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Chemical Composition of Oils and Fats from Madagascar Cockroach (*G. portentosa*), Giant Cockroach (*B. giganteus*), and Mealworm (*T. molitor*)

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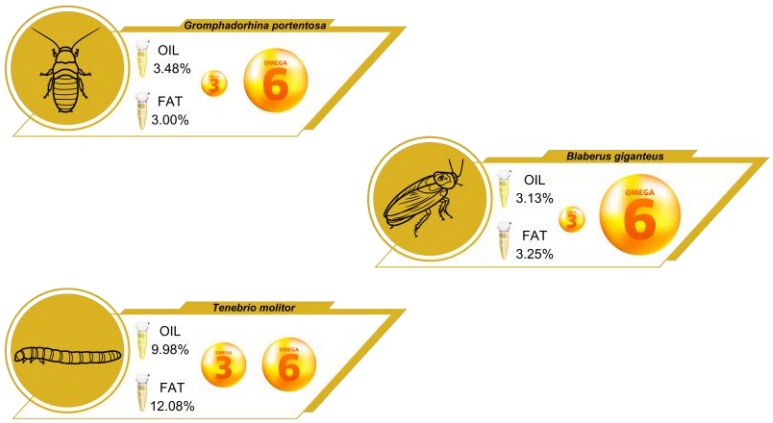
This study aimed to extract oil and fat from three insect species: *Gromphadorhina portentosa*, *Blaberus giganteus*, and *Tenebrio molitor*, analyzing its fatty acid composition and antimicrobial potential. Oils and fats were extracted from each species. Subsequent analysis focused on their fatty acid profiles, particularly examining the ratios of monounsaturated to polyunsaturated fatty acids and omega-6 to omega-3 fatty acids, including the ratios of monounsaturated to polyunsaturated fatty acids and omega-6 to omega-3 fatty acids. The broth microdilution method evaluated the antimicrobial potential against standard American Type Culture Collection (ATCC) strains. *T. molitor* yielded the highest extraction (21.98%), with major fatty acids identified as C18:1, C18:2w6, and C16:1. The oils from *G. portentosa* and *B. giganteus* also contained C18:1, C18:2w6, and C16:0. Principal component analysis distinguished *T. molitor* oil by its C15:0 and C23:0 content. All oils were rich in monounsaturated fatty acids, with *T. molitor* and *B. giganteus* showing higher levels of polyunsaturated fatty acids. Although no significant antimicrobial activity was observed, the oils exhibited high nutritional potential. Further research is needed to optimize cultivation and extraction processes for *G. portentosa* and *B. giganteus* to enhance their economic viability.

Graphical abstract



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1. Introduction

It is estimated that around 2 billion people in more than 100 countries consume insects, a practice known as entomophagy [1]. The Madagascar hissing cockroach (*Gromphadorhina portentosa*) is notable in the literature for its applications in food development. In Brazil, this species is used as a supplement in the diet of amphibians, birds, mammals, fish, and reptiles due to its high protein content [2]. Native to the island of Madagascar, off the east coast of Africa, *G. portentosa* belongs to the order Blattodea, has nocturnal habits, and displays a reddish coloration on its abdomen and black on the thoracic and head regions. Its exoskeleton is formed by a chitinous carapace [2]. However, despite its use in animal feed, studies on the bioactivity of this species still need to be completed in the literature.

Another species in Blattodea is *Blaberus giganteus*, the giant cockroach. It can grow up to 15 centimeters long, making it one of the largest cockroach species. This species is found in Central and South America [3]. Regarding morphology, *B. giganteus* has a black head with distinct spots and a pale-yellow clypeus. Its abdomen features long genitalia, with the left phallomere composed of two narrow plates that touch [4]. There needs to be more information in the literature regarding the nutritional and biological potential of this species, as well as in *G. portentosa*.

The *Tenebrio molitor*, belonging to the order Coleoptera and the family Tenebrionidae, is a species whose life cycle is divided into four stages: egg, larva, pupa, and adult, with a duration of up to five months [5]. Due to its preference for dry and warm environments, *T. molitor* consumes large amounts of grains and stored products, causing significant losses through deterioration [6]. The larval stage of this species is widely used as a non-conventional model for studies of infection and toxicity of drugs and extracts [7, 8]. Additionally, there is growing interest in the inclusion of *T. molitor* larvae in fish and poultry diets, as flour derived from the processing of these larvae contains significant amounts of proteins, fibers, and lipids, and is widely used for the development of new food products [9].

In addition to their nutritional value, insects also have a significant fat content, which plays a crucial role in their metabolism. Fat serves as an energy reserve, participates in vitellogenesis, regulates essential hormonal processes, and contributes to the synthesis of antimicrobial peptides [10]. This richness in bioactive compounds found in cockroaches and other species can be explored in new food and medicinal applications studies. When extracted and maintained in liquid form at room temperature, this substance is called oil; when solid, it is considered fat [11].

Therefore, utilizing these insects to extract fats and other bioactive compounds can open new research and innovation perspectives in various fields. Thus, the present study aimed to extract oil and fat from *G. portentosa*, *B. giganteus*, and *T. molitor* and analyze the fatty acid composition and their antimicrobial potential against bacteria and yeasts.

2. Results and Discussion

T. molitor exhibits higher fat and oil extraction yields than *B. portentosa* and *B. giganteus* (Fig. 1). The total yields of fat and oil from *T. molitor* amount to 21.98%, lower than reported in the literature, ranging from 22.90% to 43.47% [12-18]. This result may be attributed to the diet of the individuals used [13], the extraction method, and the solvents employed in the extraction process [19]. The yield of *T. molitor* appears more promising from a commercial perspective. However, this is the first study on the extraction of fat and oil from *B. portentosa* and *B. giganteus*. The results highlight a promising interest in the samples and, thus, the need for information regarding the optimization of rearing, extraction, and properties.

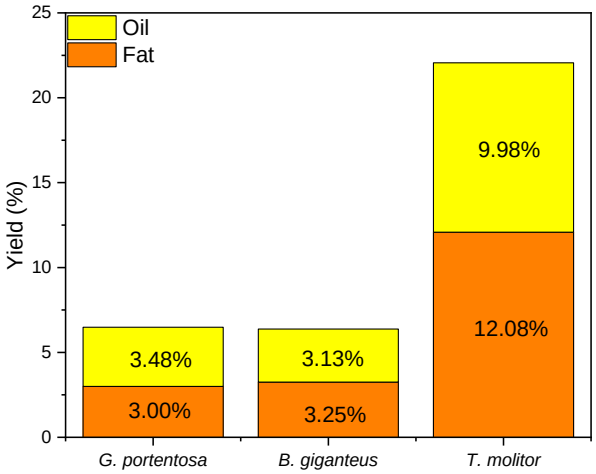

Fig. 1. Yield of oils and fats from *G. portentosa*, *B. giganteus*, and *T. molitor*. Font: Author (2024).

The presence of oils and fats in insects makes them interesting for food use, as discussed by Wee-Lek et al. [20]. The samples exhibited distinct compositions depending on the insect species and the type of lipid material. The oil from *G. portentosa* (GpO) and *B. giganteus* (BgO) showed oleic acid (C18:1), linoleic acid (C18:2), and palmitic acid (C16:0) as the major compounds. In contrast, the oil from *T. molitor* (TmO) contains C18:1, C18:2, and palmitoleic acid (C16:1). The fats from *G. portentosa* (GpF) and *B. giganteus* (BgF) present C16:0, stearic acid (C18:0), and myristic acid (C14:0) as the major compounds, whereas the fat from *T. molitor* (TmF) has C18:0, C18:1, and C16:0 as the major components (Table 1). This is the first report in the literature on the chemical composition of oils and fats from *G. portentosa* and *B. giganteus*.

The previous study of Kinsella [21] had described the fatty acid ester composition of the oil of *G. portentosa*, relating the C18:1, C16:0, and C18:0 as major compounds for males and C18:1, C16:0, and C16:1 as major compound for females. The difference in composition obtained may be a consequence of differences in insect breeding [22].

The PCA plot (Fig. 2) illustrates that Dim1 (51%) and Dim2 (22.9%) capture the primary variance among samples, distinguishing oils from fats based on their saturated and unsaturated compound profiles. The principal component analysis (PCA) separated samples into oils and fats, with saturated compounds associated with fats and unsaturated ones associated with oils (Fig. 2). The different oils exhibited a smaller Euclidean distance among themselves compared to the fat samples. TmF was the sample that differed the most due to the elevated content of pentadecanoic acid (C15:0) compared to the other samples and because it was the only

sample that contained tricosanoic acid (C23:0); however, TmO also exhibited a greater distance from the other oils. Thus, the oils and fats from *T. molitor* differ from those of *B. giganteus* and *G. portentosa*.

Table 1. Composition of fatty acids in oils and fats from *G. portentosa*, *B. giganteus*, and *T. molitor*.

Fatty Acids Esters		GpO	GpF	TmO	TmF	BgO	BgF
C12:0	Lauric Acid	0.10 ± 0.01	0.41 ± 0.04	-	0.11 ± 0.01	0.11 ± 0.01	0.41 ± 0.04
C14:0	Myristic acid	2.48 ± 0.21	**11.46 ± 1.12**	0.51 ± 0.04	7.44 ± 0.06	1.47 ± 0.09	**10.43 ± 0.08**
C14:1	Myristoleic acid	0.86 ± 0.07	0.11 ± 0.01	0.43 ± 0.41	-	0.89 ± 0.07	0.83 ± 0.09
C15:0	Pentadecanoic acid	0.39 ± 0.2	0.19 ± 0.01	0.19 ± 0.01	1.72 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
C16:0	Palmitic acid	**10.17 ± 0.09**	**32.34 ± 2.95**	7.58 ± 0.05	**35.87 ± 3.15**	**11.34 ± 0.01**	**35.76 ± 3.17**
C16:1	Palmitoleic acid	7.50 ± 0.68	0.57 ± 0.05	**10.53 ± 0.09**	0.52 ± 0.04	1.75 ± 0.10	1.62 ± 0.14
C17:0	Margaric acid	2.44 ± 0.23	0.42 ± 0.03	0.12 ± 0.01	0.41 ± 0.03	0.42 ± 0.03	0.47 ± 0.04
C18:0	Stearic Acid	7.21 ± 0.69	**27.35 ± 2.36**	3.35 ± 0.31	**27.99 ± 2.00**	7.75 ± 0.05	**31.72 ± 2.86**
C18:1	Oleic Acid	**44.72 ± 4.18**	7.57 ± 0.68	**45.55 ± 3.52**	**10.34 ± 0.10**	**41.53 ± 0.38**	7.41 ± 0.59
C18:2w6	Linoleic acid	**15.55 ± 1.32**	10.57 ± 0.09	**13.45 ± 0.08**	7.03 ± 0.07	**27.71 ± 2.15**	4.78 ± 0.33
C18:3w3	Linolenic acid	0.18 ± 0.01	0.17 ± 0.01	10.18 ± 0.09	0.73 ± 0.06	0.18 ± 0.01	0.18 ± 0.01
C20:0	Arachidic acid	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.21 ± 0.01
C20:2w3	Eicosadienoic acid	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	-	0.11 ± 0.01	0.10 ± 0.01
C20:4	Arachidonic acid	0.19 ± 0.01	0.11 ± 0.01	0.19 ± 0.01	-	0.12 ± 0.01	0.11 ± 0.01
C21:0	Heneicosanoic acid	-	-	-	0.31 ± 0.2	-	0.23 ± 0.01
C22:2	Docosadienoic acid	0.77 ± 0.06	0.80 ± 0.07	0.18±0.01	-	0.55 ± 0.04	-
C23:0	Tricosanoic acid	-	-	-	0.12 ± 0.01	-	-
Identified		92.78	92.3	92.48	92.7	94.23	94.46
Others		7.22	7.7	7.52	7.3	5.77	5.54
MUFA/PUFA ratio		3.16 ± 0.30	0.70 ± 0.06	2.35 ± 0.19	1.40 ± 0.10	1.54 ± 0.14	1.91 ± 0.20
w-6/w-3 ratio		86.39 ± 8.36	62.18 ± 5.69	1.32 ± 0.12	9.63 ± 0.86	153.94 ± 13.56	26.56 ± 2.45
CL		16.21 ± 1.43	15.50 ± 1.30	16.26 ± 1.02	15.62 ± 1.02	16.62 ± 1.43	15.78 ± 1.43
DB		0.87 ± 0.07	0.32 ± 0.02	1.15 ± 0.10	0.27 ± 0.02	1.02 ± 0.10	0.21 ± 0.02

GpO = *G. portentosa* oil; GpF = *G. portentosa* fat; TmO = *T. molitor* oil; TmF = *T. molitor* fat; BgO = *B. giganteus* oil; BgF = *B. giganteus* fat; - = Not identified. In bold are the three major compounds of each sample. SFA = Saturated compounds; MUFA = Monounsaturated compounds; PUFA = Polyunsaturated compounds; CL = Weighted average of chain length; DB = Weighted average of double bonds.

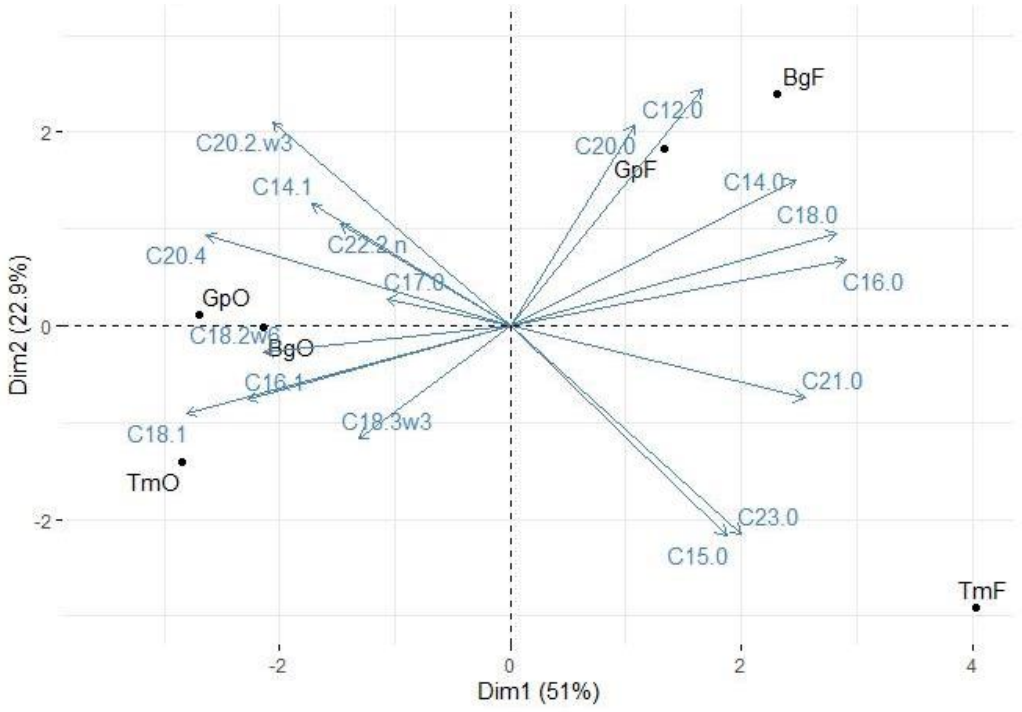

Fig. 2. Principal Component Analysis (PCA) of the composition of oils and fats from G. portentosa, B. giganteus, and T. molitor. GpO = G. portentosa oil; GpF = G. portentosa fat; TmO = T. molitor oil; TmF = T. molitor fat; BgO = B. giganteus oil; BgF = B. giganteus fat. Font: Author (2024).

Fats exhibit higher saturated fatty acids (SFA) (Fig. 3). All oils predominantly contain monounsaturated fatty acids (MUFA). TmO and BgO showed higher levels of polyunsaturated fatty acids (PUFA) compared to SFA; however, this is not the case for GpO.

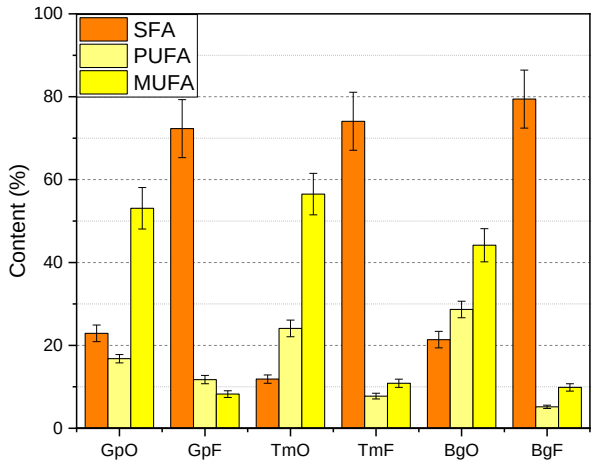

Fig. 3. Distribution of the unsaturation profile of the oils and fats from *G. portentosa*, *B. giganteus*, and *T. molitor*. SFA = Saturated fatty acid; PUFA = Polyunsaturated fatty acids; MUFA = Monounsaturated fatty acids. GpO = *G. portentosa* oil; GpF = *G. portentosa* fat; TmO = *T. molitor* oil; TmF = *T. molitor* fat; BgO = *B. giganteus* oil; BgF = *B. giganteus* fat.

There are reports in the literature of 20.37% [23], reaching up to 35.05% [24] of saturated fatty acids (SFA) for *T. molitor*. However, these values are higher than those obtained for the analyzed oil (Fig. 2). Sosa and Fogliano [11] utilized the difference in the melting point of the fats and oil from *T. molitor* for oil isolation, obtaining SFA between 21.05% and 21.85%. In comparison, the values for monounsaturated fatty acids (MUFA) ranged from 40.70% to 41.73%, and for polyunsaturated fatty acids (PUFA) between 32.45% and 32.96%, representing proportions different from those obtained in the present study (Fig. 3).

The fatty material from the Black Soldier Fly larva (*Hermetia illucens*) exhibits SFA levels between 37.7% and 72.1% [25], which are lower than those obtained for GpO, TmO, and BgO (Fig. 3). Using a centrifuge to separate the oil from the fat aided in concentrating SFA in the fatty phase. Nevertheless, the oils from the other analyzed species show higher SFA levels, with values close to those reported in the literature [23, 24] for *T. molitor* (Fig. 3).

GpO obtained the highest MUFA/PUFA ratio and the lowest average number of double bonds (DB), indicating a possible thermal stability superior to that of the other oils. The best values regarding fats in these parameters were for GpF. The oils exhibit medium carbon chain lengths (CL) and a higher MLD and MUFA/PUFA ratio compared to the fats; however, the omega-6/omega-3 ratio does not show a clear relationship (Table 1).

Kolobe et al. [26] demonstrate that oils and fats from insects generally have a predominance of chain lengths between 16 and 18 carbons. Thus, the oils studied exhibit a profile similar to that in the literature; however, the oils have, on average, shorter chains. The level of unsaturation is associated with the stability of oils and fats, as double bonds are more susceptible to oxidation [27]. In this regard, the MUFA/PUFA ratio helps to understand the oxidative tendency of these products [28]. Furthermore, the National Health Surveillance Agency (ANVISA) recommends a balanced diet in MUFA and PUFA, with a ratio close to 1:1. In this sense, GpF and TmF are close to the recommendation. It is also worth highlighting the MUFA/PUFA result of BgO, as it is a sample richer in unsaturated compounds than the oils while presenting a good balance between MUFA and PUFA.

The samples of oils and fats showed relevant levels of omega-6 (C18:2w6) and low levels of omega-3 (C18:3w3), except for TmO, which showed levels close to these two substances (Table 1). This is reflected in the omega-3 to omega-6 ratio (w-3/w-6 ratio) (Table 2). It is also possible to ascertain that omega-3, C18:1, and C16:1 levels are responsible for a smaller Euclidean distance of this sample with the other oils in the PCA (Fig. 2). Omega-3 is essential for human health due to its inverse relationship with cardiovascular diseases, rheumatoid arthritis, and asthma [29].

Omega-3 and Omega6 are associated with different functions in the human body, like maintaining homeostasis of cell membranes, brain functions, and blood plasma oxygenation [30]. Simpoulos [31] describes how human diets have become poorer in omega-3 throughout societal evolution, changing from a w-3/w-6 ratio of 0.79 in the Paleolithic to values between 4 and 16.74 today, depending on the society analyzed. Omega-3 and Omega-6 compete for enzymes involved in reactions, desaturation, and chain elongation. Therefore, the balance between these compounds is essential [30]. Thus, the consumption of TmO stands out due to its balanced availability of omega-6 and omega-3.

One possible application of the analyzed fats is substituting butter in the production of food products. Delicato et al. [32] demonstrated in their study that it is possible to substitute up to 25% of butter in baked goods with fat from *Hermetia illucens* without affecting the acceptability of the products. The oils and fats can also be applied in animal nutrition [10]; however, they are more suitable due to their lower SFA content.

All evaluated samples did not show antimicrobial potential, with MICs> 1000 µg/mL for bacteria and yeasts (Table 2). However, the scientific literature demonstrates a growing interest in new natural alternatives that can act against pathogenic bacteria and yeasts.

Table 2. Minimum inhibitory concentration (µg mL^{-1}) of oil and fat from *G. portentosa*, *T. molitor*, and *B. giganteus*.

Microorganism	GpO	GpF	TmO	TmF	BgO	BgF	AMP	FLU
Bacillus cereus	>1000	>1000	>1000	>1000	>1000	>1000	32	-
Staphylococcus aureus	>1000	>1000	>1000	>1000	>1000	>1000	0.06	-
Salmonella Typhimurium	>1000	>1000	>1000	>1000	>1000	>1000	1	-
Klebsiella pneumoniae	>1000	>1000	>1000	>1000	>1000	>1000	>128	-
Candida albicans	>1000	>1000	>1000	>1000	>1000	>1000	-	0.125
Candida glabrata	>1000	>1000	>1000	>1000	>1000	>1000	-	8
Candida krusei	>1000	>1000	>1000	>1000	>1000	>1000	-	>64
Candida tropicalis	>1000	>1000	>1000	>1000	>1000	>1000	-	1

GpO = *G. portentosa* oil; GpF = *G. portentosa* fat; TmO = *T. molitor* oil; TmF = *T. molitor* fat; BgO = *B. giganteus* oil; BgF = *B. giganteus* fat; AMP = Ampicillin; FLU = Fluconazole

However, there is a trend that values biological actions of a narrow spectrum, in which the sample in question exhibits bioactivity without causing adverse impacts in the context in which it is used [33]. Some microorganisms, classified as pathobionts, can be commensals in the human intestinal microbiota without causing disease; however, in situations of imbalance, they can become opportunistic. The ingestion of antimicrobials can affect the intestinal microbiota, leading to symptoms such as abdominal discomfort and diarrhea. In some cases, it may also contribute to the development of antimicrobial resistance [34]. Food additives are also associated with the imbalance of the intestinal microbiota. In contrast, there are reports in the literature about the positive impact of omega-3 on the improvement of the composition of intestinal microorganisms [35].

In this sense, the oils and fats from *G. portentosa*, *T. molitor*, and *B. giganteus* can be evaluated for their nutritional potential for application in foods or in the development of new products, suggesting that they may not cause a direct impact on the intestinal microbiota, as they did not demonstrate antimicrobial profiles against Gram-positive bacteria, Gram-negative bacteria, or yeasts. Future studies are necessary to evaluate the impact of these insect-derived samples on more specific intestinal microorganisms and their digestibility in association with intestinal enzymes and toxicity.

3. Material and Methods

3.1 Creation and preparation of samples

A breeding colony was established in the Biology laboratory of the Federal Institute of Mato Grosso do Sul at the Coxim-MS campus to obtain the biological material to be studied. Aiming to eliminate potential distortions related to feeding, a breeding protocol was created in which the species *Blaberus giganteus* and *Gromphadorhina portentosa* were provided with unlimited access to water and food. The breeding boxes were constructed from FORTLEV reservoirs of 500 L. The edges of each box were coated with entomological glue to prevent insect escape, and the lid was perforated with a circular drill using a power drill to ensure internal ventilation. Inside each box, a water dispenser with a capacity of 5 L and a feeding tray with a radius of 30 cm and height of 3 cm were placed. From the established breeding colony in the laboratory, 200 individuals (males and females, in equal numbers) from each species were selected and accommodated in separate boxes. This procedure was performed at 8:00 PM, as this is the time when these species begin their foraging activities. The boxes were positioned near glass windows to receive sunlight and a natural photoperiod of 12 hours of light and 12 hours of darkness. Both species were provided with a feeding load of 0.5 kg of commercial balanced feed for quails and 0.5 kg of balanced feed for laying hens every 5 days. Any leftover food was discarded, the trays were cleaned, and a new feeding load was provided. Filtered water was changed every 2 days, and the water dispensers were washed to reduce the growth of microorganisms. The breeding colony remained under this system for four months to establish population development in the new environment. After this period, the individuals were selected for use in experiments.

The breeding of *Tenebrio molitor* for experimental use was conducted following the protocol established in the Biology laboratory. Healthy pupae without visible anomalies were placed in plastic trays measuring 45x30x12 cm, with 300 g in total. Each tray contained 2 kg of feed, with 1 kg for quails and 1 kg for laying hens. After 10 days, the first adults emerged and began feeding on the ration and water provided in a container containing polyacrylate and 20 drops of Vitagold. Once all the pupae had transformed into adults, a superficial cleaning was conducted to remove the shells, and the trays were placed on steel shelves, covered with black cloth due to the photophobia of this species. After 15 days, the adults had mated and deposited eggs in the feed. At this point, a new tray with the same characteristics was prepared, and the adults were transferred to this new box to prevent overcrowding in the previous box. Six boxes were prepared in total. The larvae in the boxes developed and transformed into pupae, which were collected, and new boxes were prepared following the same procedure over 8 months. The larvae from this breeding colony were used in the experiments.

The following steps were carried out based on the adaptation of the methodology proposed by Kröncke et al. [36]. For euthanasia, the insects were subjected to a dehydration regime for 48 hours. After this process, they were euthanized by freezing at -21°C for 48 hours and lyophilized for 72 h at -56°C. After lyophilization, the samples were ground to improve granularity and surface contact with the solvent. In a ratio of 1:10 (w/v), the lyophilized material (10 g) was subjected to contact with Hexane PA (100 mL) under continuous agitation for 3 days. Subsequently, filtration and rotary evaporation were performed to remove the solvent completely. Centrifugation was conducted at 3,500 rpm for 10 minutes to separate the oil from the fat. The samples were stored at 4 ºC [35].

3.2 Determination of Fatty Acid Composition of Oils and Fats

Each sample was solubilized for this analysis and subsequently methylated with 0.25 mol L^{-1} sodium methoxide in diethyl ether–methanol (1:1 v). Analyses were performed using a gas chromatograph with a flame ionization detector (GC-FID) (Focus GC, Thermo Scientific). An SP2560 column measuring 30 m x 0.25 mm x 0.20 µm (Supelco) was utilized with a temperature gradient: 80 ºC, 0 min, increasing at 7 ºC min^{-1} to 240 ºC; injector (split 1:30) at 250 ºC and detector at 260 ºC. Hydrogen was used as the carrier gas (2.0 mL min^{-1}), and the injection volume was 1 µL. Peak identification was conducted by comparing with standards of methylated fatty acids (FAME C8-C24) (Sigma-Aldrich). Quantification was performed by area normalization. The results were utilized to calculate several parameters to assist in understanding the compositional differences among the studied oils and fats. A weighted average of the number of double bonds (DB) (Equation 1) and chain length (CL) (Equation 2) was calculated, according to Correia et al [37].

$$DB = \sum \ (DB_n \times w_i) \quad (1)$$

$$CL = \sum \ (nC_n \times w_i) \quad (2)$$

Where nC_n is the number of carbons in each fatty acid ester, w_i is the weight percentage of each fatty acid ester, and nDB_n is the number of double bonds of each fatty acid ester. Additionally, the ratio of omega-6 to omega-3 (Equation 3) and the ratio of monounsaturated fatty acids (MUFA) to polyunsaturated fatty acids (PUFA) (Equation 4) were determined.

$$ratio of omega6 omega3 = \frac{omega6}{omega3} \quad (3)$$

$$the ratio of MUFA PUFA = \frac{MUFA}{PUFA} \quad (4)$$

3.3 Analysis of Antimicrobial Potential

The bacteria and yeasts used in the antimicrobial activity assay are standard strains from the American Type Culture Collection (ATCC, Rockville, MD, USA). The bacteria included *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Salmonella* Typhimurium (ATCC 14028), and *Klebsiella pneumoniae* (ATCC 13883). The yeasts included *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 2001), *Candida krusei* (ATCC 6558), and *Candida tropicalis* (ATCC 750).

The minimum inhibitory concentration (MIC) of the samples was determined using the broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute M07-A9 [38] for bacteria and M27-A3 [39] for yeasts, with adaptations for natural products. Bacteria and yeasts were suspended in sterile saline solution (0.9% NaCl), and the suspensions were standardized using a spectrophotometer at 625 nm for bacteria and 530 nm for yeasts. The samples were diluted in water with 2% Tween 80 and subjected to successive dilutions (1:2) in 96-well microplates with Mueller-Hinton broth for bacteria and RPMI-1640 for yeasts. Sample concentrations ranged from 0.24 to 1000 μg mL^{-1}.

Negative controls for sterility of the culture medium and extracts were prepared, along with positive controls, to verify the viability of the microorganisms. Ampicillin and fluconazole were used as standard antibiotic and antifungal agents, respectively. The incubation temperatures and periods were set at 37 ºC for 24 h. Visual readings were conducted for bacteria after adding 50 μL of 0.1% triphenyl tetrazolium chloride (TTC), determining the MIC as the concentration where no color change occurred in the wells. Visual readings were also performed for yeasts, and the MIC was defined as the lowest concentration that showed no fungal growth compared to the positive control. The assays were conducted in duplicate at three different times.

3.4 Statistical Analysis

Data processing was performed in the R environment [40]. Initially, Shapiro-Wilk normality tests were conducted to verify that the use of the mean and standard deviation is representative of the results. Principal Component Analysis (PCA) with Euclidean distance was performed using the factoextra package [41] with the data obtained from GC-FID.

4. Conclusions

The oils exhibited larger average chain lengths and higher unsaturation than the fats of the analyzed insects. The oils and fats from *G. portentosa* and *B. giganteus* show greater chemical similarities than those of *T. molitor*. The oil of *B. giganteus* has the best balance of MUFA/PUFA compared to the other oils; however, the oil and fat of *T. molitor* present the best omega-6/omega-3 ratios. The oils and fats did not demonstrate antimicrobial activity, suggesting a lack of impact on the intestinal microbiota. The samples are rich in fatty acids, indicating potential as a source of these substances; however, further studies are necessary to optimize the cultivation and extraction of oils from *G. portentosa* and *B. giganteus* to enhance their economic viability, considering the low yields of oil and fat obtained. Nutritional assessments and toxicity profile evaluations also contribute to understanding the potential of these samples.

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Author Contributions

JVAS = Investigation, Visualization, and Writing - Original Draft; TLAC = Formal analysis, and Writing - Original Draft; CLAC = Investigation, Validation, and Writing - Review & Editing; KMPO = Resources, and Writing - Review & Editing; RSM = Investigation, and Methodology; SAO = Investigation, and Methodology; GST = Investigation, and Methodology; MVP = Investigation, and Formal analysis; MRC = Investigation, and Formal analysis; DCVV = Investigation, and Visualization; GRS = Investigation, and Methodology, and AK = Conceptualization, Supervision, and, Writing - Review & Editing.

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