Ten (10) g of fresh carrots were added twice a week in each container to serve as a source of moisture. After approximately three months, the TM larvae were removed from each container.

After each generation of rearing on the substrates, three (3)– five (5) kg of the TM larvae were harvested and frozen at –20 °C until needed for nutritional composition analysis. About 20 % of the larvae were allowed to pupate and thereafter the pupae were transferred into separate containers until adult emergence. Once the adults of the first generation emerged, they were allowed to mate and lay eggs in their respective substrates for three (3) weeks, after which they were removed from the rearing boxes. The second generation was then reared using a similar approach as those of the first generation.

2.2. Nutrient analysis

2.2.1. Sample preparation

One hundred (100) g of TM larvae produced from each treatment from the two generations were removed and starved for 48 h to remove all gut contents. Then washed with water, and frozen at –20 °C until required for analysis. When required for analysis, the frozen samples

were freeze-dried over a period of 48 h, ground to a powder using a pestle and mortar and sieved through a number 30 sieve (600 µm). The powdered sample was then packed in pre-labelled air-tight capped bottles until required for nutrients analysis. This was repeated for each of the two generations of TM larvae reared.

2.2.2. Proximate analysis of TM larvae fed on different substrates

Ash and crude fat contents were determined according to the Association of Official Analytical Chemists methods 942.05, and 920.39, respectively (AOAC 2006). A Memmert UM500 dry oven at 105 °C was used to determine the dry matter of the TMs. To determine the ash content, samples were incinerated by heating to 600 °C for 2 h. Crude fibre was determined by exhaustive Soxhlet extraction using petroleum ether (AOCS, 2005) while gross energy value (GEV) was determined adiabatically using an IKA oxygen bomb calorimeter (C5000; IKA Werke GmbH, Germany).

Crude protein analysis was carried out using method 968.06 of the methods of the Association of Official Analytical Chemists (AOAC 2006). Briefly, nitrogen (N₂) was freed by pyrolysis and combustion, and the residual N₂ measured and converted to equivalent protein using 4.76 (Janssen et al. 2017) as Crude protein conversion factor instead of 6.47 which overestimates the protein contents of insects.

2.2.3. Mineral analysis of TM larvae reared on different substrates

Minerals were analysed by subjecting the samples to dry-ashing at 550 °C according to the AOAC method (AOAC 2006), and content determined using inductively coupled plasma-atomic emission spectrometry and calibration curves for each mineral standard.

2.2.4. Fatty acid (FA) analysis of TM larvae reared on different substrates

Lipid were extracted using variation of Folch et al. (1957) method followed by trans-esterification of the FAs from the TMs into FAs Methyl Esters (FAMES) as described in Webster et al. (2006) and O'Fallon et al. (2007) with a slight modification. To enable quantification of the FAMES, 1 mL of 0.5 mg C15:0/mL (prepared in methanol) was added to the samples as an internal standard. The hexane layer containing FAMES was transferred into a 2 mL gas chromatography (GC) vial and 1 µL of the sample analysed on the GC.

The identification and quantification of the FAMES was achieved on a SCION 456 gas chromatograph (Bruker, UK) equipped with a flame ionisation detector and an Rt-2560 (100 m × 0.25 mm × 0.20 µm) column (Restek, USA) using methods described in Webb et al. (1999). Chromatograms were analysed using the Compass CDS Software Version 3.0.1 (Scion Instruments, UK) and FAs were identified by comparing retention times to a Supelco FAME mix standard of 37 FAMES (Merck Life Science South Africa). The FA composition for each sample was calculated based on the peak areas of the analytes relative to that of an internal standard in comparison with the FAME mix standards.

2.3. Statistical analyses

The nutrient composition of TM larvae reared on the different substrates was analysed using ANOVA with substrates as the independent variables and nutritional composition as dependent variables. Thereafter, a Dunnett's Test for multiple comparisons was used to compare between means from the different experimental groups. In order to test if there are differences in nutrient composition between the two TM generations, a Student's T-test was performed. All data analyses were conducted in R using the interface R studio (RStudio Team, 2020).

3. Results

3.1. Proximate and mineral contents of TM larvae reared on different substrates

The proximate composition of the TM larvae reared on the different

substrates are shown in Table 1. Dry matter contents of the TM larvae were between 82.25 – 87.95% and did not differ significantly between substrates (Table 1). The crude protein of the TM ranged between 28.57 - 36.24% and differed across the substrates (Table 1) with TM reared on maize flour and Lucerne having the lowest and highest crude protein content respectively. The crude fibre contents were between 4.87 - 8.67% and varied among the substrates (Table 1). Similarly, crude fat contents of TM larvae also varied significantly across substrates and was between 27.11 - 36.07% with maize flour producing larvae with highest crude fat content in F1 and highest gross energy values (Table 1). There were no variations in nutrient contents between larvae from F1 and F2 generations except for crude fibre ($t(6) = 3.8090, p > 0.05$), fat ($t(6) = 6.8819, p > 0.05$) from wheat flour, crude fibre ($t(6) = 13.2504, p > 0.05$) and GEV ($t(6) = 4.3295, p > 0.05$) from maize flour and Lucerne ($t(6) = 6.0333, p > 0.05$).

The mineral content of TM larvae reared on the four different substrates in F1 and F2 showed that those reared on wheat flour had higher sodium; potassium, calcium, phosphorus, magnesium and manganese (Table 2). Iron (0.24 and 1.27 mg/100 g) and Zinc (2.21 and 2.87 mg/100 g) were higher for TM reared on wheat bran and maize flour, respectively. While TM reared on Lucerne contained more copper (5.14 - 6.83 mg/100 g) than those from other substrates (Table 2).

3.2. FA composition of TM larvae reared on different substrates

The FA composition of TM larvae reared on the four different substrates in F1 and F2 are presented in Table 3. Larvae produced on all four substrates contains predominantly (51.92–60.70%) monounsaturated FAs (MUFA), followed by saturated FAs (SFA) and polyunsaturated FAs (PUFA) respectively (Table 3). There were no differences in the composition of MUFA ($t(6) = 0.6288, p > 0.05$), PUFA ($t(6) = -1.2778, p > 0.05$) and SFA ($t(6) = 0.4055, p > 0.05$) between F1 and F2 generations. The most abundant individual FA from the profiles were C18 FAs (oleic acid, C18:1; linolenic acid C18:2) accounting for between 49.2 – 56.67% and 16.34 – 25.95 % of the total FA contents respectively (Table 3).

4. Discussion

We evaluated the nutritional composition of *T. molitor* larvae on four substrates reared over two generations on the same substrate and found differences between rearing substrates but not between generations. Thus, confirming earlier reports by Langston et al. (2023) showing differences in the suitability of alternative substrates for rearing TM larvae and those by van Broekhoven et al. (2015) and Rumbos et al. (2020) who reported differences in nutrient composition of larvae based on the rearing substrates. The finding that nutritional composition does not differ between generations indicates the suitability and affordability of TM larvae rearing as a tool to reduce waste, as a future food and feed (Hong et al., 2020; Errico et al., 2022a), and for attaining the UN SDG

goals (1, 2, 3, 5, 8, 10, 11, 13, 15) due to its negligible impact on the environment in comparison to other animal products (Oonincx and de Boer, 2012). This is one of the reasons why *T. molitor* became the first insect to be approved by the European Food and Safety Authority as a novel food pursuant to EU Regulation 2015/2283. (EFSA NDA, 2021).

The crude protein content of our TM larvae were higher than those from silkworm (16% protein) (*Bombyx mori*) (Longvah et al., 2011) but lower than the ranges reported from other studies on *T. molitor* (Errico et al. 2022b). It is worth noting that, we used 4.76 as a nitrogen-to-protein conversion factor instead of 6.25 because the latter overestimates the protein contents in insects due to the presence of nonprotein nitrogen fraction like chitin, nucleic acids, phospholipids, residues of excretion etc. (Janssen et al. 2017). There are about two other nitrogen to protein conversion factors that have been proposed for *T. molitor*, 5.41 by Boulos et al. (2020) and 5.60 by Janssen et al. (2017). Irrespective of the conversion factor used, protein content of TM larvae is comparable to those of beef sirloin (20.1 g/100 g), chicken breast (21.5 g/100 g) and pork shoulder (16.89 g/100 g) (Orkus, 2021). And edible insects are known to be richer in proteins (Bukkens, 1997; Ramos-Elorduy, 1997) than most conventional meat sources such as beef, chicken or pork (Nakagaki and Defoliart, 1991; Oonincx et al., 2010, 2015; Orkus, 2021).

The crude fat contents we found in this study are within the ranges (19–43 %) reported previously for TM larvae (reviewed in Errico et al., 2022b). Fat is essential to living organisms, particularly humans and animals, where it plays a major role in the supply of energy and protection of body organs and cell membranes as well as absorption of vitamins to vital parts of the body. Insects store fats in the form of fat bodies that play an important role in energy storage, metabolism, and nutrient reserves (Arrese and Soulages, 2010), thus making them a rich source of fat (Rumpold and Schlüter, 2013; Dobermann et al., 2017).

Minerals are necessary to maintain normal physiological functions (Fritz et al., 2019; Kinyuru et al., 2015; Rempel et al., 2021). *Tenebrio molitor* larvae raised on the four substrates were rich in all minerals except calcium. However, TM larvae raised on wheat flour were richer in Na, K, Fe, Zn, Cu compared to those raised on the other three substrates. This explains why previous studies have recommended that substrates used to rear TM larvae should be supplemented with additional calcium to improve their performance and nutrient profile if they have to be used for food and feed (Finke, 2002; Niassy et al., 2018; van Huis et al., 2013). Although TM larvae reared on wheat bran, flour, maize flour, and Lucerne contain essential minerals, these are not at the recommended dietary intake allowances (RDA) for humans (NHI, 2018) except for magnesium. This implies that future research using these substrates should consider supplementing the mineral content of these substrates. The high value of magnesium in the TM larvae is important because magnesium has been reported to facilitate the active movement of calcium and potassium ions across cell membranes, which is essential for the transmission of nerve impulses, muscle contraction, and a regular heartbeat (Musso, 2009). Manganese, on the other hand, is essential to

Table 1

Proximate composition (mean \pm SD) of mealworm larvae *Tenebrio molitor* reared on four different substrates over two generations.

Substrate	Wheat bran F1	Wheat flour F1	Maize flour F1	Lucerne F1	Wheat bran F2	Wheat Flour F2	Maize flour F2	Lucerne F2	df	f statistic
Dry matter	87.90 \pm 4.40 ^a	82.80 \pm 4.14 ^a	84.75 \pm 4.24 ^a	86.65 \pm 4.33 ^a	82.25 \pm 4.11 ^a	84.75 \pm 4.24 ^a	85.45 \pm 4.27 ^a	82.25 \pm 4.11 ^a	7	0.722
Crude protein	34.68 \pm 1.73 ^a	32.10 \pm 1.61 ^{ab}	28.57 \pm 1.43 ^b	36.24 \pm 1.81 ^a	32.34 \pm 11.62 ^{ab}	29.25 \pm 1.46 ^a	29.64 \pm 1.48 ^a	34.81 \pm 1.74 ^b	7	9.473
Crude fibre	5.46 \pm 0.27 ^{abc}	6.01 \pm 0.30 ^b	4.87 \pm 0.24 ^c	5.63 \pm 0.28 ^{abc}	5.82 \pm 0.29 ^a	7.03 \pm 0.35 ^b	8.67 \pm 0.43 ^c	5.61 \pm 0.28 ^a	7	43.770
Crude fat	30.84 \pm 1.54 ^{ab}	27.11 \pm 1.36 ^a	34.68 \pm 1.73 ^b	27.20 \pm 1.36 ^a	27.85 \pm 1.39 ^a	36.07 \pm 1.80 ^b	3.15 \pm 0.16 ^a	22.84 \pm 1.14 ^c	7	15.340
Ash	3.35 \pm 0.17 ^a	3.45 \pm 0.17 ^a	3.15 \pm 0.16 ^a	3.25 \pm 0.16 ^a	3.25 \pm 0.16 ^a	33.74 \pm 1.69 ^b	3.25 \pm 0.16 ^a	22.07 \pm 1.10 ^{bc}	7	1.106
GEV (MJ/kg)	25.56 \pm 1.28 ^a	25.24 \pm 1.26 ^a	26.27 \pm 1.31 ^a	24.74 \pm 1.24 ^a	26.30 \pm 1.31 ^a	30.34 \pm 1.52 ^a	3.25 \pm 0.16 ^a	19.28 \pm 0.96 ^b	7	12.450

Different letters in the same rows indicates significant differences ($p < 0.05$), GEV = Gross energy value, F1 = first generation and F2 = second generation.

Table 2Mineral composition (mg/100 g) of TM larvae (*Tenebrio molitor*) reared on four different substrates with the recommended dietary allowances for adult humans.

Substrate	Wheat bran F1	Wheat flour F1	Maize flour F1	Lucerne F1	Wheat bran F2	Wheat Flour F2	Maize flour F2	Lucerne F2	RDA(mg)	df	f statistic
Minerals											
Sodium (Na)	93.15 ± 4.66 ^a	107.16 ± 5.36 ^{bc}	116.98 ± 5.85 ^{bc}	93.56 ± 4.68 ^{ac}	94.77 ± 4.74 ^a	91.72 ± 4.59 ^a	95.05 ± 4.75 ^a	97.19 ± 4.86 ^a	1500	7	9.842
Potassium (K)	97.45 ± 4.87 ^a	101.96 ± 5.10 ^a	110.48 ± 5.52 ^a	102.46 ± 5.12 ^a	87.47 ± 4.37 ^a	91.42 ± 4.57 ^a	87.04 ± 4.35 ^a	93.91 ± 4.70 ^a	2300–3400	7	8.503
Calcium (Ca)	29.57 ± 1.48 ^a	41.41 ± 2.07 ^b	20.71 ± 1.04 ^c	29.57 ± 1.48 ^a	28.81 ± 1.44 ^a	35.47 ± 1.77 ^b	41.44 ± 2.07 ^c	41.16 ± 2.06 ^c	100–1200	7	59.09
Phosphorus (P)	125.72 ± 6.29 ^{abc}	140.64 ± 7.03 ^b	120.29 ± 6.01 ^c	135.17 ± 6.76 ^{abc}	119.78 ± 5.99 ^{ab}	106.79 ± 5.34 ^a	104.46 ± 5.22 ^a	125.99 ± 6.30 ^b	700	7	12.44
Magnesium (Mg)	249.67 ± 12.48 ^{ac}	283.09 ± 14.15 ^b	277.58 ± 13.88 ^{cb}	224.64 ± 11.23 ^a	207.96 ± 10.40 ^a	128.23 ± 6.41 ^b	212.30 ± 10.62 ^{ac}	244.08 ± 12.20 ^c	320–420	7	53.15
Iron (Fe)	1.27 ± 0.06 ^a	0.98 ± 0.05 ^b	0.36 ± 0.02 ^c	0.98 ± 0.05 ^b	0.24 ± 0.01 ^a	0.37 ± 0.02 ^b	0.37 ± 0.02 ^b	0.24 ± 0.01 ^a	08–18	7	39.80
Zinc (Zn)	1.88 ± 0.09 ^a	2.20 ± 0.11 ^b	2.87 ± 0.14 ^c	1.22 ± 0.06 ^d	0.87 ± 0.04 ^a	1.88 ± 0.09 ^b	2.21 ± 0.11 ^c	1.54 ± 0.08 ^d	08–11	7	126.6
Copper (Cu)	4.02 ± 0.20 ^{ab}	4.55 ± 0.23 ^a	4.56 ± 0.23 ^a	6.83 ± 0.34 ^b	5.69 ± 0.28 ^{ab}	6.32 ± 0.32 ^a	5.46 ± 0.27 ^b	5.14 ± 0.26 ^b	900	7	37.24
Manganese (Mn)	4.57 ± 0.23 ^a	5.84 ± 0.29 ^b	5.84 ± 0.29 ^b	6.31 ± 0.32 ^b	6.51 ± 0.33 ^a	5.29 ± 0.26 ^b	5.30 ± 0.27 ^b	5.00 ± 0.25 ^b	1.8–2.3	7	16.54

*The nutrition Source, Harvard School of Public Health (<https://www.hsph.harvard.edu/nutritionsource/vitamins/>). Different letters in the same columns indicate significant differences ($p < 0.05$). F1 = first generation and F2 = second generation.

Table 3Proportions of individual Fatty acids (FAs) and saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated (PUFAs) from TM larvae (*Tenebrio molitor*) reared on four different substrates.

Fatty Acids (FA)	% of Fatty Acids- F1				% of Fatty Acids- F2			
	Wheat bran	Wheat flour	Maize Flour	Lucerne pellets	Wheat bran	Wheat flour	Maize Flour	Lucerne
Myristic acid (C14:0)	3.91	3.82	3.93	3.36	3.73	4.48	4.94	4.89
Palmitic acid (C16:0)	19.76	13.86	15.79	17.07	18.71	16.63	14.31	11.90
Palmitoleic acid (C16:1)	1.82	3.95	2.29	2.03	2.01	3.09	3.29	4.50
Heptadecanoic acid (C17:0)	0.06	0.00	2.72	0.10	0.07	0.00	1.30	0.06
Cis-Heptadecanoic acid (C17:1)	0.04	0.00	0.01	0.00	0.04	0.04	0.00	0.20
Eliadic acid (C18:1) trans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Oleic acid (C18:1) cis	50.02	56.67	55.38	56.09	50.58	55.22	54.20	49.20
Linoleic acid (C18:2)	20.42	18.46	16.34	17.56	20.76	17.10	19.69	25.95
Arachidic acid (C20:0)	0.07	0.00	0.00	0.00	0.10	0.19	0.00	0.12
Eicosenoic acid (C20:1)	0.04	0.08	0.00	0.00	0.05	0.07	0.07	0.03
Alpha-linolenic acid (C18:3)	0.34	0.01	0.01	0.49	0.48	0.11	0.01	0.66
Heneicosanoic acid (C21:0)	0.00	0.04	0.00	0.00	0.00	0.00	0.10	0.00
Eicosadienoic acid (C20:2)	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.04
Behenic acid (C22:0)	0.00	0.01	0.00	0.00	0.01	0.02	0.01	0.04
SFA	23.80	17.73	22.44	20.53	22.62	21.32	20.66	17.01
MUFA	51.92	60.70	57.68	58.12	52.68	58.42	57.56	53.94
PUFA	20.76	18.47	16.35	18.05	21.25	17.23	19.70	26.65

Abbreviations: SFA, Saturated Fatty acids, MUFA, monounsaturated Fatty acids, PUFA, polyunsaturated Fatty acids.

our bodies only in small amounts as we obtain it from food as a supplement that assists enzymes involved in breaking down carbohydrates, proteins and cholesterol. Although we found amounts of manganese that were more than the recommended RDAs, these were within the range reported by Jajić et al. (2019) for TM larvae reared on different substrates. This further emphasises the need to evaluate edible insects for their safety and potential biological and chemical risks during rearing, processing and utilisation to prevent consumption of harmful contaminants (Charlton et al., 2015; Belluco et al., 2018).

The FA content of edible insects is considerably different from those of other animal fat, with insects generally having higher FAs both in quality and quantities of individual FAs and classes (saturated and unsaturated) (Nakagaki and Defoliart, 1991; Xiaoming et al., 2010). This is consistent with the results from TM larvae we raised on all four substrates which had more MUFA than PUFA and SFA respectively as previously reported (Errico et al. 2022b). Polyunsaturated FAs (PUFA) are vital to human health, even though humans are unable to synthesise them *de novo* but have to acquire them through food. *Tenebrio molitor* larvae had high amounts of C18 FAs responsible for lowering blood

pressure and blood cholesterol in humans (Degirolamo and Rudel, 2010), linoleic acid (C18:2); responsible for the lipid component of cell membranes, and palmitic acid (C16); responsible for lung secretions and generating energy (Mancini et al., 2019), which were consistent with the results from other studies (Ravzanaadii et al., 2012). Although TM larvae cannot meet human requirements of total FAs, they can provide a good amount depending on the substrate used and environment (Errico et al. 2022b).

5. Conclusion

We demonstrated the possibility of rearing TM larvae for use as food or feed on alternative and cheaper food substrates other than the traditionally used wheat bran. We have shown that, although nutrient contents vary based on rearing substrates, they are consistent when used to rear more than one generation of TM larvae. Thus, further demonstrating the use of TM larvae for biotransformation of plant materials into other usable products or energy sources. Future studies should adapt the use of different combinations/mixture of biowastes as food for

improved TM larvae production and biomass quality for use in food and or pharmaceutical industries.

CRedit authorship contribution statement

Keandra Langston: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Lethogonolo Selaledi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Chrysantus Tanga:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis. **Abdullahi Yusuf:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Ethical statement- Studies in humans and animals

The authors declare that the research presented does not involve any animal or human study.

Funding

This research was funded in parts through the South African National Research Foundation (NRF) Incentive Funding for Rated Researchers (Grant no. 109380), NRF Research Development Grant for Y- Rated Researchers' (Grant no. 116347) and the PI grant from the South African Research Chair in Mathematical Models and Methods in Bio-engineering and Biosciences (SARChI M³B²) at the University of Pretoria.

Acknowledgements

We thank Ms Kitty Stamhuis, Joseph Mabogwane and Estme Msiza for their assistance in maintaining the stock colonies of *T. molitor*. We thank Prof Robin Crewe for his fruitful discussion and assistance with language editing.

Ethics statement

The studies does not involve humans and animals. It was on invertebrates and no ethical approval is required.

References

- Alves, A.V., Sanjinez-Argandoña, E.J., Linzmeier, A.M., Cardoso, C.A.L., Macedo, M.L.R., 2016. Food value of mealworm grown on *Acrocomia aculeata* pulp flour. *PLoS. One* 11 (3), e0151275. <https://doi.org/10.1371/journal.pone.0151275>.
- Anankware, J.P., Royal, O.O., Obeng-Ofori, D., 2019. Econometrics of domestication of the African palm weevil (*Rhynchophorus phoenicis* F.) production as small-scale business in Ghana. *Edible Insects. IntechOpen*. <https://doi.org/10.5772/intechopen.90259>.
- Anankware, P.J., Fening, K.O., Osekere, E., Obeng-Ofori, D., 2015. Insects as food and feed: a review. *Int. J. of Agric Res Rev.* 3, 143–151.
- AOAC, 2006. Official methods of Analysis of the Association of Official Analytical Chemists (Vol. 2). Association of Official Analytical Chemists.
- AOCS, 2005. Crude fibre analysis in feed by filter bag technique. American oil chemist society approved procedure Ba 6a-05. Official Methods and Recommended Practices of the AOCS, The American Oil Chemists' Society, Champaign, IL.

- Arrese, E.L., Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. *Annu. Rev. of Entomol.* 55, 207–225. <https://doi.org/10.1146/annurev-ento-112408-085356>.
- Banjo, A.D., Lawal, O.A., Songonuga, E.A., 2006. The nutritional value of fourteen species of edible insects in southwestern Nigeria. *Afr. J. of Biotech.* 5 (3), 298–301. <https://doi.org/10.5897/AJB05.250>.
- Belluco, S., Mantovani, A., Ricci, A., Halloran, A., Flore, R., Vantomme, P., 2018. Edible insects in a food safety perspective. In: Roos, N. (Ed.), *Edible Insects in Sustainable Food Systems*. Springer, Cham. https://doi.org/10.1007/978-3-319-74011-9_7.
- Boulos, S., Tännler, A., Nyström, L., 2020. Nitrogen-to-protein conversion factors for edible insects on the Swiss market: *T. molitor*, *A. domesticus*, and *L. migratoria*. *Front. in Nutr* 7, 89. <https://doi.org/10.3389/fnut.2020.00089>.
- Bukkens, S.G.F., 1997. The nutritional value of edible insects. *Ecol. of Food & Nutr.* 36 (2–4), 287–319. <https://doi.org/10.1080/03670244.1997.9991521>.
- Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Smith, R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. *J. Ins. Food and Feed* 1 (1), 7–16.
- DeFoliart, G.R., 1999. Insects as food: why the Western Attitude Is Important. *Annu. Rev. of Entomol.* 44 (1), 21–50. <https://doi.org/10.1146/annurev.ento.44.1.21>.
- Degriolamo, C., Rudel, L.L., 2010. Dietary monounsaturated fatty acids appear not to provide cardioprotection. *Curr. Atheroscler. Rep.* 12 (6), 391–396.
- Devi, W.D., Bonsyana, R., Kapesa, K., Mukherjee, P.K., Rajashekar, Y., 2023. Edible insects: as traditional medicine for human wellness. *Future Foods.*, 100219
- Errico, S., Spagnoletta, A., Verardi, A., Moliterni, S., Dimatteo, S., Sangiorgio, P., 2022a. *Tenebrio molitor* as a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects. *Comp. Rev. F. Sci & F. Saf.* 21, 148–197. <https://doi.org/10.1111/1541-4337.12863>.
- Errico, S., Spagnoletta, A., Moliterni, S., Dimatteo, S., Verardi, A., Sangiorgio, P., Baldacchino, F., Balducchi, R., 2022b. *Tenebrio molitor* as a new promising alternative in the production of feed and food. *Env. E. Mgt. J.* 21, 1657–1671. <https://doi.org/10.30638/eemj.2022.14>.
- Elemo, B.O., Elemo, G.N., Makinde, M.A., Erukainure, O.L., 2011. Chemical evaluation of African palm weevil, *Rhynchophorus phoenicis*, larvae as a food source. *J. of Ins. Sci.* 11 (1) <https://doi.org/10.1673/031.011.14601>.
- Dobermann, D., Swift, J.A., Field, L.M., 2017. Opportunities and hurdles of edible insects for food and feed. *Nutr. Bull.* 42 (4), 293–308.
- EFSA NDA, 2021. European food safety association panel on nutrition, novel foods and food allergens, scientific opinion on the safety of dried yellow mealworm (*Tenebrio Molitor* Larva) as a novel food pursuant to regulation (EU) 2015/2283. *EFSA Journal* 19 (1), 6343. <https://doi.org/10.2903/j.efsa.2021.6343>.
- Fernandez-Cassi, X., Supeanu, A., Vaga, M., Jansson, A., Boqvist, S., Vagsholm, I., 2019. The house cricket (*Acheta Domesticus*) as a novel food: a risk profile. *J. Ins. Food Feed.* 5 (2), 137–157. <https://doi.org/10.2903/j.efsa.2018.e16082>.
- Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo. Biol.* 21 (3), 269–285. <https://doi.org/10.1002/zoo.10031>.
- Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. of Biol. Chem.* 226, 497–509. [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5).
- Fritz, J., Walia, C., Elkadri, A., Pipkorn, R., Dunn, R.K., Sieracki, R., Goday, P.S., Cabrera, J.M., 2019. A systematic review of micronutrient deficiencies in pediatric inflammatory bowel disease. *Inflam. Bowel Dis.* 25 (3), 445–459. <https://doi.org/10.1093/ibd/izy271>.
- Grau, T., Vilcinskis, A., Joop, G., 2017. Sustainable farming of the mealworm *Tenebrio molitor* for the production of food and feed. *Zeitschrift für Naturforschung C* 72 (9–10), 337–349. <https://doi.org/10.1515/znc-2017-0033>, 2017c.
- Hong, J., Han, T., 2020. Mealworm (*Tenebrio molitor* Larvae) as an alternative protein source for monogastric animal: a review. *Animals* 10 (11), 2068. <https://doi.org/10.3390/ani10112068>.
- Jajić, I., Popović, A., Urošević, M., Krstović, S., Petrović, M., Guljaš, D., 2019. Chemical composition of mealworm larvae (*Tenebrio molitor*) reared in Serbia. *Contemp. Agric.* 68 (1–2), 23–27. <https://doi.org/10.2478/contagri-2019-0005>.
- Janssen, R.H., Vincken, J.P., van den Broek, L.A., Fogliano, V., Lakemond, C.M., 2017. Nitrogen-to-protein conversion factors for three edible insects: *tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *J. Agric. Food. Chem.* 22, 2275–2278. <https://doi.org/10.1021/acs.jafc.7b00471>.
- Kinyuru, J.N., Mogendi, J.B., Riwa, C.A., Ndung'u, N.W., 2015. Edible insects—A novel source of essential nutrients for human diet: learning from traditional knowledge. *Animal. Front.* 5 (2), 14–19.
- Ladrón de Guevara, O., Padilla, P., García, L., Pino, J.M., Ramos-Elorduy, J., 1995. Amino acid determination in some edible Mexican insects. *Amino Acids.* 9 (2), 161–173. <https://doi.org/10.1007/BF00805837>.
- Langston, K.L., Selaledi, T., Yusuf, A.A., 2023. Evaluation of alternative substrates for rearing the yellow mealworm *Tenebrio molitor* (L). *Inter. J. Trop. Ins. Sci.* 43, 1523–1530. <https://doi.org/10.1016/s42690-023-01061-z>.
- Longvah, T., Mangthya, K., Ramulu, P., 2011. Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricini*) prepupae and pupae. *Food Chem.* 128 (2), 400–403. <https://doi.org/10.1016/j.foodchem.2011.03.041>.
- Mancini, S., Paci, G., Ciardelli, V., Turchi, B., Pedonese, F., Fratini, F., 2019. *Listeria monocytogenes* contamination of *Tenebrio molitor* larvae rearing substrate: preliminary evaluations. *Food Micro* 83, 104–108. <https://doi.org/10.1016/j.fm.2019.05.006>.
- Mancini, S., Fratini, F., Tuccinardi, T., Degl'Innocenti, C., Paci, G., 2020. *Tenebrio molitor* reared on different substrates: is it gluten free? *Food Cont* 110, 107014. <https://doi.org/10.1016/j.foodcont.2019.107014>.

- Melo-Ruíz, V., Moreno-Bonnet, C., Sanchez, K., Díaz-García, R., Gazga-Urrioste, C., 2016. Macronutrient composition of giant water bug (*Lethocerus* sp.) edible insect in Mexico and Thailand. *J. Agric. Sci. Techn. A* 6 (5). <https://doi.org/10.17265/2161-6256/2016.05.007>.
- Meyer-Rochow, V.B., 2005. Traditional food insects and spiders in several ethnic groups of Northeast India, Papua New Guinea, Australia, and New Zealand. *Ecological Implications of Mini Livestock-Potential of Insects, Rodents, Frog and Snail*. USA. Sci. Publishers Enfiel 389–414.
- Musso, C.G., 2009. Magnesium metabolism in health and disease. *Int. Urol. Nephrol.* 41, 357–362. <https://doi.org/10.1007/s11255-009-9548-7>.
- Nakagaki, B.J., Defoliart, G.R., 1991. Comparison of diets for mass-rearing *Acheta domestica* (Orthoptera: gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. *J. Econ. Entomol.* 84 (3), 891–896. <https://doi.org/10.1093/jee/84.3.891>.
- NHI 2018. National Institutes of Health, Office of Dietary supplements Nutrient Recommendation and Databases. <https://ods.od.nih.gov/HealthInformation/nutrientrecommendations.aspx>.
- Niassy, S., Ekesi, S., Hendriks, S.L., Haller-Barker, A., 2018. Legislation for the use of insects as food and feed in the South African context. *Edible Insects Sustain. Food Syst.* 457–470. https://doi.org/10.1007/978-3-319-74011-9_29.
- Ooninx, D.G.A.B., de Boer, I.J.M., 2012. Environmental impact of the production of mealworms as a protein source for humans – A life cycle assessment. *PLoS. One* 7 (12), e51145. <https://doi.org/10.1371/journal.pone.0051145>.
- Ooninx, D.G.A.B., van Broekhoven, S., van Huis, A., van Loon, J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS. One* 10 (12). <https://doi.org/10.1371/journal.pone.0222043>.
- Ooninx, D.G.A.B., van Itterbeeck, J., Heetkamp, M.J.W., van Den Brand, H., van Loon, J. J.A., van Huis, A., 2010. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS. One* 5 (12). <https://doi.org/10.1371/journal.pone.0014445>.
- O'Fallon, J.V., Busboom, J.R., Nelson, M.L., Gaskins, C.T., 2007. A direct method for FA methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *J. Anim. Sci.* 85, 1511–1521. <https://doi.org/10.2527/jas.2006-491>.
- Orkus, A., 2021. Edible Insects versus Meat—Nutritional Comparison: knowledge of Their Composition Is the Key to Good Health. *Nutrients.* 13, 1207. <https://doi.org/10.3390/nu13041207>.
- Petrescu-Mag, R.M., Rastegari Kopaei, H., Petrescu, D.C., 2022. Consumers' acceptance of the first novel insect food approved in the European Union: predictors of yellow mealworm chips consumption. *Food Sci. Nutr.*, 10 (3), 846–862. <https://doi.org/10.1002/fsn3.2716>.
- Ramaswamy, S.B., 2015. Setting the table for a hotter, flatter, more crowded earth: insects on the menu? *J. Ins. Food Feed* 1 (3), 171–178. <https://doi.org/10.3920/JIFF2015.0032>.
- Ramos-Elorduy, B.J., 1997. The importance of edible insects in the nutrition and economy of people of the rural areas of Mexico. *Ecol. Food Nutri* 36 (5), 347–366. <https://doi.org/10.1080/03670244.1997.9991524>.
- Ravzanaadi, N., Kim, S.H., Choi, W.H., Hong, S.J., Kim, N.J., 2012. Nutritional value of mealworm, *Tenebrio molitor* as food source. *Int. J. Ind. Entomol.* 25 (1), 93–98. <https://doi.org/10.7852/ijie.2012.25.1.093>.
- Rempel, J., Grover, K., El-Matary, W., 2021. Micronutrient deficiencies and anemia in children with inflammatory bowel disease. *Nutrients.* 13 (1), 236. <https://doi.org/10.3390/nu13010236>.
- RStudio Team (2020) RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL <http://www.rstudio.com/>.
- Rumbos, C.L., Karapanagiotidis, I.T., Mente, E., Psafakis, P., Athanassiou, C.G., 2020. Evaluation of various commodities for the development of the yellow mealworm, *Tenebrio molitor*. *Sci. Rep.* 10, 11224. <https://doi.org/10.1038/s41598-020-67363-1>.
- Rumpold, B.A., Schlüter, O.K., 2013. Nutritional composition and safety aspects of edible insects. *Mol. Nutri. Food Res.* 57 (5), 802–823. <https://doi.org/10.1038/s41598-020-67363-1>.
- Tanga, C.M., Ekesi, S., 2024. Dietary and therapeutic benefits of edible insects: a Global perspective. *Annu. Rev. Entomol* 69, 303–331.
- Usman, H.S., Yusuf, A.A., 2021. Legislation and legal frame work for sustainable edible insects use in Nigeria. *Int. J. Trop. Ins. Sci.* 41 (3), 2201–2209. <https://doi.org/10.1007/s42690-020-00291-9>.
- van Broekhoven, S., Ooninx, D.G.A.B., van Huis, A., van Loon, J.J.A., 2015. Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: tenebrionidae) on diets composed of organic by-products. *J. Insec. Physiol.* 73, 1–10. <https://doi.org/10.1016/j.jinsphys.2014.12.005>.
- van Huis, A., 2020. Importance of insects as food in Africa. African edible insects as alternative source of food. *Oil, Protein Bioact. Components* 1–17. https://doi.org/10.1007/978-3-030-32952-5_1.
- van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., Vantomme, P., 2013. Edible insects: Future prospects for food and feed security. *Food and Agriculture Organization of the United Nations*, p. 171.
- Webb, E.C., Casey, N.H., Bosman, M.J.C., 1999. Dietary influences on lutein pigments and carcass fat quality in wethers of different maturity types. *S. Afr. J. Anim. Sci.* 29, 83–91. <https://doi.org/10.4314/sajas.v29i2.44216>.
- Webster, L., Walsham, P., Yusuf, A., Richards, S., Hay, S., Heath, M., Moffat, C.F., 2006. Development of an analytical method for the determination of storage lipids, FAs and fatty alcohols in *Calanus finmarchicus*. *J. Sep. Sci.* 29, 1205–1216. <https://doi.org/10.1002/jssc.200500378>.
- Xiaoming, C., Ying, F., Hong, Z., Zhiyong, C., 2010. Review of the nutritive value of edible insects. *Humans Bite Back* 85.