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PEDRO PAULO LORDELO GUIMARÃES TAVARES

ENRIQUECIMENTO NUTRICIONAL DE LARVAS DE *Zophobas atratus* A PARTIR DE SUBSTRATOS MODIFICADOS COM COPRODUTO DE LINHAÇA

SALVADOR
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Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos (PGAli) da Universidade Federal da Bahia, como requisito parcial para a obtenção do título de Doutor em Ciência de Alimentos.

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A Comissão Julgadora dos trabalhos de defesa de Tese de Doutorado do candidato ***Pedro Paulo Lordelo Guimarães Tavares***, em sessão pública realizada em 30/09/2024.

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Salvador, 30 de setembro de 2024.

Dedico este trabalho,

À minha família, amigos, professores e colegas pesquisadores que participaram desta caminhada.

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“The future has not been written. There is no fate but what we make for ourselves.”

John Connor

RESUMO

Os insetos comestíveis são fontes alternativas de proteínas, lipídios, minerais e vitaminas, representando grande potencial para a indústria alimentícia. A utilização de resíduos agroindustriais, a exemplo da torta de linhaça, como parte de sua dieta, é capaz de auxiliar na transformação de materiais geralmente descartados em biomassa nutricionalmente valiosa. O objetivo do estudo foi avaliar a viabilidade de enriquecimento nutricional das larvas de *Zophobas atratus* Fabricius (Coleoptera: Tenebrionidae) através da substituição de substrato convencional por torta de linhaça. Inicialmente, foi desenvolvido um estudo de revisão para explorar as aplicações emergentes de tecnologias e conhecimentos científicos relacionados ao uso de insetos comestíveis na indústria alimentícia. Utilizou-se o Espacenet como ferramenta de busca, com os termos Insect, Pupa, Larva ou Nimph e os códigos A23L33 e A23V2002. A busca resultou em 1.139 documentos encontrados, dos quais 341 foram considerados relevantes. Para avaliar domínios tecnológicos e agrupamentos de conceitos, foi empregado o software Orbit®. Adicionalmente, uma busca na base de dados Scopus com o termo "edible and insect*" foi realizada para identificar a prevalência de pesquisas na área. Quanto ao estudo experimental, 100 larvas de *Zophobas atratus* foram alocadas em caixas plásticas contendo substrato (Controle e substituição de 25%, 50%, 75% e 100% por torta de linhaça (Tratamentos 0 (Controle), 25, 50, 75 e 100). Após 90 dias de criação, os insetos foram abatidos e avaliados quanto a composição centesimal e valor energético; eficiência de conversão alimentar e taxa de mortalidade; perfil de ácidos graxos e índices de qualidade lipídica; e teor de minerais. Os principais insetos identificados na revisão incluíram bichos-da-seda, abelhas, besouros, larvas de farinha, grilos e cigarras. A tecnologia predominante envolvia isolados proteicos, utilizados como ingredientes em alimentos ou suplementos. Quanto aos resultados do experimento conduzido, a inclusão de até 50% de torta de linhaça no substrato manteve a massa larval e a eficiência de conversão alimentar estatisticamente iguais ao Controle. As larvas 100 apresentaram maior teor proteico (46,68% vs 41,38% - Controle). A concentração de ácido alfa-linolênico aumentou estatisticamente, chegando a até 28,66% (100) em comparação com 1,03% (Controle), auxiliando na modulação da razão ômega-6/ômega-3 para valores mais próximos das recomendações de consumo (de 23,61 no Controle para 0,58 (100)). Os parâmetros de qualidade lipídica apresentaram resultados mais seguros à saúde cardiovascular humana no tratamento 100 (H/H 4,06; HPI 3,68; UI 147,27; COX 8,17; PI 74,61; IA 0,27 e IT 0,21). As larvas podem ser consideradas como ricas em ferro (>2,4 mg/100g - Controle), fósforo (>210 mg/100g), manganês (>0,69 mg/100g), zinco (>3,3 mg/100g) e magnésio (>120 mg/100g – tratamentos Controle e 100); e fonte de magnésio (>60 mg/100g - tratamentos 25, 50 e 75) e ferro (> 1,2 mg/100g - tratamentos 25, 50, 75 e 100). Os resultados do presente estudo sugerem que a criação de larvas de *Zophobas atratus* pode se beneficiar com o uso de torta de linhaça como forma de enriquecimento nutricional, principalmente quanto ao teor proteico e de ácido graxo alfa-linolênico larval.

***Palavras-chave**: Entomofagia. Novo ingrediente. Larva da farinha. Composição nutricional. Modulação lipídica.*

ABSTRACT

Edible insects are alternative sources of proteins, lipids, minerals, and vitamins, presenting significant potential for the food industry. The use of agro-industrial waste, such as linseed cake, in their diet can help convert materials that are typically discarded into nutritionally valuable biomass. The objective of the study was to evaluate the viability of nutritionally enriching *Zophobas atratus* Fabricius (Coleoptera: Tenebrionidae) larvae by substituting their conventional substrate with linseed cake. Initially, a review study was developed to explore the emerging applications of technologies and scientific knowledge related to the use of edible insects in the food industry. Espacenet was used as a search tool with the terms “Insect,” “Pupa,” “Larva,” or “Nymph” and the codes A23L33 and A23V2002. This search yielded 1,139 documents, of which 341 were deemed relevant. The Orbit® software was used to analyze technological domains and concept clusters. Additionally, a search in the Scopus database with the term “edible and insect*” was conducted to identify the prevalence of research in this area. In the experimental study, 100 *Zophobas atratus* larvae were placed in plastic boxes containing different substrates: a control substrate and substrates where 25%, 50%, 75%, and 100% of the conventional substrate were replaced by linseed cake (Treatments 0 (Control), 25, 50, 75, and 100). After 90 days, the insects were harvested and analyzed for their centesimal composition, energy value, feed conversion efficiency, mortality rate, fatty acid profile, lipid quality indices, and mineral content. The review identified silkworms, bees, beetles, mealworms, crickets, and cicadas as the primary insects of interest. The predominant technology involved protein isolates used as ingredients in food or supplements. Experimentally, up to 50% inclusion of linseed cake in the substrate maintained larval mass and feed conversion efficiency statistically comparable to the control. The 100 larvae had higher protein content (46.68% vs. 41.38% in the control). Alpha-linolenic acid concentration increased significantly, reaching 28.66% (100) compared to 1.03% (control), improving the omega-6/omega-3 ratio towards recommended levels (from 23.61 in control to 0.58 in treatment 100). The lipid quality parameters in treatment 100 showed improved results for human cardiovascular health (H/H 4.06; HPI 3.68; UI 147.27; COX 8.17; PI 74.61; AI 0.27; and TI 0.21). The larvae were found to be rich in iron (>2.4 mg/100g in control), phosphorus (>210 mg/100g), manganese (>0.69 mg/100g), zinc (>3.3 mg/100g), and magnesium (>120 mg/100g in control and treatment 100), and a source of magnesium (>60 mg/100g in treatments 25, 50, and 75) and iron (>1.2 mg/100g in treatments 25, 50, 75, and 100). The findings suggest that rearing *Zophobas atratus* larvae with linseed cake can serve as a form of nutritional enrichment, particularly enhancing the larvae's protein content and alpha-linolenic acid levels.

Keywords*: Entomophagy. New ingredient. Mealworm. Nutritional composition. Lipid modulation.*

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LISTA DE ABREVIATURAS E SIGLAS

AFSCA	Agência Federal Belga para a Segurança da Cadeia Alimentar
ALA	Ácidos alfa-linolênico
AOAC	Associaton of Official Analytical Chemists
COX	Índice de Oxidabilidade
DHA	Ácido docosahexaenoico
DRI	Ingestão Dietética de Referência
ECI	Eficiência de Conversão do Alimento Ingerido
EFSA	Autoridade Europeia para a Segurança dos Alimentos
EPA	Ácido eicosapentaenoico
EPO	Escritório Europeu de Patentes
FAO	Organização das Nações Unidas para a Alimentação e a Agricultura
FCR	Razão de Conversão Alimentar
FDA	Food and Drug Administration
H/H	Razão Hipocolesterolêmico/Hipercolesterolêmico
HPI	Índice de Promoção da Saúde
IA ou AI	Índice de Aterogenicidade
IFAD	Fundo Internacional de Desenvolvimento Agrícola
IT ou TI	Índice de Trombogenicidade
MUFA	Ácidos graxos monoinsaturados
NAAS	Instituto Nacional de Ciências Agrárias
ODS	Objetivos do Desenvolvimento Sustentável
OMS ou WHO	Organização Mundial da Saúde
ONU ou UN	Organização das Nações Unidas
PI	Índice de Peroxidabilidade
PUFA	Ácidos graxos poli-insaturados
SEM	Erro Padrão da Média
SFA	Ácidos graxos saturados
EU/EU	União Europeia
UNICEF	Fundo das Nações Unidas para a Infância
WFP	Programa Mundial de Alimentos

SUMÁRIO

1 INTRODUÇÃO

A população mundial apresenta tendência crescente, o que exige um aumento da produção atual de alimentos. A terra é escassa e a área dedicada à agricultura raramente é uma opção viável ou sustentável. As mudanças climáticas e escassez de água podem ter implicações profundas na produção de alimentos. Para enfrentar os desafios alimentares e nutricionais de hoje, há necessidade de desenvolvimento de modelos de produção de alimentos mais sustentáveis (Garcia; Osburn; Jay-Russell, 2020).

Entomofagia é o termo usado para descrever o consumo alimentar de insetos. Os insetos estão incluídos na dieta regular de cerca de 2 bilhões de pessoas, com cerca de 2.000 espécies de insetos comestíveis (Olivadese; Dindo, 2023). Com o crescimento da população humana e exigências nutricionais se tornando uma preocupação crescente, a entomofagia tem sido proposta como uma futura fonte sustentável de alimentos (Olivadese; Dindo, 2023). Os insetos comestíveis são um recurso alimentar natural para muitos grupos étnicos da Ásia, África, México e América do Sul, onde a entomofagia pode ser sustentável e gera benefícios econômicos, nutricionais e ecológicos (Abril *et al*., 2022).

Os insetos podem ser consumidos *in natura*, ou coccionados e em diferentes estágios de desenvolvimento (ovos, larvas, pupas, adultos). Não apenas isso, eles podem também estar presentes em preparações culinárias tradicionalmente consumidas, como biscoitos, bolos e bebidas (Bisconsin-Júnior *et al*., 2022). Uma grande vantagem associada a produção e consumo de insetos é o seu menor impacto ambiental quando comparado com os métodos de produção convencionais da pecuária (Abril *et al*., 2022). Oonincx *et al*. (2010) relatam que a criação de insetos produz menores emissões de gases de efeito estufa, e que as espécies *Tenebrio molitor, Blaptica dubia, Acheta domesticus* e *Locusta migratoria* apresentam produção de metano entre 0,00 e 0,16 g / kg de massa corporal / dia, enquanto o gado de corte 0,239 g / kg de massa corporal / dia. Miglietta *et al*. (2015) também relataram uma pegada hídrica de 4,341m^3 por tonelada de produção de insetos comestíveis, especialmente para larvas-de-farinha, inferior ao registrado para a carne de bovino que exigia 154,115 m^3 por tonelada.

Dentre os insetos comestíveis, podem ser destacadas as larvas-da-farinha, que são amplamente utilizadas como ração animal de répteis, aves e macacos (Van Huis *et al*., 2013). São insetos pertencentes a ordem *Coleoptera* e família *Tenebrionidae,* representados por espécies como *Tenebrio molitor* e *Zophobas atratus* (Cacchiarelli *et al*., 2022). Os insetos desta ordem são amplamente distribuídos devido ao ciclo de vida curto, fácil manuseio e recentemente, por efeitos antrópicos como mudança de habitat e intensa comercialização de alimentos (Siddiqui *et al*., 2024). Além disso, o conteúdo nutricional e a composição de larvas-da-farinha são viáveis para uso como alimento, podendo ser considerados como nutritivos ao serem capazes de fornecer proteínas, gorduras, vitaminas, minerais e fibras (Ordoñez-Araque; Quishpillo-Miranda; Ramos-Guerrero, 2022). No entanto, baixos níveis de ácidos graxos poli-insaturados (PUFA n-3) foram observados nestes insetos (Mattioli *et al*., 2024).

Pesquisas recentes também descobriram que as larvas-da-farinha são capazes de biodegradar plásticos à base de petróleo, incluindo poliestireno e polietileno (Yang *et al*., 2018; Wang *et al*., 2024), sugerindo que estes insetos possuem sistemas digestivos extremamente funcionais. Atualmente, a produção comercial da biomassa de larva-da-farinha utiliza farelo de trigo ou aveia como matéria-prima principal. Por outro lado, por conta de sua capacidade de metabolizar diversos componentes, convém avaliar a viabilidade de substituir as rações tradicionais por alternativas sustentáveis, como coprodutos agroindustriais (Brandon *et al*., 2018).

Dentre os coprodutos da agroindústria, destacam-se as tortas. São assim denominados os materiais obtidos após a prensagem de nozes e sementes para obtenção de óleo. Em 2021 foram produzidas 700.000 toneladas de óleo de linhaça (Yang *et al*., 2023), o que gera, em contrapartida, elevados teores de torta, seu coproduto de menor interesse comercial, já que apenas 30% da composição centesimal da semente de linhaça é composta de lipídios. Isto representa uma grande preocupação do ponto de vista ambiental, considerando que estes produtos devem ser destinados a algum uso. As tortas apresentam alto valor de nutrientes, com teor protéico entre 32,20 e 35,90%, além de elevada concentração de compostos fenólicos e ácidos graxos poli-insaturados, como ômega-3. São comumente utilizadas como ração para animais, como ruminantes e peixes (Rakita *et al*., 2023). Por outro lado, as tortas de prensagem podem apresentar usos alternativos devido ao valor nutricional agregado. Um destes uso pode ser a fortificação da dieta de larvas-da-farinha.

Alguns estudos têm investigado o uso da linhaça na alimentação de larvas de Tenebrionidae. Exemplos incluem: o uso de seu óleo como forma de melhorar o perfil lipídico de *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) e *Tenebrio molitor*, obtendo redução significativa na relação ômega-6/ômega-3 (Oonincx *et al*., 2019; Rossi *et al*., 2022); uso de seu coproduto (torta), substituindo a alimentação convencional de larvas de *Tenebrio molitor* em 50%, resultando em larvas com 17,00% de ácido alfa-linolênico (C18:3n3) em comparação com 1,73% no Controle, e também com melhor relação ômega-6/ômega-3, de 1,71 para 20,55 no Controle (Bordiean *et al*., 2022). Esses estudos indicam o potencial de uso da linhaça e seus coprodutos para alimentar larvas de besouros da farinha, com o objetivo de melhorar seu perfil nutricional de ácidos graxos.

2 OBJETIVOS

2.1 Objetivo geral

- ✓ Avaliar o potencial do uso de torta de linhaça como alimento alternativo no enriquecimento nutricional de larvas de *Zophobas atratus*.

2.2 Objetivos específicos

- ✓ Explorar as aplicações emergentes de tecnologias e conhecimentos científicos relacionados ao uso de insetos comestíveis na indústria alimentícia através de uma prospecção tecnológica;
- ✓ Avaliar a composição nutricional da torta de linhaça;
- ✓ Elaborar dietas contendo diferentes porcentagens de substituição de substrato convencional por torta de linhaça;
- ✓ Avaliar o desempenho de crescimento das larvas de *Zophobas atratus* alimentadas com diferentes concentrações de torta de linhaça;
- ✓ Avaliar a composição centesimal e valor energético das larvas de *Zophobas atratus* alimentadas com diferentes concentrações de torta de linhaça;
- ✓ Avaliar o perfil de ácidos graxos e índices de qualidade lipídica das larvas de *Zophobas atratus* alimentadas com diferentes concentrações de torta de linhaça;
- ✓ Avaliar o teor de minerais das larvas de *Zophobas atratus* alimentadas com diferentes concentrações de torta de linhaça;
- ✓ Avaliar o potencial nutricional para inserção na alimentação humana.

3 FUNDAMENTAÇÃO TEÓRICA

3.1 Demanda global por alimentos e suas repercussões ambientais

O uso adequado da terra enfrenta vários desafios interconectados. Estima-se que até 2050 a população mundial irá atingir os 9 bilhões de pessoas, o que indica uma sobrecarga futura dos ecossistemas terrestres, que desempenham um papel fundamental no sistema climático global e fornecem serviços essenciais para os seres humanos, incluindo alimentos, energia, purificação de água e ar, regulação do microclima e proteção contra riscos naturais (Garcia; Osburn; Jay-Russell, 2020). Estima-se que a produção de alimentos deve aumentar em 70% até 2050 para atender às necessidades da população em crescimento (van Dijk *et al*., 2021). Esse aumento na produção de alimentos exigirá avanços significativos nas práticas agrícolas, na tecnologia e na infraestrutura. O uso da água, da terra e as emissões de gases de efeito estufa são muito altos em sistemas de criação de animais, especialmente tratando-se de carne bovina, o que causa pressão ambiental quando os sistemas de produção são especializados na entrega de produtos em grandes volumes (Misselbrook *et al*., 2016).

A urbanização é outro fator que contribui para o aumento da demanda por alimentos. À medida que mais pessoas se mudam para áreas urbanas, seus hábitos alimentares mudam. As populações urbanas tendem a consumir mais alimentos processados e com alto teor calórico, o que leva a uma maior demanda por carne, laticínios e outros produtos de origem animal. Espera-se que os hábitos alimentares até 2050 apresentem mudanças significativas com aumento do consumo de carnes e laticínicios em 31% e 58%, respectivamente. Essa mudança nas preferências alimentares exigirá mais recursos, como terra, água e energia, para produzir alimentos de origem animal (Cazcarro; López-Morales; Duchin, 2019).

3.1.1 Impactos ambientais dos sistemas tradicionais de produção alimentar

A agricultura é responsável por aproximadamente 70% das retiradas globais de água doce (Wu *et al*., 2022). A extração excessiva de água para irrigação não só afeta a disponibilidade de água para outros fins, mas também tem graves consequências ecológicas, incluindo a perda de habitats aquáticos. A produção de gado é um grande consumidor de recursos hídricos. A pegada hídrica dos produtos de origem animal inclui a água usada para beber, produzir ração e processar. Diferentes espécies animais têm necessidades de água variadas, influenciadas por fatores como dieta, clima e sistemas de produção. Por exemplo, a produção de carne bovina é uma das formas de criação de gado que mais consome água. De acordo com Rodrigues e Dziedzic (2021), a produção de um quilo de carne bovina requer aproximadamente 13,074 litros de água. Esse alto consumo de água se deve principalmente às grandes quantidades de água necessárias para o cultivo de rações, como milho e soja.

A produção de laticínios também apresenta uma pegada hídrica significativa. A produção de um litro de leite requer cerca de 805 litros de água (Grossi; Vitali; Lacetera, 2022). Isso inclui água para beber, para alimentar as plantações e para o processamento. O uso de água para produtos lácteos pode variar dependendo do sistema de produção, sendo que os sistemas baseados em pastagens geralmente têm uma pegada hídrica menor em comparação com os sistemas intensivos alimentados com grãos.

A produção de alimentos à base de plantas geralmente requer menos água em comparação com a produção de gado. No entanto, a pegada hídrica de diferentes culturas pode variar muito. Fatores como o tipo de cultura, as práticas de irrigação e as condições climáticas desempenham um papel significativo na determinação do uso da água. As culturas de cereais, como trigo, arroz e milho, são alimentos básicos para uma grande parte da população mundial. De acordo com Shikun *et al*. (2015), a produção de um quilo de trigo requer aproximadamente 1.800 litros de água.

A pecuária é uma das formas de agricultura mais intensivas em terra. São relatados por González-Quintero valores máximos de uso de terra de cerca 25,20 m^2 para a produção de 1 litro de leite e 108,60 m^2 para a produção de 1 kg de peso animal para gado bovino. Tais números podem variar de acordo com a qualidade do pasto e o clima local. Em regiões com pastagens de baixa qualidade, a necessidade de terra pode ser significativamente maior. Além disso, a criação de gado geralmente envolve o cultivo de rações, como milho e soja, o que aumenta ainda mais o uso de terra (Herrero *et al*., 2021).

De acordo com Sentelhas *et al*. (2015), o rendimento do cultivo de soja pode variar entre 500 kg a 2 toneladas por hectare de terra. A necessidade de terra para a soja pode variar dependendo de fatores como qualidade do solo, clima e práticas agrícolas. O estudo também destaca o impacto ambiental do cultivo da soja, incluindo o desmatamento e a perda de habitat em regiões como a floresta amazônica.

A produção de gado é uma das maiores fontes de emissões de metano na agricultura. O metano é produzido principalmente por meio da fermentação entérica, um processo digestivo em animais ruminantes, como vacas, ovelhas e cabras. Estima-se que este setor emita 7,1 gigatoneladas de dióxido de carbono equivalente por ano, representando 14,5% de todas as formas de emissões (Eisen; Brown, 2022). A produção de carne e leite bovino é responsável pela maioria das emissões, contribuindo respectivamente com 41 e 19% das emissões do setor agropecuário (Eisen; Brown, 2022). As principais fontes de emissões são: produção e processamento de ração, fermentação entérica de ruminantes e decomposição de esterco (Smith; Reay; Smith, 2021).

A produção agrícola também contribui para as emissões de gases de efeito estufa, principalmente por meio do uso de fertilizantes sintéticos, práticas de manejo do solo e arrozais. A aplicação de fertilizantes à base de nitrogênio é uma das principais fontes de emissões de óxido nitroso. Quando esses fertilizantes são aplicados aos solos, eles passam por processos de nitrificação e desnitrificação que liberam óxido nitroso na atmosfera. Estima-se que as práticas de manejo do solo agrícola são responsáveis por cerca de 60% das emissões globais de óxido nitroso (Hassan *et al*., 2022).

3.2 Entomofagia

A entomofagia é definida como a prática de consumir insetos. Trata-se de um hábito alimentar que faz parte da civilização humana há milênios. Embora possa parecer pouco convencional para muitos no mundo ocidental, a entomofagia é uma prática comum em várias culturas ao redor do mundo. Olivadese e Dindo (2023) indicam que as civilizações antigas, incluindo os gregos, romanos e egípcios, incorporavam insetos em suas dietas. Os insetos não eram apenas uma fonte de nutrição, mas também tinham significado cultural e medicinal.

De acordo com estudo de van Huis *et al*. (2013), mais de 2.000 espécies de insetos são consumidas por humanos em todo o mundo. Esse amplo consumo é apoiado pelos benefícios nutricionais que os insetos oferecem. Os insetos são ricos em proteínas, vitaminas e minerais, o que os torna uma valiosa fonte de alimento. A comunidade científica tem reconhecido cada vez mais o potencial da entomofagia para contribuir com os sistemas alimentares sustentáveis (Tavares *et al*., 2022).

De acordo com catalogação de Jongenma (2017), existem 2096 espécies de insetos comestíveis (Tabela 1). Entre a sua vasta gama, algumas espécies são particularmente populares. Grilos, larvas-da-farinha, gafanhotos e besouros são alguns dos insetos mais comuns. Os grilos, por exemplo, são altamente considerados por seu alto teor de proteína e versatilidade em aplicações culinárias (Abril *et al*., 2022). As larvas-da-farinha, por outro lado, podem ser facilmente cultivadas em grande escala (Vrontaki *et al*., 2024). Gafanhotos e besouros também são favorecidos por seu valor nutricional e facilidade de colheita. Esses insetos são frequentemente incorporados em pratos tradicionais e até mesmo processados em barras e em forma de farinha (van Huis *et al*., 2013).

Tabela 1 – Nomes comuns, ordem taxonômica e número de espécies de insetos comestíveis.

Nome comum	Ordem	Número de espécies
Besouros	Coleoptera	659
Lagartas	Lepidoptera	362
Formigas, abelhas e vespas	Hymenoptera	321
Gafanhotos e grilos	Ortoptera	278
Cigarras, percevejos, pulgões e cochonilhas	Hemiptera	237
Libélulas	Odonata	61
Cupins	Isoptera	59
Moscas	Diptera	37
Baratas	Blattodea	37
Outros	-	45

Fonte: Jongenma (2017).

A entomofagia é predominante em muitas regiões, principalmente na Ásia, África e América Latina. Em países como Tailândia, Camboja e China, os insetos são comumente encontrados em mercados e barracas de comida de rua. Na África, a entomofagia é praticada em países como Nigéria, Uganda e República Democrática do Congo, onde insetos como cupins e lagartas são considerados iguarias (van Huis *et al.*, 2013). Os países da América Latina, inclusive o México e o Brasil, também têm uma rica tradição de consumo de insetos (Chantawannakul, 2020). Em contrapartida, a entomofagia é menos comum nos países ocidentais, onde as percepções culturais e a falta de familiaridade com insetos comestíveis representam barreiras à aceitação (Toti *et al.*, 2020).

Os insetos são uma fonte de alimento altamente nutritiva, oferecendo uma variedade de nutrientes essenciais. Eles são particularmente ricos em proteínas, sendo que algumas espécies contêm até 80% de proteína por peso seco (Rumpold & Schlüter, 2013). Os insetos também fornecem aminoácidos essenciais, vitaminas (como a B12 e a riboflavina) e minerais (incluindo ferro, zinco e magnésio). A entomofagia também oferece benefícios ambientais significativos (Orkusz *et al.*, 2020).

A criação de insetos requer menos recursos em comparação com a criação de gado tradicional. Os insetos têm uma alta eficiência de conversão alimentar, o que significa que eles podem converter alimentos em massa corporal com mais eficiência do que os animais convencionais (van Huis *et al*., 2013). Além disso, a criação de insetos produz menos emissões de gases de efeito estufa e requer menos terra e água. Essas vantagens ambientais tornam a entomofagia uma alternativa sustentável às fontes convencionais de proteína animal, contribuindo para a redução da pegada ambiental da produção de alimentos (Vauterin *et al*., 2021).

3.3 Família Tenebrionidae

A família Tenebrionidae, comumente conhecida como besouros escuros, é um grupo diversificado de insetos que inclui mais de 20.000 espécies em todo o mundo. Esses besouros são encontrados em vários habitats, desde desertos até florestas, e desempenham funções importantes nos ecossistemas como decompositores. Nos últimos anos, tem havido um interesse crescente no potencial das espécies de Tenebrionidae como fonte de alimento sustentável para os seres humanos (Li *et al*., 2022).

Uma das espécies mais conhecidas da família Tenebrionidae é o *Tenebrio molitor*. Outras espécies incluem o *Alpithobius diaperinus* e *Zophobas atratus* (Nascimento *et al*., 2022; Roncolini *et al*., 2020, Selaledi; Mabelebele, 2021). São denominadas de larvas-da-farinha pois são consideradas como praga em silos de farinhas e o seu estágio larval é amplamente utilizado como alimento para animais devido ao seu alto teor de proteína. As larvas-da-farinha são fáceis de cultivar, têm baixo impacto ambiental e são ricas em nutrientes essenciais, o que as torna uma opção atraente para a produção sustentável de alimentos. Como visto na Tabela 2, as 3 espécies apresentam composição nutricional semelhante, com ênfase no elevado teor protéico (entre 41,90% e 58,40% da base seca) (Nascimento *et al*., 2022; Roncolini *et al*., 2020, Selaledi e Mabelebele, 2021). Por outro lado, o *Zophobas atratus* se destaca pelo seu maior teor lipídico, possivelmente, por alcançar maior massa durante a fase larval, período de grande estocagem energética sob forma de gordura (Nascimento *et al*., 2022).

Tabela 2 – Composição nutricional das espécies comuns de Tenebrionidae.

	Espécie		
	Alphitobius diaperinus	*Tenebrio molitor*	*Zophobas atratus*
Energia (Kcal)	494,82	494,76	577,06
Lipídios (g/100g)	26,25	28,21	43,91
Proteínas (g/100g)	58,40	56,97	41,90
Carboidratos (g/100g)	2,52	3,24	3,58
Cinzas (g/100g)	5,38	4,63	3,02
Fibra bruta (g/100g)	7,46	6,93	7,60
Referência	Roncolini *et al*. (2020)	Selaledi e Mabelebele (2021)	Nascimento *et al*. (2022)

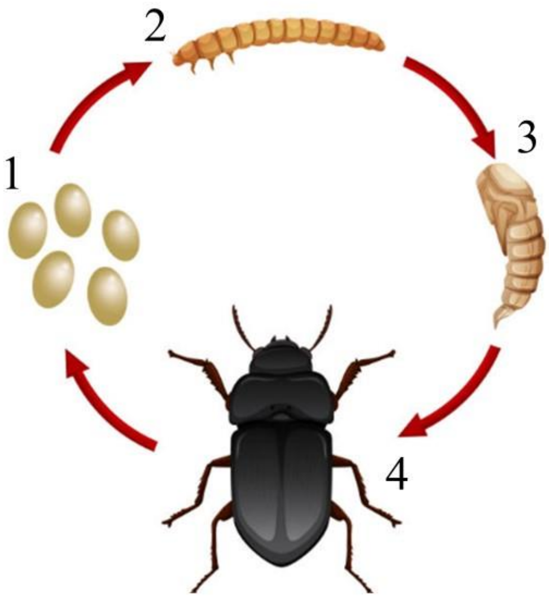
Nutrientes apresentados em base seca.

3.3.1 Fisiologia e criação da família Tenebrionidae

Os insetos Tenebrionidae passam por metamorfose completa (Figura 1), que consiste em quatro estágios de vida: ovo, larva, pupa e adulto. O estágio larval, especialmente, é de grande interesse devido ao seu alto teor de proteína e facilidade de cultivo. As larvas são geralmente cilíndricas, segmentadas e possuem mandíbulas bem desenvolvidas para alimentação. A duração de cada estágio da vida pode variar dependendo das condições ambientais, como temperatura e umidade (van Huis *et al*., 2013).

As larvas de *Zophobas atratus* geralmente atingem comprimentos de 50 a 60 milímetros ao longo de um período de 90 dias de criação (Nascimento *et al*., 2022). As larvas de *Tenebrio molitor* são menores do que as larvas de *Zophobas atratus*, geralmente medindo cerca de 15 milímetros de comprimento (Wang *et al*., 2022). Além disso, o *Tenebrio molitor* tem um ciclo de vida mais curto em comparação com o estágio larval mais longo do *Zophobas atratus*, que pode durar vários meses. As larvas do *Alphitobius diaperinus* são menores do que as do *Zophobas atratus* e do *Tenebrio molitor*, geralmente atingindo comprimentos menores que 15 milímetros. O *Alphitobius diaperinus* possui um ciclo de vida ainda mais rápido, se completando em cerca de 40 dias (Cucini *et al*., 2022).

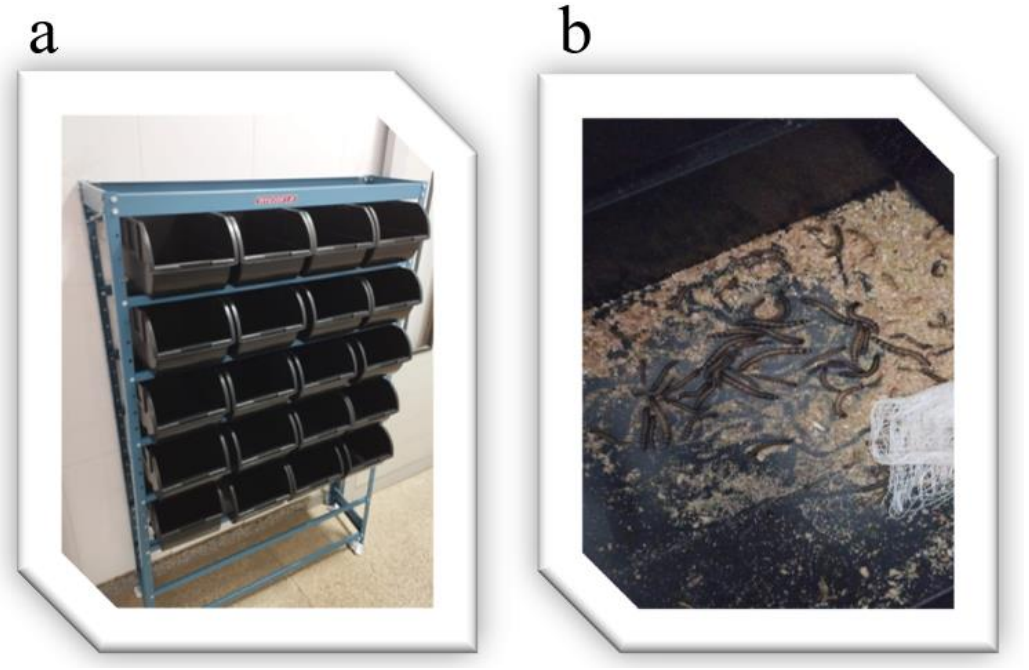
Figura 1 – Estágios de vida de tenébrios: (1) ovos, (2) larva, (3) pupa e (4) adulto.



Fonte: Istock (2018).

Considerando uma criação em massa, os substratos de alimentação comuns incluem farelo de trigo, aveia e outros grãos de cereais. Esses substratos fornecem os nutrientes necessários para o desenvolvimento das larvas e estão prontamente disponíveis, o que os torna econômicos para a produção em larga escala. A criação, geralmente, ocorre em bandejas (Figura 2a) com um substrato e uma fonte de água, que pode ser batata, ou cenoura (Figura 2b). Este processo é altamente dependente do trabalho manual, pois os restos de alimentação e os resíduos devem ser separados dos insetos peneirando o conteúdo das bandejas. A otimização dos custos de produção pode ser alcançada através do uso de substratos de baixo custo, técnicas de criação automatizadas e condições ideais de crescimento (Ribeiro; Abelho; Costa, 2018).

Figura 2 – Modelo de criação laboratorial de larvas-da-farinha em bandejas (a) e interior da bandeja, contendo larvas, substrato alimentar e gaze com fonte de umidade (b).



Fonte: Autoria própria.

As larvas-da-farinha são também particularmente eficientes na conversão de resíduos orgânicos de baixo valor em proteínas de alta qualidade. Essa capacidade chama a atenção por seu potencial em sistemas sustentáveis de produção de alimentos (Nascimento *et al*., 2022). Vários estudos demonstraram a capacidade da *Zophobas atratus* de metabolizar coprodutos agroindustriais. Por exemplo, um estudo realizado por van Broekhoven *et al*. (2015) os alimentou com coprodutos provenientes da fabricação de cerveja, panificação/biscoitos, processamento de batata e produção de bioetanol. Além disso, em outros estudos, essas larvas demonstraram a capacidade de biodegradar farelo de centeio, farelo de colza, palha de arroz e bagaço de uva (Bordiean *et al*., 2022, Montalbán *et al*., 2022, Nascimento *et al*., 2022). Os resultados revelaram que a *Z. atratus* poderia utilizar esses coprodutos de forma eficaz, apoiando ainda mais sua função no gerenciamento sustentável de resíduos.

3.4 Principais benefícios da criação de insetos

3.4.1 Melhor eficiência de conversão alimentar

Uma das principais vantagens da criação de insetos é sua alta eficiência de conversão do alimento ingerido (ECI). A ECI mede a eficiência com que um organismo converte o alimento ingerido em massa corporal. Logo, quanto maior for o valor em percentual (%), melhor. De acordo com um estudo realizado por Nascimento *et al*. (2022), o *Zophobas atratus* possui um ECI de aproximadamente 25%, significativamente maior do que o do gado, com relato de 6% (Berry; Crowley, 2013), além do fato de que, aproximadamente, 100% da massa corporal da larva é comestível. Essa maior eficiência significa que é necessário menos ração para produzir a mesma quantidade de biomassa comestível, tornando a *Zophobas atratus* uma opção mais sustentável.

A taxa de conversão alimentar (FCR) é outra métrica essencial para avaliar a eficiência dos sistemas de produção de alimentos. A FCR é a quantidade de ração necessária para produzir uma unidade de massa corporal (usualmente, g ou kg). Apresenta, então, relação oposta ao ECI. Ou seja, quanto menor for seu valor, melhor. Os animais de criação tradicionais, como o gado bovino, têm uma FCR de cerca de 8,8, o que significa que são necessários 8,8 g de ração para produzir 1 g de massa corporal (Wilkinson, 2011). Em contraste, o *Zophobas atratus* possui uma FCR de aproximadamente 3,0 (Nascimento *et al*., 2022). Essa menor FCR indica que o *Zophobas atratus* é mais eficiente na conversão de ração em biomassa comestível, reduzindo a demanda geral por recursos de ração.

A Tabela 3 apresenta estudos que desenvolveram experimentos com uso de resíduos ou coprodutos para a criação de insetos Tenebrionidae. Os estudos analisaram a eficiência de conversão alimentar sob forma de indicadores como FCR, ECI, ganho de massa larval e/ou massa larval ao fim do experimento. Gourgouta *et al*. (2024a) verificou que para as larvas de *Zophobas atratus*, a substituição de até 30% do substrato Controle por resíduos pós-destilação de extração de óleos essenciais não afetou significativamente a massa larval individual nem o FCR. Adicionalmente, o uso de substratos contendo torta de girassol, coprodutos de cevada e de aveia utilizados por Gourgouta *et al*. (2024b), foram estatisticamente iguais entre o Controle. Em contrapartida, a substituição de outros coprodutos resultou em pior desempenho alimentar. Nascimento *et al*. (2022) também relataram que até 50% de substituição do substrato Controle por resíduos de produção de suco de uva (compostos por 40% de cascas e 59% de sementes de uva) não comprometeu o ganho de massa larval de *Zophobas atratus*.

Para as larvas de *Tenebrio molitor*, Vrontaki *et al*. (2024) mostraram que a inclusão de coprodutos provenientes da produção de cerveja, arroz e aveia no substrato não alterou significativamente o FCR e o ECI, enquanto a utilização de outros coprodutos (como alfafa e milho) apresentou resultados inferiores. Yakti *et al*. (2023) observaram que a substituição de até 33% do substrato Controle por resíduos de cânhamo (incluindo talos, caules secundários, folhas e brotos de baixa qualidade) não afetou a massa larval. Já Ruschioni *et al*. (2020) destacaram que a adição de farelo de trigo orgânico enriquecido com até 25% de torta de azeitona orgânica não causou diferença significativa no peso larval individual.

De maneira geral, os dados sugerem que alguns resíduos e coprodutos podem ser utilizados como fontes alternativas na dieta de larvas de *Zophobas atratus* e *Tenebrio molitor* sem prejudicar o crescimento e a eficiência alimentar, desde que em níveis específicos de inclusão. Essa abordagem é relevante para a sustentabilidade, pois promove o aproveitamento de resíduos agroindustriais, reduzindo custos e contribuindo para a economia circular. No entanto, é importante considerar que o desempenho dos insetos pode variar dependendo do tipo e da quantidade de resíduo ou coproduto utilizado, destacando a necessidade de uma avaliação criteriosa para otimizar a formulação das dietas e garantir o equilíbrio nutricional adequado.

Tabela 3 – Artigos experimentais que utilizaram resíduos/coprodutos agroindustriais para criação de insetos Tenebrionidae.

Referência	Espécie(s)	Resíduo / Coproduto	Conversão Alimentar
Gourgouta *et al*. (2024a)	*Zophobas atratus*	Resíduos pós-destilação da extração de óleo essencial, obtidos de *Crithmum maritimum* L., *Origanum vulgare*, *Cannabis sativa* L.), *Linum usitatissimum* e *Olea europaea*.	Sem diferenças estatísticas para massa larval individual e FCR com substituição de até 30%.
Gourgouta *et al*. (2024b)	*Zophobas atratus*	Fluxos secundários do processo de limpeza de sementes de cevada, aveia, ervilha e ervilhaca e da produção de algodão, beterraba sacarina e girassol.	FCR e ECI estatisticamente iguais entre Controle e torta de girassol, coproduto de cevada, coproduto de aveia. Demais coprodutos apresentaram resultados piores.

Vrontaki *et al*. (2024)	*Tenebrio molitor*	Coprodutos provenientes da produção de cerveja, arroz, aveia, cevada, alfafa e milho.	FCR e ECI estatisticamente iguais entre Controle e grãos gastos / farelo de arroz. Demais coprodutos apresentaram resultados piores.
Yakti *et al*. (2023)	*Tenebrio molitor*	Resíduos de cânhamo (talos/caules secundários e folhas e brotos de baixa qualidade).	Sem diferença estatística para massa larval com até 33% de substituição.
Nascimento *et al*. (2022)	*Zophobas atratus*	Resíduo de produção de suco de uva (40% de casca, 59% de semente de uva e 1% de impurezas).	Sem diferença estatística para ganho de massa larval com até 50% de substituição.
Ruschioni *et al*. (2020)	*Tenebrio molitor*	farelo de trigo orgânico enriquecido com 25%, 50% e 75% de torta de azeitona orgânica.	Sem diferença estatística para peso larval individual com até 25% de substituição.

3.4.2 Menor impacto ambiental

De acordo com a Organização das Nações Unidas para Agricultura e Alimentação (FAO), a pecuária é responsável por 14,5% das emissões globais de gases de efeito estufa (FAO, 2013). Em contrapartida, Oonincx *et al*. (2010) descrevem que todas as espécies de insetos estudadas por eles produziram quantidades muito menores de NH_3 (3,0 a 5,4 mg/kg/dia para *A. domesticus*, *L. migratoria* e *B. dubia*) do que o gado convencional (4,8–75 mg/kg/dia para porcos e 14–170 mg/kg/dia para gado bovino). O uso da terra é outro fator crítico na avaliação da sustentabilidade dos sistemas de produção de alimentos. A pecuária tradicional exige vastas extensões de terra para pastagem e cultivo de ração. Em contrapartida, insetos podem ser criados em ambientes compactos e controlados, reduzindo significativamente o uso da terra (Van Huis *et al*., 2013).

A criação de gado é um grande impulsionador do desmatamento, particularmente em regiões como a floresta amazônica. A criação de insetos não contribui para o desmatamento em tal escala, tornando-a muito menos prejudicial aos ecossistemas naturais (Van Huis *et al*., 2013). Desta forma, fica evidente que a criação de insetos requer apenas uma fração da terra necessária para a criação de gado tradicional. Em relação a pegada hídrica, larvas-da-farinha exigem cerca de 23 de litros/g de proteína, comparado ao do frango que é 34 e ao da carne bovina que é 112 (Miglietta *et al*., 2015).

3.5 Legislação sobre insetos como alimento humano

O uso de insetos, incluindo espécies de Tenebrionidae, como alimento humano está ganhando força em todo o mundo. No entanto, a legislação referente à sua aplicação varia de acordo com a região. Na União Europeia (UE), a Autoridade Europeia para a Segurança dos Alimentos (EFSA) vem avaliando a segurança dos insetos como alimento. Em termos de insetos como alimento para consumo humano, as disposições que os colocam no âmbito do Regulamento (UE) 2015/2283 sobre novos alimentos são aplicáveis desde 2018 (Turck *et al*., 2021). Sob este novo Regulamento, os produtos alimentares de insetos só podem ser comercializados quando autorizados após uma avaliação de segurança pela Autoridade Europeia para a Segurança dos Alimentos (EFSA). Em 2020, foram submetidos pedidos de produtos alimentares com grilo doméstico (*Acheta domesticus*), grilo doméstico tropical (*Gryllodes sigillatus*), larva-da-farinha menor (*Alphitobius diaperinus*), mosca soldado negra (*Hermetia illucens*), abelha (*Apis mellifera*), gafanhoto (*Locusta migratoria*) e larva-da-farinha (*Tenebrio molitor*) (Lähteenmäki-Uutela; Marimuthu; Meijer, 2021). Em 2021, a EFSA aprovou o uso da larva-da-farinha seca (*Tenebrio molitor*) como um novo alimento, marcando um passo significativo para a aceitação de insetos no mercado alimentício europeu (Turck *et al*., 2021).

Nos Estados Unidos, a Food and Drug Administration (FDA) regulamenta o uso de insetos como alimento. Embora não exista uma legislação específica para as espécies de Tenebrionidae, a FDA tem diretrizes para o uso de insetos em produtos alimentícios. Os insetos devem ser criados em condições sanitárias, livres de contaminantes e seguros para o consumo humano. As empresas interessadas em comercializar alimentos à base de insetos devem garantir a conformidade com essas diretrizes (Lähteenmäki-Uutela; Marimuthu; Meijer, 2021).

Na Ásia, países como a Tailândia e a China têm um longo histórico de consumo de insetos, inclusive de espécies de Tenebrionidae. Na Tailândia, os insetos são considerados um alimento tradicional, e há mercados estabelecidos para insetos comestíveis. O governo tailandês implementou regulamentações para garantir a segurança e a qualidade dos alimentos à base de insetos e da criação de insetos, com ênfase nas fazendas domésticas de grilos. Da mesma forma, na China, o consumo de insetos é culturalmente aceito, e há regulamentações em vigor para reger seu uso como alimento (van Huis *et al*., 2013).

Na África, o uso de insetos como alimento também é comum em muitas culturas. Países como Uganda e Nigéria têm práticas tradicionais de consumo de insetos, incluindo espécies de Tenebrionidae. Entretanto, a legislação formal relativa ao uso de insetos como alimento ainda está em desenvolvimento em muitos países africanos. Estão sendo feitos esforços para estabelecer regulamentações que garantam a segurança e a qualidade dos alimentos à base de insetos (Kelemu *et al*., 2015).

Na América Latina, alguns países se destacam quanto a presença cultural da entomofagia. Dentre eles, México, Brasil, Colômbia, Venezuela, Equador apresentam grande diversidade biológica e étnica. Por outro lado, a regulamentação sobre o uso de insetos como alimento humano nesta região é limitada, pela sua percepção como pragas ou contaminantes em alimentos. A ausência de interesse governamental em desenvolver legislações específicas reflete essa percepção (Bermúdez-Serrano, 2020).

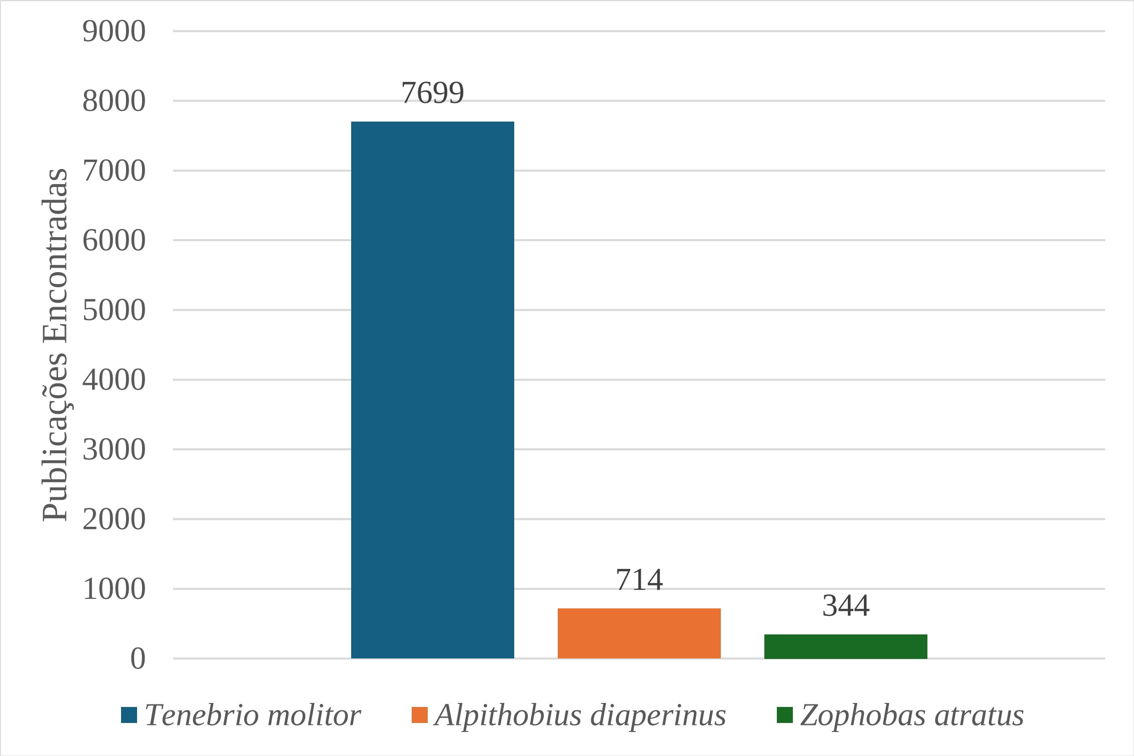
Muitos países latino-americanos adotam o Codex Alimentarius, que não inclui normas específicas que regulamentem o uso de insetos como alimento ou ração, tratando-os apenas como impurezas a serem eliminadas. Essa lacuna regulatória cria obstáculos significativos para empreendedores que buscam formalizar e expandir a produção de insetos comestíveis na região (Bermúdez-Serrano, 2020).

3.6 Produção Científica com insetos da família Tenebrionidae

Uma análise da literatura científica no ScienceDirect em 20 de Agosto de 2024 (Figura 3) revela níveis variados de interesse de pesquisa nas três espécies de besouros Tenebrionidae mais conhecidas. O *Tenebrio molitor* foi objeto de um número significativo de estudos, com mais de 7000 produções científicas. Esse alto volume de pesquisas pode ser atribuído ao uso da espécie como novo ingrediente na indústria alimentícia de forma legalizada em diversas regiões do mundo. Do total de estudos, 4432 estavam na área de ciências biológicas e agriculturais, com destaque para estudos em alimentos inovadores incorporando: proteína larval para substituir parcialmente carne magra no processamento de salsichas híbridas (Zhang *et al*., 2024); filme contendo proteína de larvas e quitosana para embalagens sustentáveis de alimentos (Liu *et al*., 2024); emulsões tipo maionese sem ovos estabilizadas com proteínas da larva (Gkinali *et al*., 2024).

Em contrapartida, o *Zophobas atratus* recebe menos atenção, com aproximadamente 344 produções científicas presentes no ScienceDirect. O número relativamente menor de estudos pode ser devido ao seu surgimento mais recente como um assunto de interesse na pesquisa científica. Além disso, ainda não há regulamentação para esta espécie a nível de aplicabilidade de consumo para humanos. Dentre as áreas de pesquisa, o destaque de publicações se encontra na bioquímica, genética e biologia molecular, onde há estudos diversos avaliando a capacidade desta espécie em metabolizar materiais, como plásticos poliolefínicos (Wang *et al*., 2024), polietileno e poliestireno (Miravalle *et al*., 2024) e espuma (Luo *et al*., 2021). Sendo assim, há uma lacuna quanto a produção científica do *Zophobas atratus* como novo alimento para humanos, o que não é encontrado para o *Tenebrio molitor*. O *Alphitobius diaperinus* apresenta densidade de produção científica intermediária entre as outras espécies. Tem sido o foco de vários estudos, especialmente na área de ciências biológicas e agriculturais (538), principalmente no contexto do manejo de pragas em granjas avícolas (Galán; Gourgouta; Athanassiou, 2024, Kavallieratos *et al*., 2024, Chen; Ma, 2024).

Figura 3 – Publicações contabilizadas na ScienceDirect até 20 de Agosto de 2024 referentes às diferentes espécies de tenébrios.



Desta forma, fica claro que maioria das pesquisas se concentra em *Tenebrio molitor* (Tavares *et al*., 2022). No entanto, *Zophobas atratus* possui algumas características distintas e interessantes, como sua dependência de isolamento durante a metamorfose e sua capacidade de atingir tamanhos maiores (Kim *et al*., 2015), com maior rendimento de biomassa, atingindo mais que o dobro do peso de *Tenebrio molitor* (Harsányi *et al*., 2020).

3.7 Perfil de ácidos graxos em larvas de *Zophobas atratus*

As larvas de *Zophobas atratus* apresentam elevado teor de ácidos graxos saturados, seguido de monoinsaturados e poli-insaturados (Tabela 4). Além disso, os ácidos graxos essenciais ômega-6 e ômega-3 se apresentam em concentrações desequilibradas considerando a recomendação para consumo humano da FAO/OMS (2009). São definidos como essenciais pela incapacidade do organismo humano de sintetizá-los, havendo necessidade de sua ingestão alimentar ou suplementação. Os ácidos graxos ômega-6, como o ácido linoleico, estão envolvidos nas respostas inflamatórias e na sinalização celular, enquanto os ácidos graxos ômega-3, como o ácido alfa-linolênico (ALA), o ácido eicosapentaenoico (EPA) e o ácido docosahexaenoico (DHA), são conhecidos por suas propriedades anti-inflamatórias e benefícios para a saúde cardiovascular e neurológica. O equilíbrio entre os ácidos graxos ômega-6 e ômega-3 é crucial, pois um desequilíbrio pode levar a vários problemas de saúde. Desta forma, a razão ômega-6/ômega-3 presente nesta larva proporcionaria um efeito pró-inflamatório para o organismo humano (FAO/OMS, 2009).

Tabela 4 – Somatório de ácidos graxos saturados, monoinsaturados, poli-insaturados e perfil de ômega-6 e ômega-3 de larvas de *Zophobas atratus*.

Ácido graxo	%
ΣSFA*	44,60
ΣMUFA*	32,10
ΣPUFA*	23,20
Ácido linoleico*	21,20
Ácido alfa-linolênico*	0,90
Ômega-6/ômega-3*	23,55/1,00
Recomendação Ômega-6/ômega-3**	5,00/1,00 - 10,00/1,00

*Fontes: * Adámková et al. (2016). **FAO/OMS (2009).*

3.7.1 Capacidade de modulação do perfil lipídico em larvas de tenébrios

Um estudo realizado por Nascimento *et al*. (2022) examinou os efeitos de diferentes proporções de resíduo de uva sobre a composição de ácidos graxos das larvas de *Zophobas atratus*. Os resultados mostraram que a composição de ácidos graxos saturados, monoinsaturados e poli-insaturados das larvas poderia ser significativamente alterada pelo substrato. Outro estudo realizado por van Broekhoven *et al*. (2015) explorou os efeitos de diferentes substratos à base de coprodutos na composição de ácidos graxos de *Zophobas atratus*, *Tenebrio molitor* e *Alphitobius diaperinus*. Os resultados indicaram que o tipo de substrato fornecido aos insetos influenciou o perfil de ácidos graxos das larvas, com ênfase na possibilidade de modulação da razão ômega-6/ômega-3. Os autores dissertam que a extensão em que a razão ômega-6/ômega-3 pode ser reduzida para as espécies de larvas-da-farinha usadas neste estudo continua sendo um tópico de investigação. Já Lawal *et al*. (2021), ao usar proporções variáveis de linhaça no substrato de larvas de *Tenebrio molitor*, descobriram que o conteúdo de ácido alfa-linolênico (C18:3n3) aumentou de 0,36% nas larvas Controle para 6,40% nas larvas alimentadas com 20% de linhaça como substituto do farelo de trigo.

Segundo o artigo de Čaloudová *et al*. (2023), há evidências de que a alimentação das larvas de *Zophobas atratus* com substratos à base de milho e torta de linhaça fermentados pode modular de forma significativa nos níveis de ácido alfa-linolênico, ácido eicosapentaenoico e ácido gama-linolênico, sugerindo uma modificação positiva na composição de ácidos graxos das larvas. Além disso, de acordo com o estudo de Rossi *et al*. (2022), para *Tenebrio molitor* foi observada uma correlação entre o conteúdo larval de ácidos palmitoleico, oleico, alfa-linolênico e eicosapentaenoico com a composição dessas substâncias nos substratos. De acordo com o mesmo estudo, os resultados sugerem uma importância grande da composição lipídica do substrato na modulação dos ácidos graxos poli-insaturados (PUFA) n-3 e a razão ômega-6/ômega-3 nas larvas.

Boukid *et al*. (2021) apoiam a hipótese de que os SFA e MUFA são principalmente sintetizados pelo *Tenebrio molitor*, enquanto os PUFA são provavelmente obtidos na alimentação, o que apoiaria a ideia da sua modulação através do perfil de ácidos graxos do substrato (Boukid *et al*., 2021). Entretanto, o mecanismo pelo qual modificações nos teores de ácidos graxos larvais de espécies Tenebrionidae ocorrem ainda não está bem elucidado (Rossi *et al*., 2022). No estudo de Szymczak-Cendlak *et al*. (2021), foi destacado que as sulfacininas, neuropeptídeos presentes em artrópodes, desempenham um papel crucial na modulação dos níveis e no perfil de ácidos graxos. Essas moléculas são estrutural e funcionalmente análogas à gastrina-colecistocinina encontrada em vertebrados. O estudo revelou que as sulfacininas influenciam a composição de ácidos graxos em enócitos de *Zophobas atratus*. Os enócitos, por sua vez, são células essenciais para a manutenção da homeostase corporal e desempenham um papel na regulação do metabolismo intermediário, especialmente no lipídico. Segundo o estudo de Slocińska *et al*. (2015), os lipídios dos insetos atuam como armazenador principal de energia, nutrientes e no metabolismo energético. Durante a fase larval, o teor de lipídio representa grande parte do corpo do inseto e os autores sugerem que esse tecido pode ser um alvo da ação das sulfacininas, ou pelo menos desempenhar um papel intermediário nas vias de transdução das sulfacininas (Slocińska *et al*., 2015).

3.7.2 Implicações da modulação de ômega-3 larval para humanos e outros animais

A capacidade de modular o perfil de ácidos graxos do *Zophobas atratus* pode ter implicações significativas para a saúde humana uma vez que estes insetos sejam inseridos em produtos alimentícios. Como a demanda por fontes de proteína sustentáveis continua a aumentar, com a otimização da composição de ácidos graxos desses insetos, é possível aumentar seu valor nutricional e fornecer uma fonte mais equilibrada de ácidos graxos essenciais (Aiking; de Boer, 2019).

Um dos benefícios mais bem documentados do ômega-3 é seu impacto positivo na saúde cardiovascular. Os ácidos graxos poli-insaturados desempenham um papel importante na regulação de processos inflamatórios no organismo, onde o ômega-3, em termos gerais, pode desempenhar um papel anti-inflamatório e o ômega-6, um papel pró-inflamatório. O consumo excessivo de ômega-6 pode causar um desequilíbrio fisiológico e gerar uma série de efeitos adversos, incluindo um estado inflamatório crônico e, a longo prazo, processos neoplásicos (Orkusz, 2021). Os ácidos graxos ômega-3 são capazes de reduzir significativamente os principais eventos cardiovasculares adversos, infarto do miocárdio e mortalidade por problemas cardíacos. A Autoridade Europeia para a Segurança Alimentar (EFSA, 2010) propõe uma ingestão adequada de ácido alfa-linolênico (C18:3n3 ou ALA) de 0,5% da ingestão energética diária (ou aproximadamente 1,1 g para uma dieta de 2000 kcal).

A inflamação é uma resposta natural do corpo a lesões ou infecções, mas a inflamação crônica pode levar a várias doenças, inclusive doenças cardíacas, câncer e distúrbios autoimunes. Foi demonstrado que o ALA possui propriedades anti-inflamatórias, por reduzir a produção de marcadores inflamatórios no corpo (James *et al.*, 2000). Isso torna o ALA um nutriente valioso para o controle de condições inflamatórias crônicas. Em termos gerais, os ácidos graxos poli-insaturados ômega-3 são incorporados à bicamada fosfolipídica das membranas celulares, influenciando a fluidez da membrana e a sinalização transmembrana. Desta forma, são capazes de modular a função de canais iônicos de Na^{+} e os canais de Ca^{2+}, auxiliando na prevenção de arritmias letais. Além disso, os ômega-3 inibem a conversão do ácido araquidônico em eicosanoides pró-inflamatórios, atuando como substrato alternativo para as enzimas ciclooxigenase ou lipoxigenase, resultando na produção de metabólitos menos inflamatórios. De acordo com Endo e Arita (2016), um conjunto de metabólitos oxigenados enzimaticamente derivados de ácidos graxos ômega-3 foram identificados, os quais atuam como mediadores anti-inflamatórios e podem apresentar funções preventivas em uma série de patologias inflamatórias crônicas.

A modulação do perfil de ácidos graxos essenciais nas larvas de *Zophobas atratus* apresenta também implicações possíveis e de grande interesse para a criação de animais. O enriquecimento de ovos em galinhas poedeiras com ômega-3 é alcançado por meio da modificação da dieta das aves. A adição de sementes de linhaça na dieta das aves pode elevar significativamente o conteúdo de ALA na gema, sem afetar negativamente as qualidades sensoriais dos ovos. De forma semelhante, maiores níveis de ALA na dieta das galinhas levaram a concentrações aumentadas de DHA na gema (Alagawany *et al*., 2021).

Kamely, Torshizi e Khosravinia (2016) relataram que a adição de óleo de peixe à dieta das galinhas melhorou diversos parâmetros de produção, incluindo a produção diária de ovos, a taxa de conversão alimentar e o peso dos ovos. O estudo também indicou que o aumento dos níveis totais de imunoglobulina G nos embriões durante a incubação, alcançado pelo aumento da ingestão de ALA poderia melhorar as capacidades de defesa imunológica dos pintos.

Considerando a produção de pescados, a inserção de larvas de *Zophobas atratus* ricas em ácido alfa-linolênico (ALA) pode representar uma estratégia eficaz para otimizar o crescimento e melhorar a qualidade muscular de tilápias, bem como para potencializar a resposta imunológica e reduzir a inflamação, de acordo com estudo de Huang *et al*. (2022). Considerando que as tilápias possuem uma capacidade de biossíntese de ácidos graxos ômega-3 de cadeia longa (EPA e DHA) a partir do ALA, o fornecimento de dietas ricas em ALA pode promover a deposição eficiente desses ácidos graxos essenciais nos tecidos musculares, melhorando características de textura como tenacidade, elasticidade e mastigabilidade, o que aumentaria o valor comercial destes peixes. Além disso, o consumo de ALA elevado também contribui para o fortalecimento da resposta imune, essencial para a resistência a infecções e ao estresse ambiental (Huang *et al*., 2022).

3.8 Sementes de linhaça: Uma visão geral

A linhaça (*Linum usitatissimum*) é um membro da família Linaceae e é cultivada em várias partes do mundo, inclusive no Canadá, Rússia, China, Índia e Estados Unidos. As sementes da planta da linhaça são ricas em ácidos graxos ômega-3, lignanas e fibras alimentares, o que as torna uma commodity valiosa no setor alimentício e de produtos funcionais (Langyan *et al*., 2023). A semente de linhaça também é usada na produção de óleo de linhaça, que tem aplicações no setor alimentício, bem como na fabricação de tintas, vernizes e outros produtos industriais (Langyan *et al*., 2023). A produção mundial de sementes de linhaça para o ano agrícola de 2023-24 é estimada em três milhões de toneladas (Tridge, 2023).

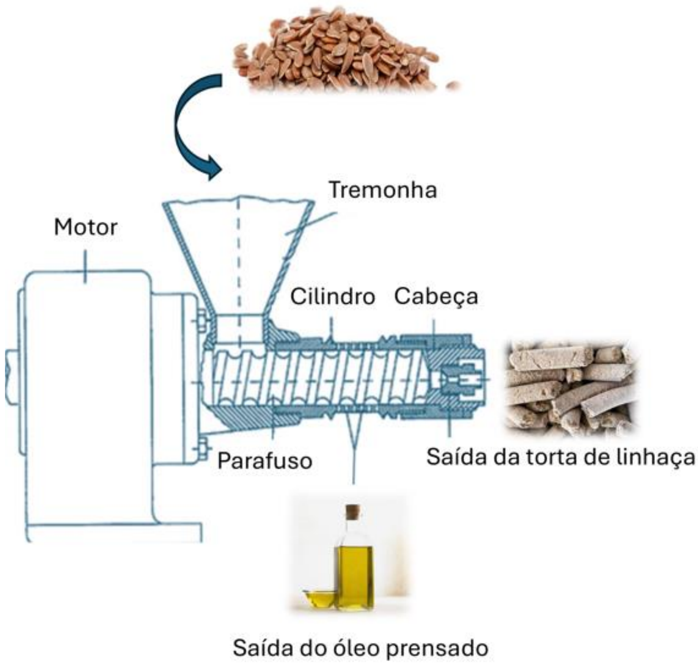
O óleo de linhaça é extraído das sementes da planta de linhaça por meio de um processo conhecido como prensagem a frio ou extração por solvente (Qiu *et al*., 2020). O óleo é conhecido por seu alto teor de ácido alfa-linolênico (ALA), um ácido graxo ômega-3 essencial que traz inúmeros benefícios à saúde. A produção global de óleo de linhaça também tem aumentado, impulsionada por suas aplicações nos setores alimentício, cosmético e farmacêutico (Qiu *et al*., 2020). Em 2021, a produção global de óleo de linhaça foi estimada em cerca de

700.151 toneladas (DiversityTimes, 2023). A crescente conscientização sobre os benefícios dos ácidos graxos ômega-3 para a saúde levou a uma crescente demanda por óleo de linhaça na indústria alimentícia, onde é usado como suplemento dietético e como ingrediente em vários produtos alimentícios (Kokić; Rakita; Vujetić, 2024).

3.9 Torta de linhaça: coproduto de grande potencial

A torta de linhaça é o coproduto do processo de extração do óleo de linhaça (Figura 4). Trata-se da parte sólida prensada restante. O óleo pode ser extraído da semente de linhaça aplicando-se pressão e forças de cisalhamento com prensas mecânicas em um processo denominado prensagem. A prensagem a frio é uma alternativa ao processo tradicional com aplicação de calor e/ou solventes. Na prensagem a frio é obtido um óleo com sua qualidade lipídica mantida, porém, a eficiência na extração do óleo é menor que demais processos. Desta forma, a depender das condições de extração, é possível verificar percentuais entre 1,1 e 16,2% de lipídios neste coproduto (Bhatty; Cherdkiatgumchai, 1990; Shim *et al*., 2015). Além disso, trata-se de uma fonte valiosa de proteína e fibra, comumente usada como ração animal, especialmente para animais de criação, como gado, aves e suínos. A produção global de torta de linhaça está intimamente ligada à produção de óleo de linhaça, pois a torta é obtida do material residual após a extração do óleo (Kokić; Rakita; Vujetić, 2024).

Figura 4 – Modelo de extrator de óleo baseado em prensagem de sementes a frio.



Fonte: Adaptado de Shim et al. (2015).

Em 2020, a produção global de torta de linhaça foi estimada em aproximadamente 2,0 milhões de toneladas métricas (FAO, 2021). O alto valor nutricional da torta de linhaça faz dela uma escolha popular para ração animal, contribuindo para a demanda e a produção gerais. Apesar do processamento, ainda é uma rica fonte de proteína (27,80 a 39,40%) e fibra (7,60 a 12,80%), além de conter níveis moderados de lipídios (até 16,90%), mantendo o perfil de ácidos graxos da semente, rico em ácido alfa-linolênico (C18:3n3; 42,90 a 68,60% do total de ácidos graxos) (Kokić; Rakita; Vujetić, 2024).

Apesar de seus possíveis benefícios, há também algumas possíveis desvantagens no uso da torta de linhaça como fonte de alimento para as larvas de *Zophobas atratus*. Uma das principais preocupações é a presença de fatores antinutricionais, como glicosídeos cianogênicos e ácido fítico, que podem interferir na absorção e utilização de nutrientes (Khare *et al.*, 2021). Esses fatores antinutricionais podem potencialmente reduzir o crescimento e o desenvolvimento das larvas se não forem adequadamente gerenciados.

3.10 uso de coprodutos agroindustriais na criação de insetos e os Objetivos de Desenvolvimento Sustentável (ODS)

Os Objetivos de Desenvolvimento Sustentável (ODS) são uma agenda global estabelecida pela ONU, composta por 17 metas que visam erradicar a pobreza, proteger o meio ambiente e garantir que todas as pessoas desfrutem de paz e prosperidade até 2030 (Hák; Janoušková; Moldan, 2016). Entre os principais objetivos estão a promoção de agricultura sustentável, a garantia de segurança alimentar e a luta contra a mudança climática. A busca por métodos inovadores que contribuam para a sustentabilidade tem levado à exploração de alternativas na produção de alimentos, como a criação de insetos. Esta prática se mostra promissora para atender vários dos ODS, especialmente no que diz respeito à segurança alimentar, à produção sustentável e à mitigação dos impactos ambientais (Hák; Janoušková; Moldan, 2016).

A criação de insetos utilizando coprodutos agroindustriais, como resíduos de alimentos e coprodutos agrícolas, oferece uma abordagem sustentável e eficiente para a produção de proteína animal. Insetos podem ser alimentados com materiais que, de outra forma, seriam descartados, convertendo-os em fontes nutritivas de proteína e outros nutrientes essenciais. O uso de coprodutos agroindustriais na criação de insetos exemplifica uma abordagem de economia circular, onde o desperdício de uma indústria é transformado em insumo para outra, maximizando o uso dos recursos naturais disponíveis. Isso alinha-se diretamente com os ODS 12 (Consumo e Produção Responsáveis), 13 (Ação Contra a Mudança Global do Clima) e 15 (Vida Terrestre), criando um ciclo em que a produção alimentar sustentável reduz os impactos ambientais e protege os ecossistemas (Nações Unidas Brasil, 2024).

Além disso, a prática também pode impulsionar o crescimento econômico sustentável, especialmente em comunidades rurais e países em desenvolvimento, alinhando-se, então, com o ODS 2 (Fome Zero e Agricultura Sustentável), ao promover a disponibilidade de alimentos de elevado valor nutricional para populações mais vulneráveis (Nações Unidas Brasil, 2024). Dessa forma, os benefícios ambientais, econômicos e sociais reforçam-se mutuamente, promovendo uma transição para práticas agrícolas mais sustentáveis e justas. A interconectividade entre esses objetivos mostra que esforços direcionados para melhorar a sustentabilidade da produção alimentar, como a criação de insetos com coprodutos, têm um impacto positivo em múltiplos ODS simultaneamente (Hák; Janoušková; Moldan, 2016).

Entretanto, a implementação desta prática em larga escala ainda enfrenta desafios. A aceitação cultural do consumo de insetos e a necessidade de regulamentação adequada são barreiras que precisam ser superadas. Além disso, o impacto ambiental real da criação de insetos, comparado a outras formas de produção animal, ainda requer mais estudos para garantir que a prática contribua positivamente para os ODS (Moruzzo; Mancini; Guidi, 2021). Se esses desafios forem superados, a criação de insetos pode desempenhar um papel significativo na construção de um sistema alimentar mais sustentável e na promoção de uma economia circular, contribuindo de maneira eficaz para o cumprimento dos Objetivos de Desenvolvimento Sustentável (Dicke, 2018).

4 MATERIAL E MÉTODOS

4.1 Metodologia da revisão

A metodologia consistiu em uma busca por documentos de patentes na base de dados internacional Espacenet em agosto de 2022. O Espacenet é uma base de dados do Escritório Europeu de Patentes (EPO), onde podem ser encontrados documentos de patentes de mais de 100 países. A estratégia de busca utilizou palavras-chave a serem encontradas nos títulos e/ou resumos e dois códigos diretamente associados ao objetivo da pesquisa. A escolha das palavras-chave (Insect, Pupa, Larva, or Nymph) foi feita para considerar todos os estágios de desenvolvimento dos insetos e evitar subestimação dos dados. Além disso, foram selecionados os códigos A23L33 (Modificação das qualidades nutritivas dos alimentos; Produtos dietéticos; Preparação ou tratamento dos mesmos) e A23V2002 (Composições alimentares, função de ingredientes alimentares ou processos para alimentos ou produtos alimentares), para que a busca recuperasse apenas documentos diretamente relacionados à indústria alimentícia. Os operadores booleanos (OR) e (AND) foram utilizados para garantir que pelo menos um código e uma palavra-chave estivessem presentes nos documentos avaliados. O operador de truncamento (*) foi empregado para encontrar derivações das palavras-chave. Os documentos selecionados para o processamento de dados foram aqueles que apresentavam maior proximidade com o tema proposto.

As informações referentes aos documentos disponíveis foram exportadas para o Microsoft 365 Excel utilizando o software CSVed, para posterior análise. O Orbit® foi utilizado em associação com o Espacenet para avaliar domínios tecnológicos e grupos de conceitos mais prevalentes em documentos de patentes. Uma busca na base de dados científica Scopus foi realizada para avaliar a prevalência de pesquisas sobre insetos até agosto de 2022. O termo "edible and insect" foi utilizado, para ser encontrado nos títulos, resumos ou palavras-chave dos documentos.

4.2 Material experimental biológico

4.2.1 Larvas

A empresa SuperBugs - Alimentos Funcionais (Salvador-BA, Brasil) doou 2000 larvas de *Z. atratus* com até 15 dias de eclosão (peso médio larval unitário de 39,58 mg ± 4,50 e comprimento entre 0,5 e 1,0 cm). Previamente à condução do experimento, as larvas foram exclusivamente alimentadas com farelo de trigo (Relva Verde, Ibiporã-SC, Brasil).

4.2.2 Material para formulação das dietas

A torta de linhaça foi doada pela empresa Vital Âtman Ltda (São Paulo-SP, Brasil), resultante do processo de prensagem a frio para extração de óleo.

A dieta Controle foi desenvolvida com mistura de: 70% de ração comercial para crescimento de aves (Imbramil, São Paulo-SP, Brasil) adquirida em comércio local na cidade de Salvador-BA, Brasil, composta por: milho integral moído, farelo de soja, farelo de trigo, farinha de carne e ossos e mistura mineral/vitamínica; e 30% de farelo de trigo (Relva Verde, Ibiporã-SC, Brasil), também adquirido em comércio local na cidade de Salvador-BA, Brasil.

4.3 Métodos

4.3.1 Formulação das dietas

A dieta Controle e a torta de linhaça passaram por um processo de trituração em moedor de grãos (80393 Hamilton Beach, Estados Unidos da América) até atingirem granulometria menor que 0,71mm (25 mesh), para posterior confecção de 5 dietas, as quais compuseram os tratamentos deste estudo: 0 (Controle) (70% de ração comercial para crescimento de aves + 30% de farelo de trigo); 25 (75% Controle + 25% torta de linhaça); 50 (50% Controle + 50% torta de linhaça); 75 (25% Controle + 75% torta de linhaça); e 100 (100% torta de linhaça).

4.3.2 Criação de larvas

Cem larvas foram acondicionadas em caixa plástica medindo 25,5 cm x 11,5 cm x 15 cm (comprimento x altura x largura), com abertura na parte superior coberta por tela. Cada caixa continha a respectiva dieta na proporção de 2 g de dieta para 1 g de peso larval (Boukid *et al*., 2021). Além disso, 0,3 g de batata inglesa fresca (*Solanum tuberosum*) por 1 g de peso larval foi ofertada, em camadas de algodão e substituídas duas vezes na semana, como fonte de água (Ruschioni *et al*., 2020). O experimento foi conduzido por 90 dias com 4 repetições por tratamento. As caixas foram dispostas em prateleira vertical de forma randomizada e mantidas em sala climatizada com controle de temperatura (25 °C ± 1) e umidade relativa (50% ± 5), através de termo-higrômetro digital (KR42 Instrubras, Brasil) e 12h de fotoperíodo diurno/noturno.

Quinzenalmente, dietas frescas eram renovadas (considerando o peso larval por caixa) e a dieta não consumida e as fezes eram pesadas. As pesagens das larvas foram feitas semanalmente em balança analítica (AY220 Shimadzu, Japão). Ao fim do experimento, as larvas foram separadas das dietas para esvaziamento do trato digestivo por 24 horas (Kulma *et al*., 2020), seguido de congelamento a -80 °C e liofilização (Lyophilizer L101 Liobras, Brasil) por 48 horas. As larvas abatidas e liofilizadas foram moídas em moedor de grãos (80393 Hamilton Beach, Estados Unidos da América) até atingirem granulometria homogênea e armazenadas em recipientes plásticos a -80 °C para posterior análise.

4.3.3 Composição centesimal

A composição centesimal foi realizada de acordo com as normas da AOAC (2019). A umidade foi determinada por secagem em estufa (Tecnal, TE-394/I, Brasil) a 105 °C até obtenção de peso constante, as cinzas foram determinadas em forno mufla (Lavoisier 402-D, Brasil) por incineração a 550 °C, fibra bruta foi determinada por extração ácida e alcalina. Para determinação de lipídios totais, o método de extração a frio (Bligh; Dyer, 1959) foi utilizado. Os carboidratos foram calculados através da diferença dos demais macronutrientes (Bhattacharjee *et al*., 2013). O valor energético foi calculado considerando 4 kcal/g para carboidratos e proteínas e 9 kcal/g para lipídios (Bhattacharjee *et al*., 2013). A proteína bruta foi determinada pelo método Kjeldahl (AOAC, 2019), onde o fator de conversão de nitrogênio 4,76 foi utilizado para as larvas (Janssen *et al*., 2017).

4.3.4 Perfil de ácidos graxos e qualidade lipídica

A identificação e quantificação de ácidos graxos foi conduzida de acordo com metodologia proposta por Souza *et al*. (2017), adaptada de López-López, Castellote-Bargalló e López-Sabater (2000). Para tanto, uma alíquota dos lipídios totais foi submetida à reação de saponificação com NaOH em metanol (0,5 N), seguida de metilação com BF_3 (12% em metanol) e extração com isooctano. Os ésteres metílicos de ácidos graxos extraídos foram acondicionados em vial âmbar em atmosfera inerte (N_2). Para separação dos ésteres metílicos de ácidos graxos foi utilizado um cromatógrafo gasoso (Perkin Elmer Clarus 680) com detector de ionização de chama e coluna DB – Fast FAME (30 m × 0, 25 mm × 0, 25 μm). Hélio foi utilizado como gás de arraste com vazão de 1,0 mL/min, e injeções de 1 μL foram realizadas em modo split (1:50). A identificação dos ácidos graxos foi feita através da comparação dos tempos de retenção dos picos apresentados nas amostras com os tempos de retenção de uma mistura padrão (189-19, Sigma Aldrich, EUA). A quantificação dos ácidos graxos das amostras foi realizada por normalização das áreas dos picos (% área). Os somatórios de ácidos graxos saturados totais (∑SFA), ácidos graxos monoinsaturados totais (∑MUFA) e ácidos graxos poli-insaturados totais (∑PUFA) foram calculados, assim como a razão ômega-6/ômega-3. Os seguintes índices de qualidade lipídica foram calculados para as larvas de *Zophobas atratus*: Índice de Aterogenicidade (IA, Eq. 1), Índice de Trombogenicidade (IT, Eq. 2), Razão Hipocolesterolêmico/Hipercolesterolêmico (H/H, Eq. 3), Índice de Promoção da Saúde (HPI, Eq. 4), Grau de Insaturação (UI, Eq. 5), Índice de Oxidabilidade (COX, Eq. 6) e Índice de Peroxidabilidade (PI, Eq. 7). Suas respectivas equações estão apresentadas abaixo (Chen; Liu, 2020; Duarte *et al*., 2022):

Eq.1 $\mathrm{IA} = (\mathrm{C12{:}0} + 4 \times \mathrm{C14{:}0} + \mathrm{C16{:}0}) / (\Sigma\mathrm{MUFA} + \text{ômega} - 6 + \text{ômega} - 3)$

Eq.2 $\mathrm{IT} = (\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}) / ((0.5 \times \Sigma\mathrm{MUFA}) + (0.5 \times \text{ômega} - 6) + (3 \times \text{ômega} - 3) + \text{ômega} - 3/\text{ômega} - 6)$

Eq.3 $\mathrm{H/H} = (\mathrm{cis} - \mathrm{C18{:}1} + \Sigma\mathrm{PUFA}) / (\mathrm{C12{:}0} + \mathrm{C14{:}0} + \mathrm{C16{:}0})$

Eq.4 $\mathrm{HPI} = \Sigma\mathrm{UFA} / (\mathrm{C12{:}0} + (4 \times \mathrm{C14{:}0}) + \mathrm{C16{:}0})$

Eq.5 $\mathrm{UI} = (1 \times \%\ \text{monoenoicos}) + (2 \times \%\ \text{dienoicos}) + (3 \times \%\ \text{trienoicos}) + (4 \times \%\ \text{tetraenoicos}) + (5 \times \%\ \text{pentaenoicos}) + (6 \times \%\ \text{hexaenoicos})$

Eq.6 $\mathrm{COX} = ((\mathrm{C18{:}1}) + (10.3 \times \mathrm{C18{:}2}) + (21.6 \times \mathrm{C18{:}3}))/100$

Eq.7 $\mathrm{PI} = (0{,}025 \times \mathrm{C18{:}1}) + (\mathrm{C18:2}) + (2 \times \mathrm{C18{:}3})$

4.3.5 Eficiência de conversão alimentar

Para avaliar a adaptação das larvas às dietas e a eficiência na conversão do alimento em biomassa larval, os seguintes parâmetros foram calculados: Eficiência de Conversão de Alimentos Ingeridos (ECI (%), Eq. 8 – base seca), Taxa de Conversão Alimentar (FCR, Eq. 9 – base úmida) e Taxa de Mortalidade (TM (%) Eq. 10) (Oonincx *et al*., 2015; Zhang *et al*., 2019) de acordo com as seguintes equações:

Eq. 8 $ECI\ (\%) = (peso\ ganho)/(peso\ do\ alimento\ ingerido) \times 100$

Eq. 9 $FCR = (peso\ do\ alimento\ ingerido)/(peso\ ganho)$

Eq. 10 $\mathrm{TM}\ (\%) = (\text{número de insetos mortos})/(\text{número de insetos iniciais}) \times 100$

4.3.6 Análise de metais

A determinação de metais foi realizada por espectrometria de emissão óptica com plasma indutivamente acoplado (ICP OES; Agilent Technologies, série 720, Estados Unidos da América). Os elementos analisados foram: cobre, sódio, manganês, magnésio, selênio, ferro, cálcio, zinco, potássio, fósforo, cobalto, arsênio, cádmio e níquel. A precisão do método foi estabelecida através da análise de material de referência e folhas de macieira certificadas (NIST 1515) nas mesmas condições de análise dos tratamentos.

4.4 Análise estatística

Para avaliar diferenças estatísticas entre tratamentos, One-way ANOVA e Teste de Tukey foram aplicados para análises com distribuição normal. Já os Testes de Kruskall-Wallis e U de Mann-Whitney foram conduzidos para análises com distribuição não-normal. A correlação de Spearman foi conduzida entre os dados das larvas e das dietas. Para todos os testes, considerou-se nível de significância de 5%. A massa larval unitária ao longo do período de experimento foi ajustada no programa JMP pro12 através de aplicação do modelo de crescimento de Gompertz com três parâmetros.

5 RESULTADOS

Como resultado da presente tese foi produzido um artigo publicado no periódico *Foods* (Fator de Impacto 4.7 - DOI: 10.3390/foods11233792) e outro manuscrito em processo de submissão no periódico *Future Foods* (Fator de Impacto 7.2).

5.1

Artigo: Innovation in Alternative Food Sources: A Review of a Technological State-of-the-Art of Insects in Food Products

Innovation in Alternative Food Sources: A Review of a Technological State-of-the-Art of Insects in Food Products

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Maior percentil (Scopus):	*98 (Health Professions (miscellaneous))*

Abstract: Insects present great potential for the food industry due to their easier rearing conditions and high nutritional value, in comparison with traditional livestock. However, there is a lack of evaluation of the technological status of food products developed with edible insects. Therefore, this study aims to analyze the emergent technological and scientific applications of edible insects in the food industry through a prospective study of patent documents and research articles. Espacenet was used as a research tool, applying the terms Insect, Pupa, Larva, or Nymph and the codes A23L33 and A23V2002. 1139 documents were found - 341 were related to the study. Orbit® was used to evaluate technological domains and clusters of concepts. Scopus database research was performed to assess the prevalence of insect research, with the term "edible and insect*”. The main insects used were silkworms, bees, beetles, mealworms, crickets, and cicadas. Protein isolates were the predominant technology, as they function as ingredient in food products or supplements. A diverse application possibility for insects was found due to their nutritional composition. The insect market is expected to increase significantly in the next years, representing an opportunity to develop novel high-quality/sustainable products.

Keywords: Patent study; Market trend; Edible insect; Food industry; Prospection; Novelty

1. Introduction

World hunger, sustainable food production, and waste reduction are global challenges for current and future generations. It is estimated that approximately one-third of the food produced in the world is lost [1]. In a conjunction report by FAO; IFAD; UNICEF; WFP; WHO [2], from 702 to 828 million people were suffering from hunger in 2021. Moreover, the COVID-19 pandemic resulted in a growing number of malnutrition by around 150 million since its beginning up until recent days [2]. Another recent and aggravating situation refers to the conflict between Russia and Ukraine, relevant countries regarding the exportation of agricultural products. With the ceasing of Ukrainian exports, commodities have highly increased in price worldwide and future harvests may not compensate for this issue. This may incur an increase in food insecurity by about 47 million people, compared with a context without war [3]. Therefore, malnutrition has been growing considerably in the last few years, which indicates a situation of urgency,that needs to be reversed . Research in the innovation area has focused on producing alternative sources that can combine efforts aimed at food and nutrition security [3,4]. Considering that foods of animal origin generally have protein quality superior to plants [5], insects are presented as potential ingredients for the food industry [3].

Edible insects are considered excellent sources of proteins, lipids, minerals, and vitamins, [6–8]. They also contain antioxidant compounds [9], essential amino acids [10], and polyunsaturated fatty acids [11]. Insects can be consumed whole or used as ingredients to fortify food formulations [12], to fulfill nutritional needs in countries that present a high prevalence of malnutrition and food insecurity [12]. They can also serve as nutritious food for healthy populations [13], using a source whose environmental impact is lower compared to conventional animal and plant proteins [14].

Studies indicate an increase in interest regarding the use of insects as alternative ingredients in the preparation of traditional foods, such as bread [15–17], pasta [18], cereal bars [19], soup [20], biscuits [21], sausage [22] and extruded snacks [23]. The insertion of 10% flour/powder of the cinereous cockroach (*Nauphoeta cinerea*) in bread, increased protein intake by 12.90% [24]. Moraes *et al*. [25] when developing cereal bars with flour/powder of *Tenebrio molitor* larvae, observed significant increases in lipid content (from 20.39 to 30.58%) and total protein (from 13.68 to 21.08%) for the elaborated product.

Edible insects are already produced on a commercial scale in several countries around the world. Following the report encouraging the production and consumption of insects by FAO [26], countries in North America, Europe, Africa, and Asia have installed insect production systems for human and animal consumption, called insect farms. These are structures that must meet specific requirements for proper operation, considering that each species has its intrinsic characteristics [27]. European agencies have published regulations for the production and consumption of edible insects as human food, such as the Federal Agency for the Safety of the Food Chain of Belgium (AFSCA), which released general standards for the rearing and marketing of insects and derivatives in 2014; and the marketing permission published by the Swiss Federal Food Safety and Veterinary Office (FSVO) in 2017, which now allows the marketing of products based on crickets, grasshoppers and larvae of *Tenebrio molitor* [28,29].

The European Commission for Food Safety, through Regulation 2015/2283, authorized the use of *Tenebrio molitor* larvae in food for human consumption and guaranteed its safety [30].

It is known that edible insects are an alternative to replace traditional food sources. There are several other potential benefits of its creation, such as environmental, economic, and social [31]. The concept of biorefinery, for example, deals with the conversion of biomass from agroindustrial waste into various products with higher added value [32,33]. An insect biorefinery corresponds to an integrated technology that allows the valorization of organic waste through its transformation into biomass rich in nutrients and bioactive compounds [34].

The global edible insects market presented in 2018 a value close to US$ 400 million, with a forecast to grow approximately three times by 2023, indicating great investment potential [35]. The mass production of edible insects, on the other hand, was predicted at approximately 200,000 tons in 2020 and should increase to 1.20 million tons in 2025 [36– 38]. Considering that this is a market with great potential, the development of a technological prospection is important to identify trends and potential investments in the area.

Patent documents represent an invention deposited in a specific technological field, being, therefore, one of the main sources of information regarding new technologies [39]. The analysis of patent documents generates valuable information for organizations, by enabling the determination of the originality of their inventions, as well as the verification of technologies protected by competing organizations [38]. It is also an important tool in decision-making and can facilitate the appropriation with quality of Intellectual Property, by increasing the critical sense and broadening the vision of existing technologies and opportunities [40].

The present study, therefore, aims to evaluate the application of edible insects in the food industry through a prospective study, focusing on elements such as insects most predominantly used in patents, main countries holding technologies, types of products developed, and claims/functions associated with these products.

2. Review Methodology

The methodology consisted of searching for patent documents in the international database Espacenet in August 2022. Espacenet is a database of the European Patent Office (EPO), where patent documents from more than 100 countries can be found. The search strategy used keywords to be found in the titles and/or abstracts and two codes directly associated with the search objective. The choice of keywords (Insect, Pupa, Larva, or Nymph) was made to consider all stages of insect development and avoid underestimation of the data. In addition, the codes A23L33 (Modifying nutritive qualities of foods; Dietetic products; Preparation or treatment thereof) and A23V2002 (Food compositions, function of food ingredients or processes for food or foodstuffs) were selected so that the search would retrieve only documents directly associated with the food industry. The Boolean operators (OR) and (AND) were used so that at least one code and one keyword were present in the documents evaluated. The truncation operator (*) was used to find derivations of the keywords. The documents selected for data processing were those that presented greater proximity to the proposed theme. The information regarding the available documents was exported to Microsoft 365 Excel using the CSVed software, for later analysis. The Orbit® was used in association with Espacenet to evaluate technological domains and clusters of concepts more prevalent in patent documents. A search in the scientific database Scopus was performed to assess the prevalence of insect research until August 2022. The term "edible and insect*" was used, to be found in the titles, abstracts, or keywords of documents.

The descriptors selected in the search for patent documents are highlighted in bold in Table 1. The search resulted in a total of 1139 patent documents. After reading the titles and abstracts, only 341 documents were within the proposed theme and were selected.

Table 1. Scope of prospection of patent documents by keywords and IPC/CPC codes in the Espacenet database.

Insect *	*Pupa* *	*Larva* *	*Nymph* *	A23L33	A23V2002	Results
X						> 10.000
	X					3.137
		X				> 10.000
			X			898
X				X		341
X					X	359
	X			X		227
	X				X	151
		X		X		215
		X			X	137
			X	X		36
			X		X	19
X	X	X	X	X		751
X	X	X	X		X	621
X	**X**	**X**	**X**	**X**	**X**	**1139**

3. Annual Evolution in the Deposit of Patent Documents and Scientific Articles

Figure 1 shows the deposit of patent documents and scientific articles related to the development of food products containing edible insects. In 1991, the first patent registration occurred in China - CN1055644A [41]. This invention refers to the development of food with health claims that use cicadas as an ingredient.

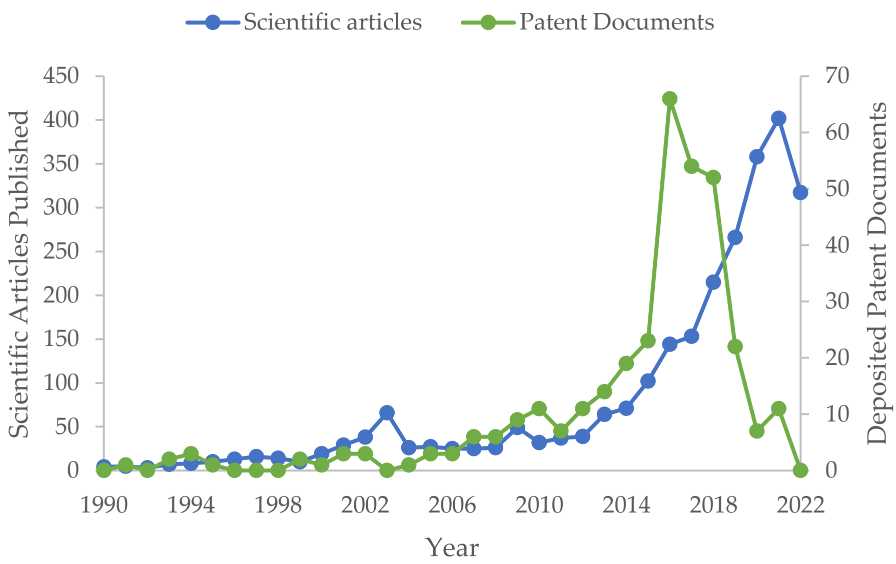

Figure 1. Global trend in the publication of patent documents and scientific papers* associated with edible insects. *Number of scientific articles published between 1990-2022 on edible insects, according to the Scopus database.

In the 1990s, the number of patent document filings related to the evaluated technology was low. However, as of 2007, it is possible to observe a slight increase in the average number of documents filed. In 2010, functional foods with insects represented the majority of patented technologies (36.36%), followed by food supplements (27.27%). As an example of technologies developed in 2010, patent CN102370666A [42] refers to a powdered energetic supplement for athletes, obtained from freeze-dried water beetle (*Cybister tripunctatus orientalis*). The patent KR20110121223A [43] deals with a functional food powder, encapsulated, containing silkworms, with the ability to control the effects of menopause. In 2010, the Food and Agriculture Organization (FAO) published the document entitled "Forest insects as food: humans bite back" [44], reporting the proceedings of an international workshop held in Chiang Mai, Thailand, in 2008. The workshop was developed by FAO Regional Office for Asia and the Pacific, which invited experts in the field of entomophagy to discuss topics such as management, processing, marketing, and consumption of edible forest insects, in addition to assessing the economic potential of insect production by local farmers [44].

As of 2013, the technology showed an exponential increase in annual average deposits, a trend that may be associated with the dissemination of a document entitled "Edible insects: future prospects for food and feed security" by FAO [26]. This document is a collaborative production between FAO and the Entomology Laboratory of Wageningen University in the Netherlands and aimed to conduct a comprehensive assessment of the contributions of edible insects to food security and livelihoods in developed and developing countries, and stimulate the national and international agencies to invest in the scientific research in sustainability, and food security [26]. Still, in 2013, the researcher Arnold van Huis published the review article "Potential of Insects as Food and Feed in Assuring Food Security" [31], indicating a trend in the use of edible insects as alternative nutritional sources of quality and sustainable, thus arousing the interest of the scientific community in conducting new research in the area.

The facts that occurred in the year 2013 may represent milestones responsible for the subsequent peak in the filing of documents that occurred in 2016 (66 patents filed). Most patents filed this year had their origin in Asia (97.40%), where 61.04% of the filings occurred in China and 36.36% occurred in the Republic of Korea, countries with high technological development and consolidated entomophagy culture [11,45].

The growth in the number of patent document filings has also been accompanied by an increase in the number of published scientific articles. Van Huis and Oonincx [13] demonstrated that the interest in insect research is illustrated by the many articles referenced. In 2010, there were 32 articles listed in the Scopus database using the term "edible and insect*", in 2016 this number increased by over 346% (143 articles), peaking in the years 2020 and 2021 with 358 and 401 articles respectively (query on 14 August 2022) (Figure 1). Finally, in the years 2020, 2021, and 2022, a reduction in the number of patent deposits is perceived. This may be associated with the secrecy period, which can last up to 18 months.

In general, there is a progressive increase in the number of patent documents and scientific articles, which indicates that the technology is still recent and has room for growth and development, not yet presenting scientific-technological maturity. Entomophagy is an ancient practice [46], however, only after FAO's efforts, it was noticed a stimulus in the production of technologies and research within the theme, which have been developed very recently. Some authors discuss the presence of several gaps to be solved by conducting more scientific studies, mainly focused on nutritional quality, management, cultivation, and ethical issues in insect production, which are still scarce [47,48].

4. Main Technology Domains

Figure 2 deals with the technological domains pertaining to the patent documents selected in Orbit. Food chemistry presented the greatest contribution, with 65.80%, followed by the pharmaceutical area, with 14.60%. As for the areas of study present in the scientific articles evaluated in Scopus, there is a greater prevalence of Agricultural & Biological Sciences (42.08%) and Biochemistry, Genetics & Molecular Biology (11.71%). The predominance of these sectors can be recognized by the fact that most patent documents and scientific studies are dedicated to the use of insects to develop nutritionally rich food products (emphasis on proteins and lipids) or as sources of bioactive compounds with various beneficial effects to the human body [49]. Considering that the use of insects in the food industry is a subject still in expansion, from the identification of technological domains less studied it is possible to verify opportunities for the development of new technologies [39]. In this case, the domains of Organic fine chemistry, Biotechnology, Other special machines, and Basic materials chemistry are highlighted as less explored areas. As examples of applications of these domains: the document CN111000021A [50] deals with equipment capable of producing insect protein powder for human consumption. It is composed of the main base, feeding device, dryer, crusher, supercritical extraction machine, and freeze-dryer. Document KR102098079B1 [51] is about a fermentation process of *Protaetia brevitarsis* by *Bacillus subtilis* to develop a digestible food formula with medicinal properties associated with liver function improvement.

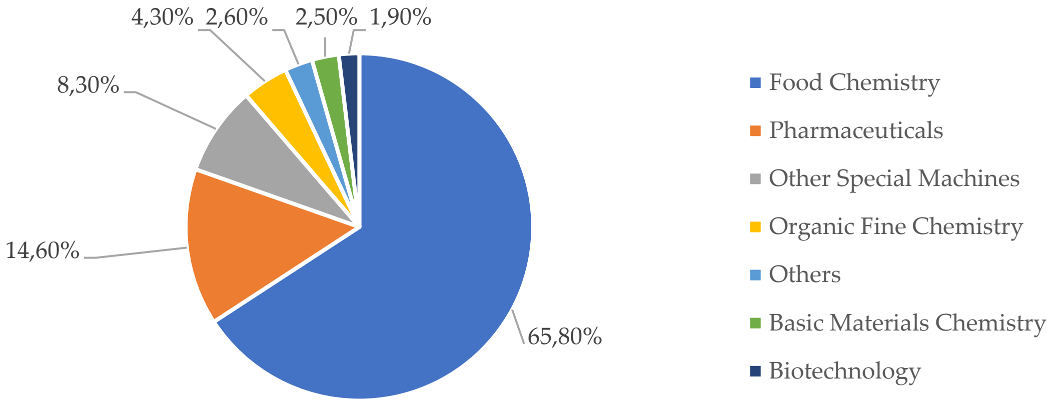

Figure 2. Technological domains present in patent documents based on the use of edible insects and food products. Source: Q. Orbit, ®.

5. Main Depositors

Private companies predominate the sector (Figure 3), with 38.87% of the deposits of patent documents, followed by independent inventors (34,58%), universities (14.75%), and government institutions (11.80%). It is possible to note that the sum of patent deposits by universities and governmental institutions does not exceed the deposits of the private sector. It is then emphasized the need to increase the interactions between universities and the industrial sector. The establishment of this partnership can generate financing, and exchange of knowledge, in addition to stimulating the process of technology transfer in a cooperative system and the commercial use of the results achieved [52,53]. To this end, the training of teachers and researchers about the protection of intellectual property, its applications in the market, and the stimulus of communication/cooperation with companies through internship programs and Research and Development actions, are essential to result in a greater insertion of universities on patent filing [54]. Another relevant point is the establishment of technology transfer offices, designed by universities which can disseminate research, commercialize inventions for revenue generation and facilitate interrelationships with other agents of the innovation system (industries and government) [55].

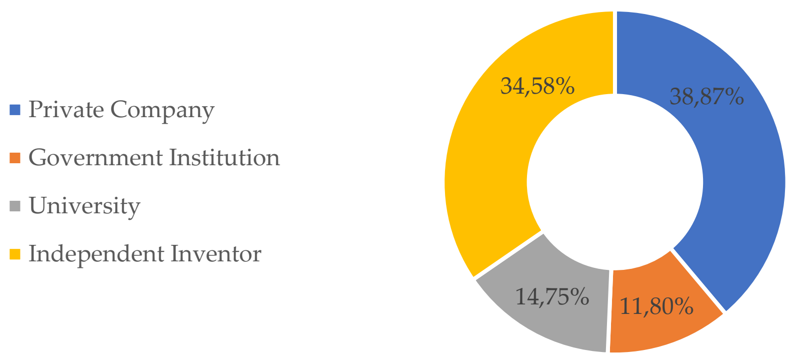

Figure 3. Distribution of applicants of patent documents.

The Korean Rural Development Administration (RDA) holds 18 out of 341 documents from patents assessed (Figure 4). The RDA is a government institution based in Jeonju City - Republic of Korea. The company is known for fostering research with edible insect species, relevant genetics research, and material extraction, among other topics. Since its establishment in 1962, it has been playing a crucial role in the development of agricultural technologies in South Korea [56].

Most of its patents (15) relate to food compositions with functional/health claims: antithrombotic (4 - KR102150121B1, KR101828294B1, KR101962008B1, KR101758718B1 [57–60]), anti-inflammatory (3 - KR102169046B1, KR101424125B1, KR101382400B1 [61–63]), anti-obesity (3 - KR102159019B1, KR20180075888A, KR101491771B1 [64–66]) antioxidant (1 - KR102150122B1 [67]), sexual function improvement (1 - KR100462166B1 [68]), internal organ protection (1 - KR102121807B1 [69]), hangover improvement (1 - KR101055252B1 [70]) and hepatoprotective (1 - KR101702053B1 [71]). The document KR101758718B1 [71] has as an object of the invention a food with the claim of antithrombotic effect, developed with compounds isolated from *Protaetia brevitarsis*. These compounds were identified by [72] to be indolic alkaloids with the potential to inhibit platelet aggregation.

Hwang Jae Sam, Kim Mi Ae, and Yun Eun Young were the most frequent inventors and coinventors in RDA filings, with 9, 8, and 9 documents each, respectively. These inventors also developed scientific studies in recent years that evaluated insect bioactive compounds with beneficial functions for the body, such as glycosaminoglycan derived from *Gryllus bimaculatus*, a substance with potential adjuvant for the treatment of chronic arthritis [73]; and ethanolic extract of *Tenebrio molitor* larvae with anti-obesity effect [74], which is in line with technologies patented by RDA in the area of edible insects.

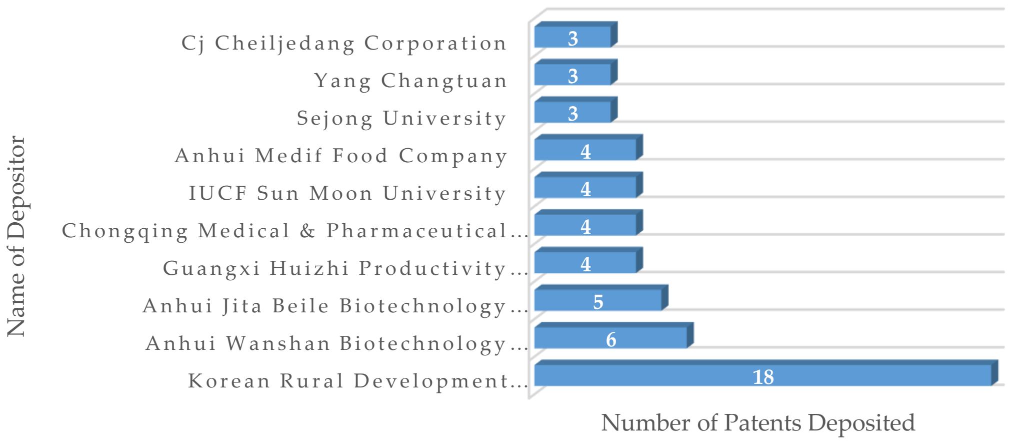

Figure 4. Main applicants of patent documents associated with the development of food products with edible insects.

The Chinese company Anhui Wanshan Biotechnology, located in the city of Hefei, submitted 06 patent applications regarding insects and food products. All the patents associated with the company report the production of enriched bee larval protein powder with minerals.

An example, the patent CN103622038A [75] discloses a multifunctional bee larvae protein powder rich in zinc. The high content of the mineral, according to the patent, would be associated with the dietary modulation of bee larvae. Some studies have shown that the nutritional composition of edible insects can be modified through their diet and can even make them sources of nutrients that commonly they would not be if they consumed a natural diet [76–78]. Regarding mineral composition, differences are attributed almost exclusively to diet, as minerals are not synthesized in the animal body, but rather accumulated after dietary intake [76]. Moreover, bee protein seems to present potential from a nutritional and environmental point of view, and can serve as a substitute ingredient in preparations, such as hamburgers [79], with a balanced amino acid composition [80].

Chongqing Medical and Pharmaceutical College was one of the leading universities to file patents associated with the subject of this study, with a total of 04 filings. It is a university established in 1948, located in the university town of Shapingba, China, a cultural district in Chongqing. Its structure is composed of 19 colleges, including the Food Nutrition and Testing College [81]. The documents deposited by this University are associated with the application of dragonfly nymphs in foods, such as sausage - CN108077797A [82], canned food - CN108077805A [83] and dumplings - CN108142798A [84] and CN108175042A [85]. According to the documents, dragonfly nymphs have a unique and pleasant flavor, are widely accepted by consumers and their application represents a way to enhance the country's food culture. In China, the consumption of dragonflies is relatively common in preparations such as vegetable soup, scrambled eggs, or even roasted dragonflies [86].

6. Key Inventors

As for the applicants, there is a prevalence of inventors of Asian origin, with the top 10 having at least co-ownership in 4 patents filed (Figure 5).

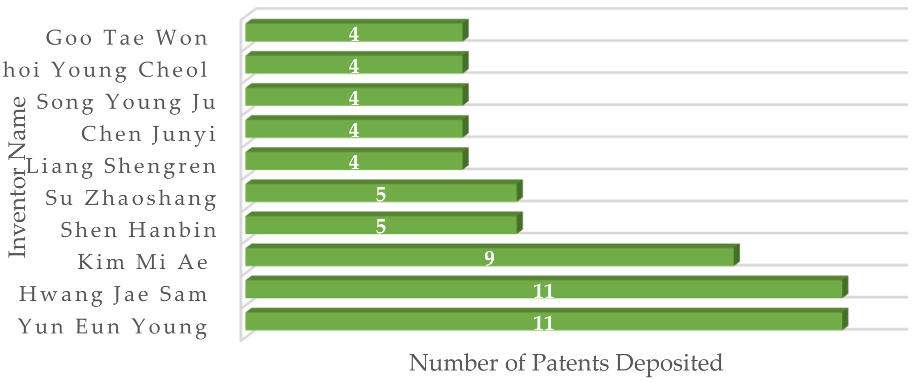

Figure 5. Main inventors of patent documents associated with the development of food products with edible insects**.**

The Korean inventor Eun Young Yun, presented the largest number of patents (11), most of which were filed on behalf of RDA or through partnerships between the RDA and research institutions, such as Sejong University (Seoul, South Korea), where he teaches and researches with edible insects in the Department of Integrative Bioindustrial Engineering [49]. Most of the patented technologies deal with foods with functional and health potential (9). As an example, the patent KR101702053B1 [71] refers to the development of a food ingredient consisting of *Allomyrina dichotoma* (beetle) powder. As possible uses, the patent states that the powder can be inserted in meats, sausages, bread, chocolates, sweets, confectionery products, pizza, noodles, gums, ice cream, and dairy products, resulting in foods with hepatoprotective and anticancer potential. In a study developed by Lee *et al*. [87], in which the inventor Eun Young Yun participated as a co-author, *Allomyrina dichotoma* powder was able to reduce hepatotoxicity in rats, considering the acute and chronic signs. In addition, the fractionation of *Allomyrina dichotoma* extract with ethyl acetate showed cytotoxicity to various tumor cells through the induction of apoptosis and necrosis [87].

Jae Sam Hwang also submitted 11 stored documents, all of them had the RDA as the applicant, possibly because he develops his activities as a researcher at the National Institute of Agricultural Science (NAAS), an institute inserted in the RDA. Most of his patents (9) presented cooperation with researcher Eun Young Yun in the invention process, indicating the presence of a partnership between the university and government agency. As an example of technology produced, the patent KR101424125B1 [62] presents *Tenebrio molitor* as a food ingredient in powder or suspension form to be used in the preparation of gums, teas, vitamin complexes, functional foods, tablets, capsules, or beverages in order to confer the antioxidant and anti-inflammatory capacity to the product. In a study developed by the same inventor [88], the extract of *Tenebrio molitor* showed DPPH radical capture activity of 81.17% and nitrite capture of 43.69%, confirming the antioxidant potential described in the patent.

7. Origin of Technology

Of the total patent documents reviewed, China presented 210 deposits, which represents 61.58%, followed by the Republic of Korea with 111 (32.55%), and the remainder distributed among other countries (Figure 6). Together, these countries were responsible for 94.13% of the patent applications related to the use of edible insects in food products. This arrangement is not the same observed for the number of scientific articles related to edible insect technology. According to data evaluated in the Scopus database, although the development of the technology is mainly in China, the U.S. ranks first in terms of scientific publications, demonstrating that China is more focused on developing technologies with market applications.

The entomophagous culture in China has been reported from at least 3,000 years ago. In addition, approximately 300 species of edible insects have already been classified in this country [86]. Compared to other countries in the world, China has invested more intensively in green technologies. For example, in 2007, the Chinese government proposed the National Climate Change Program, which focused on controlling greenhouse gas emissions, overhauling agricultural systems, and stimulating research and development [89]. Therefore, between 2000-2015, China represented the forefront of technological innovation related to green technologies, with a considerable increase in patents filed in the area [89].

In China, insects are bred for human food, medicine, and animal feed through domestication and partial or full captive breeding [90]. An example, the Chinese company Gooddoctor-Panxi Pharmaceutical is responsible for cultivating *Periplaneta americana* cockroaches for developing medicinal formulations with healing properties [91]. On average, 6 billion cockroaches are raised by the company, annually, which generated a total of US$684 million in revenue during its period of activity [92]. In 2018, the company Bugsolutely launched the first snack containing silkworm powder in China [93]. It is a snack containing high protein content and uses one of the main byproducts of sericulture: the silkworm, after silk collection [94].

The edible insect and insect protein industry in China accounted for USD 49.1 million in 2018. An increasing population adhering to a healthy lifestyle may account for higher market revenue in the future. The economic forecast is expected to increase to US$ 115.7 million/year by 2023, which would generate a compound annual growth rate of 18.7% [95].

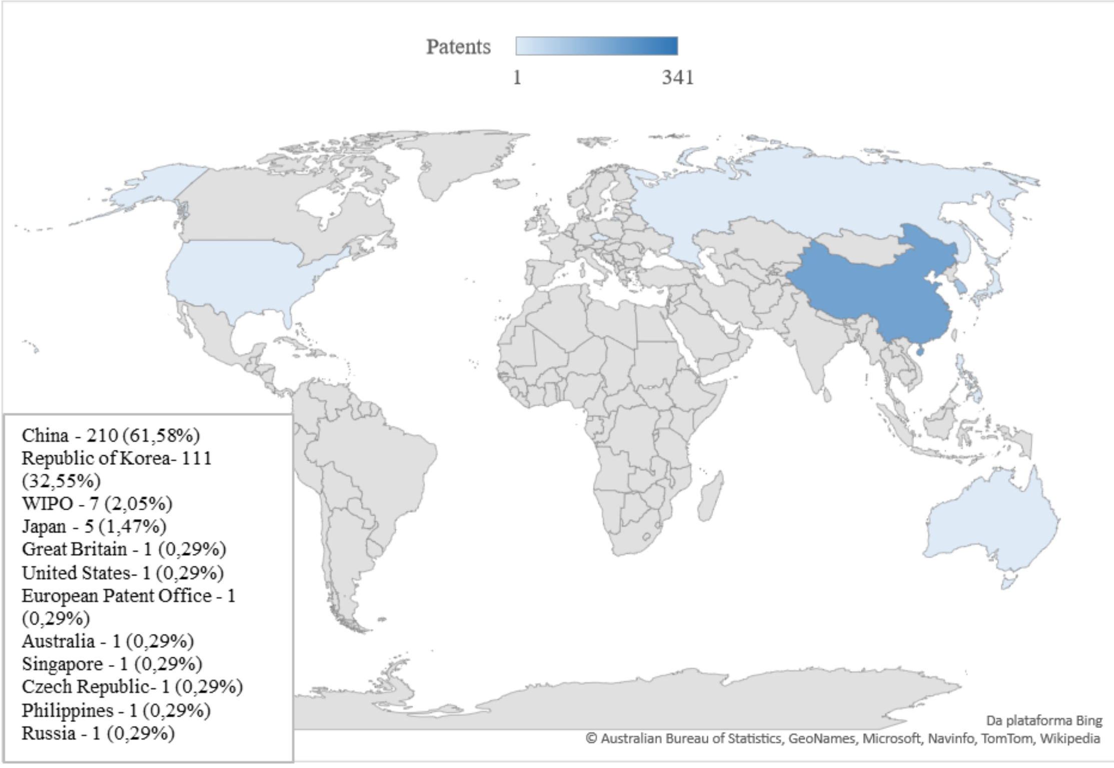

Figure 6. Worldwide distribution of patent documents associated with the development of food products with edible insects.

Much of Korean scientific and technological productions can be associated with governmental incentives. As an example, in 2006 the "Bio-Vision 2016", a national investment program in the biotechnology area, was launched with the purpose of making the country one of the largest biotechnology producers in the world. As a result, there was the development of many research institutes acting in conjunction with large companies and the country met a significant stimulus in technological production through patents filed in this area and scientific productions of relevance. [96,97].

The Korean government, in 2010, released the Act on Fosterage and Support of the Insect Industry [98], aiming to increase the income of the families of insect breeders, stimulate the national industry, and basic formation for the development of this area. Since then, the production of insects has grown intensively [99]. As an example, Jeju Gold Larva Co., Ltd, a farm established in 2017, is responsible for breeding *Protaetia brevitarsis* beetles. The enterprise developed a product with this insect, which had been patented (KR102056108B1 [100]). It is a formula for hangover control and liver function improvement. The product, called Bengjooya, is for sale on the company's website [101].

At the Korean market level, the company named Korean Edible Insect Laboratory (KEIL) is a pioneer in the research and development of insect-derived products. The company has been responsible for the large insertion of edible insects in the Republic of Korea in recent years and presents market dominance [102]. KEIL has developed several partnerships with other enterprises, such as Jeongpoong, in the development of products such as soups and ice cream with insects; CoffeeNie Café, where cookies and energy bars are distributed to approximately 200 stores in the country; and Korea Matsutani Corporation, which prepares confectionery products [102]. The Global Food company has also been developing insect-based products, such as Korean sausage with powdered mealworms [103]. The annual revenue of the edible insect market in South Korea was estimated at $16.3 million in 2018. It is projected to increase to US $32 million by 2023, with a compound annual growth rate of 14.4% [104].

This information corroborates the idea that the edible insect market presents great investment potential. There is still plenty of room for growth, even in countries with more consolidated markets, such as China and South Korea.

8. Type of Product Developed

Figure 7 indicates the types of products developed. In general, foods with functional claims were very present in the patent documents. A total of 230 documents dealt with food products with some functional claims. As an example, the patent CN107997142A [105] reports a porridge with a constipation improvement claim, containing bee pupa powder. Consumers around the world have become aware of the impact of food on their health and, therefore, have been increasingly consuming functional foods [106]. The global functional food market in 2018 had a total value of US$ 161.49 billion, with a forecast to grow to US$ 275.77 billion by 2025 [107,108]. Edible insects are important sources of bioactive compounds to be used as ingredients in food products, to generate various health functions, such as antihypertensive, antimicrobial, and antioxidant [109].

Figure 7. Main types of products developed in patent documents associated with the development of food products with edible insects.

Protein isolate powders have been patented 48 times. Patent CN101124936A is intended to provide a method for isolating the protein from *Tenebrio molitor* through refining, filtration, separation, and freeze-drying processes to result in a powdered product. Patent CN109170118A [110] deals with the production of silkworm protein powder. The innovation is the use of a simple extraction process, with high protein yield and little environmental pollution, resulting in a fine, white, odorless powder with high protein content. The protein content of insects is known to be higher than that of beef or pork [111,112]. Insects can show variation from 40 to 75% protein in dry matter, depending on the species and stage of development [113]. Insect protein also presents excellent quality, being a source of enough essential amino acids to meet FAO / WHO requirements for adults [114,115]. In a study developed by Lee *et al*. [116], the protein digestibility of *Protaetia brevitarsis* larvae was significantly higher than the bovine loin, confirming the feasibility of using insects as a protein source in the food industry.

Protein isolates are acquired through some general technological steps, such as pretreatment, defatting, solubilization, protein recovery and purification, and drying [117]. The resultant can be used as an ingredient in food or as a supplement, to enrich food products, and complement or supplement protein intake in healthy or malnourished people. The use of insect protein isolate can have advantages by masking its odor and other undesirable characteristics, favoring its acceptance as an ingredient in food products; besides having better absorption by the body by separating the protein fraction from components that compromise its digestibility [114,118].

Various preparations were identified in 34 documents. The types of preparations varied from Asian dishes to dumplings. Preparations with rice stand out by their presence in 8 patents. Rice is a cereal present in the diet of half the world's population. Moreover, approximately 90% of the production and consumption of rice is in Asia, which may justify the findings of this study [119]. For example, patent CN103494094A [120] deals with the development of protein rice containing silkworm and bee powder as ingredients, which according to the document, confer a greater nutritional contribution, in addition to good digestibility; and the patent KR20200004161A [121] presents a method for preparing tofu using an edible insect, citing cricket as one possibility. The patent reports that by adding the insect to the tofu preparation, the protein content can be enriched by approximately 60%.

The high prevalence of products categorized as nutritional supplements in this study (36 times) may be associated with the fact that edible insects have high energy density and high fat, protein, and mineral content [122]. For example, patent CN110150653A [123] deals with the development of a protein bar, containing peanut protein powder, silkworm pupa protein powder, olive oil, modified soy phospholipid, stevioside, konjac flour, glycerin, multivitamin powder, milk, potassium sorbate and vanilla powder. The ingredients are mixed, formatted as a roll and vacuum packed. Patent KR101622784B1 [124], on the other hand, relates to a diet energy bar containing quinoa, nuts, and the whole powder of an edible insect, thereby taking advantage of the nutritional richness of whole insect use.

Edible insects are already used in several regions of the world as an affordable nutritional supplement because of their quality and higher concentrations of nutrients, despite the perception that they are considered as delicacies or exotic products [122]. Flours and/or insect powder, are excellent options for consumption and incorporation as an ingredient in products because the ingestion of whole insects or parts of them, still causes repulsion in consumers, because they consider entomophagy as a primitive and repugnant practice [125,126]. In the development of vegetable soup enriched with termite (*Macrotermes bellicosus*) based flour, authors observed that the insertion of 23% of the insect flour resulted in an increase of approximately twice the protein content (29.94%) compared to the control soup (15.03%) [20]. Oat biscuits enriched with 5, 10 and 15% *Acheta domesticus* flour showed increased protein content by 18.35, 36.81, and 55.16%, respectively, compared to the control [127]. The increase in nutritional contribution observed in products with insects occurs because these are important sources of macronutrients such as proteins, lipids, and carbohydrates, thus, the use of insects in its processed form (such as flour/powder) can add more nutrients to products and favor the sensory acceptance [31].

Some studies have shown that acceptance of foods containing processed insects is good and tends to vary with their proportion in the products. As an example, bread produced with grasshopper (*Schistocerca gregaria*) flour [17] showed higher sensory acceptance when the grasshopper flour was present at a lower concentration. Muffins developed with cricket powder [128] had wheat flour replacement with 2, 5, and 10% insect flour. Samples with 2 and 5% were found to have higher sensory acceptance with higher taste scores compared to the control. The products with 2%, 5%, 10%, and the control obtained, respectively, scores of 7.1, 7.0, 6.9, and 6.2 on a hedonic scale of acceptance ranging from 1 to 9. Thus, it is evidenced that the addition of insects to food products is not harmful to their sensory characteristics. This indicates future trends and the feasibility of food products with insects since from sensory studies it is possible to reach an insect proportion acceptable to consumers, increasing thus, the consumption of a product potentially richer in nutrients.

9. Nutrients Associated with Patented Products

In relation to the nutrients described in the patent documents (Figure 8), the protein fraction has the highest value, being cited in 165 documents. The average protein content is variable, ranging from 40% ± 14% for insects of the order *Isoptera* (termites) to 64%± 20% for insects of the order *Blattodea* (cockroaches) when evaluated on a dry matter basis [129]. Insect protein has good digestibility when compared to casein or soybean, however, it can be improved by removing chitin, the main component of the exoskeleton [130]. Factors that may contribute to variation in the protein content of edible insects of the same species include differences in diet, developmental stage, location, and season of insect collection [129].

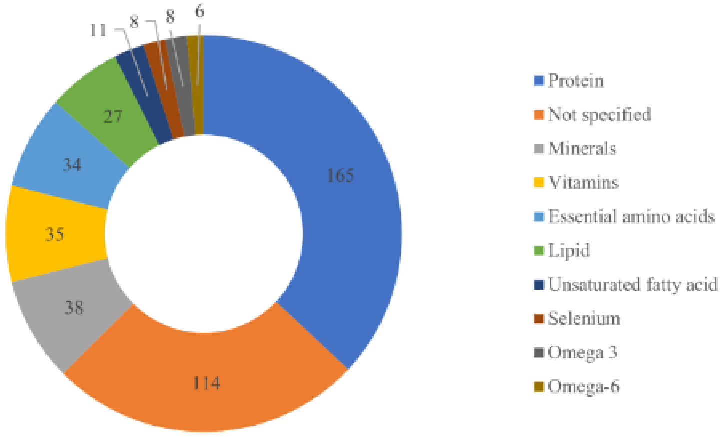

Figure 8. Main nutrients cited as components of the products described in the patent documents.

Essential amino acids were also among the nutrients in evidence (34 citations). The quality of a protein source is determined primarily by its amino acid composition. Thus, essential amino acids are key parameters in the evaluation of food quality. According to Akhtar & Isman [130], a large proportion of insects possess enough of the essential amino acids to meet the daily intake requirements of adults. In a study by Mba *et al*. [131], all essential amino acids showed a score above 1 in the protein of *Rhynchophorus phoenicis* (beetle) larvae, indicating that they were present in sufficient amounts, considering the protein reference standard for human consumption [115]. The sum of total essential amino acids was two times higher than the WHO reference protein standard and leucine was the most abundant essential amino acid in this insect [115,131]. Ademola *et al*. [132] found variation in essential amino acid concentration between 44.2 and 46.8% when analyzing the protein quality of *Apis mellifera* (honeybee), *Macrotermes bellicosus* (termite), *Rhynchophorus phoenicis* (beetle) and *Anaphe infracta* (silkworm), which indicates adequacy with WHO [115] requirements for adults. The limiting amino acids in the study were valine in termites and silkworms, threonine in bees and lysine in beetle larvae.

Köhler *et al*. [10], when analyzing the nutritional composition of grasshopper (*Patanga succincta*), beetle (*Holotrichia* sp.), house cricket (*Acheta domesticus*), and silkworm (*Bombyx mori*), found that only silkworm met FAO / WHO requirements, considering a minimum of 40% essential amino acids and 0.6 essential/non-essential amino acid ratio [133]. In addition, tryptophan was found to be the limiting amino acid in grasshopper and cricket, lysine in beetle, and leucine in silkworm. From these studies, there is great variability between species, however, in general, insects show good protein quality. Also, the amino acid gap present in diets rich in cereals, usually poor in leucine, lysine, and tryptophan, can be overcome by the consumption of insects [10].

Insects are also considered lipid sources. According to this study, 27 patents described their products as a lipid source. A study developed by Ray & Gangopadhyay [134] verified total lipid content between 24.16 and 26.21 g/100g dry matter from the indian silkworm (*Samia ricini*). In addition to this, α-linolenic acid was the most abundant in this species, making up between 38.97 and 40.20% of the total fatty acids. Paul *et al*. [135] identified a lipid content of 15% in *Acheta domesticus* (cricket) and 32% in *Tenebrio molitor* (mealworm larvae) on a dry basis, with a predominance of α-linolenic acid in *Acheta domesticus* (41.39%) and oleic acid in *Tenebrio molitor* (35.83%). An advantage of insects is the plasticity of their fat composition, which can vary depending on the species and the type of diet [136]. A study developed by Lehtovaara *et al*. [137] found that the type of diet can significantly modify the fatty acid composition of grasshoppers *Ruspolia differens*, mainly in relation to polyunsaturated fatty acids. Diets rich in linoleic and α- linolenic acids were able to increase tenfold the content of these fatty acids in the insect.

10. Insect Species Used in Patent Documents

According to Figure 9, the silkworm was the insect most cited in the patent documents (105). The silkworm (*Bombyx mori* L.) has been bred in China for a long time [138], because of the market for silk obtained from its cocoon. Since the beginning of its rearing 5,000 years ago, China remains the largest producer of silk and, therefore, the largest producer of silkworms, with an annual production of about 500,000 tons of pupae, accounting for 70% of world production [139].

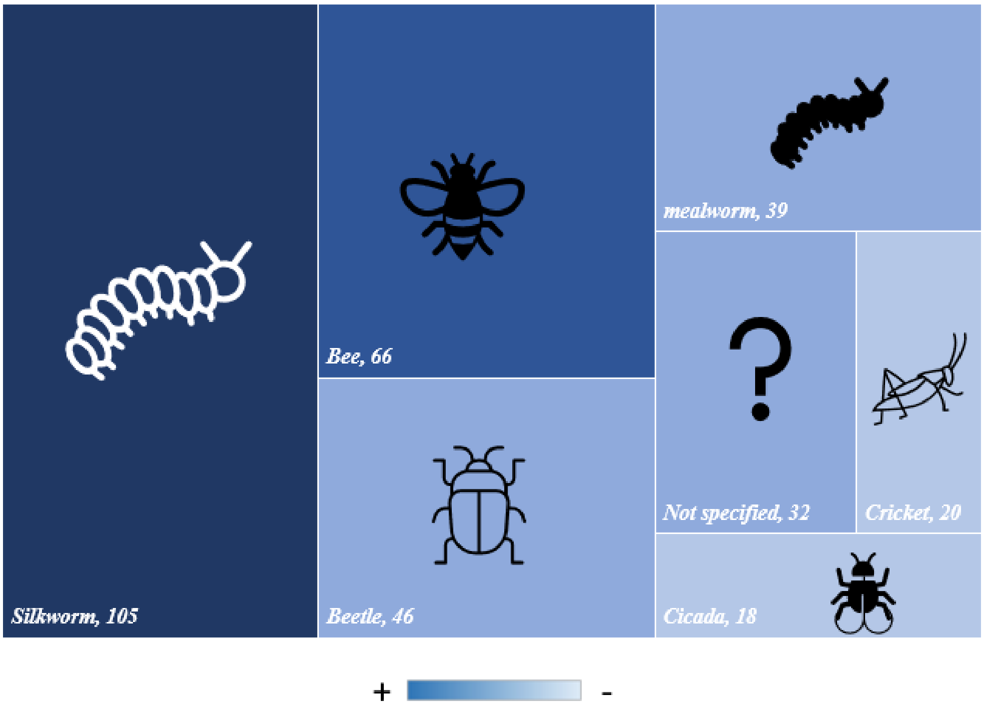

Figure 9. Insects used in the products described in patent documents.

Silkworm pupa contains about 51% protein and 30% lipids. Products such as canned and powdered silkworm pupa are also available in the Chinese market [139]. In addition to this, the Chinese Ministry of Health has recently started to consider silkworm pupae as a new source of protein for Chinese consumers, which has generated great interest in research with this insect [139]. Park *et al*. [140] investigated the physicochemical properties of a meat product developed with the replacement of pork by 5%, 10%, and 15% of silkworm (*Bombyx mori*) pupae powder. As a result, higher contents of protein were found, from 18.25% in the control to 26.58% with the incorporation of 15% silkworm powder. Akande *et al*. [141] demonstrated that silkworms can serve as an alternative to meat in the production of pie and pastry fillings for their high protein and mineral content. The amino acid profile also showed that the protein is of high quality, highlighting the contents of lysine (8.42%), leucine (7.63%), and total essential amino acids (54.44%). In addition, sensorially, there was no statistical difference between the control sample and the one stuffed with the insect.

Another insect with representation in the documents, was the bee, with 66 patents. The bee is also an insect of millennial breeding, known for the high nutritional and therapeutic value of the honey produced by it. Similarly, to the silkworm, it is also common to consume bee larvae consumption of bee larvae and pupae, especially in Latin America and Thailand [31]. Bees can be considered good sources of protein (35% and 46-57% in larvae and pupae, respectively), and have a lipid profile composed of the fatty acids oleic, linoleic, linolenic, myristic, palmitic, and stearic in large amounts [80]. Ulmer *et al*. [79] verified the feasibility of burgers by replacing meat with bee biomass. As a main result, it was proposed that to achieve the average protein content of a hamburger (30%), it would be necessary only 462g of bee biomass, a value much lower than that of meat (1kg), indicating better yield and, consequently, lower environmental impact.

Mealworm was described in patent documents 39 times, and *Tenebrio molitor* was specifically mentioned 18 times. Among the mealworms, three species are most produced at the commercial level: *Tenebrio molitor*, *Zophobas atratus*, and *Alphitobius diaperinus* [142]. A study developed by van Broekhoven *et al*. [142] verified lipid content between 18.9 - 27.6% of dry matter for *Tenebrio molitor*; 32.8 - 43.5% for *Zophobas atratus* and between 13.4 - 24.3% for *Alphitobius diaperinus*, rich in palmitic, oleic, and linoleic acids in the three species. The highest protein content was found for *Alphitobius diaperinus* (65.0%), followed by *Tenebrio molitor* (48.6%) and *Zophobas atratus* (42.5%). Concerning products made with mealworms, García-Segovia *et al*. [143] developed extruded snacks with the addition of 5% *Alphitobius diaperinus* or *Tenebrio molitor* powder and verified an increase in protein content of 33.57% and 25.73%, respectively. In addition, *Tenebrio molitor* powder was able to significantly increase the selenium content in the snack compared to the control. Breads fortified with 5% and 10% *Tenebrio molitor* were developed and have presented an increase of up to 27% in protein content compared with control bread (wheat flour). In addition, bread fortified with 10% *Tenebrio molitor* showed higher amounts of tyrosine, methionine, isoleucine, and leucine [16]. In 2018, the European Food Safety Authority (EFSA) was invited to provide an opinion on the use of *Tenebrio molitor* in the food industry, in its whole form or as an ingredient in food product development. As a final opinion, the use of *Tenebrio molitor* was considered safe for food application in Europe [30]. In the Republic of Korea, the Korean Food and Drug Administration (KFDA) authorized the use of *Tenebrio molitor* as a safe food ingredient in 2015 [11].

According to the present study, cricket was cited in 20 patent documents. The species *Acheta domesticus* L., known as house cricket, has been used as a food source worldwide [144]. Thailand, in specific, is one of the countries with the largest breeding of crickets in the world, containing, approximately, 20,000 insect factories [145] and even developed the guidelines for the rearing of crickets to standardize production methods and, consequently, maintain a minimum quality [146]. In addition, the Korean Food and Drug Administration (KFDA) also authorized the use of *Gryllus bimaculatus*, another known and globally produced cricket species, as a safe and applicable ingredient in the food industry [11]. Kulma *et al*. [147] found the lipid content for *Acheta domesticus* L. between 12.9 and 21.7 g/100g dry matter and proteins of 61.2 - 69.6 g/100g. Udomsil *et al*. [148] when evaluating the composition of *Acheta domesticus* and *Gryllus bimaculatus*, reported protein contents of 71.7% and 60.7% and lipids of 10.4% and 23.4%, respectively. The predominant fatty acids in both species were palmitic, oleic, stearic, and linoleic. All essential amino acids were present, with higher values of valine, leucine, isoleucine, and lysine. Pasta developed with cricket powder (0, 5, 10, and 15%) as a substitute for semolina, showed increases in lipid concentrations (1.31, 2.45, 3.59, and 4.73% and protein (9.96, 12.27, 14.60, and 16.92) as a function of increasing the concentration of insect powder [149]. Bawa *et al*. [150] incorporated cricket powder in bread and biscuits and found higher contents of protein, iron, and phosphorus. In addition, consumer sensory acceptability was found to be comparable to control products.

11. Functions Associated with the Consumption of Patented Products

Nutritional supplementation was most frequently cited as a function for the products described in the patent documents (59 citations), according to Figure 10. As an example, patent CN111296833A [151] developed nutritional tablets containing bee extract to supplement athletes.

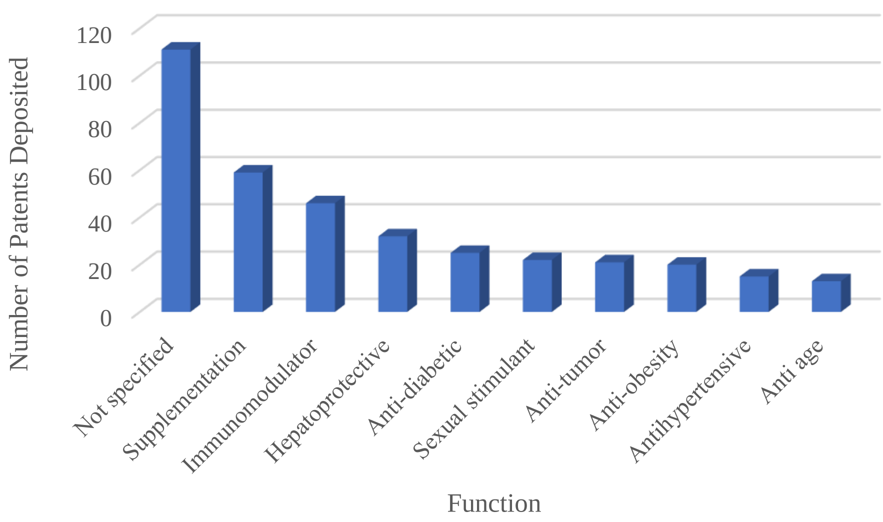

Figure 10. Main Functions Associated with the Consumption of the Patented Products.

In general, insects have a strong connection with traditional medicine in several countries around the world. According to Seabrooks & Hu [152], more than one hundred molecules isolated from insects with bioactive potential have been identified in recent years, some of which are described in Table 2.

Immunomodulation was cited 46 times as a function associated with the consumption of insect-containing products. As an example, patent CN105942197A [153] deals with the development of pasta containing insect powder. As a claim, the patent brings that the consumption of pasta can strengthen the immune system. Chitin is a component of the exoskeleton of crustaceans and insects that can generate stimulation in cells of the innate immune system [154]. One of the justifications for the function of immune stimulation would be the presence of insect chitin in patented food products.

Ali *et al*. [155] identified a bioactive polysaccharide (Table 2) in the larvae of the black soldier fly (*Hermetia illucens*) as a molecule that activates the mammalian innate immune response. The molecule, named dipterose-BSF, is capable of stimulating cytokine production in macrophages via the TLR signaling pathway.

The hepatoprotective function was cited 32 times in patent documents. As an example, the patent KR101852840B1 [156] describes a formulation containing larvae extract, which, after enzymatically treated with peptidases, alkalases, and proteases, ensures hepatic protection potential. In a study developed with *Tenebrio molitor* (Table 2), it was observed that alkalase hydrolysate from this insect can protect hepatocytes from cytotoxicity induced by reactive oxygen species, through a mechanism of upregulation of antioxidant genes dependent on nrf-2 [157]. The administration of Cricket extract demonstrated the ability to improve alcohol-induced acute liver injury in mice, possibly by controlling oxidative stress [158].

The hypoglycemic function is described in 25 patent documents evaluated. Patent CN105661547A [159] relates to a nutritional supplement containing active probiotic microcapsules, aminobutyric acid, xyllo-oligosaccharide, zinc-rich yeast, mulberry leaf polysaccharide, silkworm pupa peptide, and dietary fiber. The formulation, according to the patent, confers an anti-diabetic effect, by the synergistic action of silkworm peptides with other bioactive substances present. Lee *et al*. [160] were able to isolate fractions with inhibitory activity of the enzyme α-glucosidase from the E5K6 peptide (Table 2), found in the silkworm cocoon (*Bombyx mori*). The peptide would be useful in reducing postprandial hyperglycemia in patients with diabetes.

Another function cited in the patent documents was anti-tumor (21 documents). The CN101683166A patent [161], for example, deals with the preparation of dehydrated mealworms (*Tenebrio molitor*) ready for consumption. As an advantage, the patent states that the ingestion of dehydrated larvae would improve the body's metabolism and confer an anticancer anticancer effect. Wu *et al*. [162], found caspase 3-mediated cytotoxicity in human hepatocellular carcinoma and colorectal adenocarcinoma, caused by an oil extract of mealworm (*Tenebrio molitor*), which may corroborate the anticancer function associated with the consumption of this insect. A similar study was able to isolate a new oxazole from the insect *Aspongopus chinensis*, in addition to three known N-acetyldopamine derivatives (Table 2) and verify variable cytotoxicities of these molecules against different types of tumor cells [163].

Anti-inflammatory and antioxidant functions were cited 12 and 11 times, respectively. Patent KR20190050540A [164] deals with the method for preparing formulation containing cricket extract. The formulation can be presented in powder, granule, or liquid form, and, by undergoing the action of proteolytic enzymes, presents high content of free amino acids, in addition to antioxidant and anti-inflammatory potential. When evaluating In vitro protein digestion of *Gryllodes sigillatus*, *Tenebrio molitor* and *Schistocerca gragaria* bioactive peptides were found with anti-radical activity via ABTS and DPPH analyses, in addition to LOX and COX-2 inhibitory activity and Fe chelating capacity2 + [165]. In addition, chitin and chitosan extracted from *Calliptamus barbarus* and *Oedaleus decorus* presented antioxidant activity and antimicrobial function against pathogenic microorganisms, indicating the potential for use in the food industry [166]. Tang *et al*. [167] developed an experiment that was able to isolate 13 non-peptide nitrogen compounds from *Polyrhachis dives*. Most of these substances are composed of pyridine moieties and three are alkaloids, which were evaluated for the potential of kidney protection, T and B lymphocyte proliferation, and inhibition of TNF-α, COX-1, COX-2, and Jak3 kinase. It was noticed that part of the molecules presented activity in one or more of these assays, indicating anti-inflammatory potential. Yan *et al*. [168] were able to isolate four new compounds possessing a 2,3-dihydrobenzo [b] [1,4] dioxin group, together with five N-acetyldopamine dimers from *Blaps japanensis*. All compounds were found to exhibit inhibitory effects for COX-2. Ahn *et al*. [73] evaluated the possible anti-inflammatory effects of glycosaminoglycan obtained from *Gryllus bimaculatus* and found the molecule useful for the treatment of inflammatory diseases, such as chronic arthritis.

The use of insects for medicinal purposes is described in most medical systems in various regions of the world. The historical record shows that the use of insect-based remedies is an ancient practice, which can be found in ancient Egyptian pharmacological texts, as well as being present in Mesopotamian, Roman, and Greek medical conducts [169]. Other bioactive molecules have been isolated from insects, such as: residual polysaccharide extracted from *Periplanata americana* [170] with healing potential through stimulation of collagen deposition, polarization of M2 macrophages, and angiogenesis; new isoflavone present in *Periplaneta* *americana*, with inhibitory effect against the bacterium *Bacillus subtilis* [171] and indolic alkaloids present in *Protaetia brevitarsis seulensis* with antithrombotic and platelet aggregation inhibition potential [72].

Table 2. Molecules isolated from insects and their respective biological functions.

Reference	Insect	bioactive molecule	Function
Cho & lee [157]	*Tenebrio molitor*	Peptides: Ala-Lys-Lys-His-Lys-Glu and Leu-Glu	Hepatic protection
Wang et al. [170]	*Periplanata americana*	Residual Polysaccharide	Wound healing
Ali et al. [155]	*Hermetia illucens*	Polysaccharide "dipterose-BSF"	Immunomodulator
Zielińska, Baraniak & karaś [165]	*Gryllodes sigillatus, Tenebrio molitor* and *Schistocerca gragaria*	Bioactive peptides	Antioxidant and anti-inflammatory
Gao et al. [171]	*Periplaneta americana*	Isoflavone 13,13-dimethyl,12-(16-hydroxy,16,16-dimethyl)-propanol-Δ11,12-hydrogenated pyranyl-7,8[benzo]-4′-methoxyisoflavone	Antibacterial
Lee et al. [72]	*Protaetia brevitarsis seulensis*	Indolic alkaloids	Inhibition of platelet aggregation
Kaya et al. [166]	*Calliptamus barbarus* and *Oedaleus decorus*	Chitin and chitosan	Antimicrobial and antioxidant
Tang et al. [167]	*Polyrhachis dives*	13 non-peptide nitrogen compounds	Anti-inflammatory, immunosuppressive and renoprotective
Yan et al. [168]	*Blaps japanensis.*	four new compounds having a 2,3-dihydrobenzo [b] [1,4] dioxin group, together with five known dimers of N-acetyldopamine	Anti-inflammatory
Ahn et al. [73]	*Gryllus bimaculatus*	Glycosaminoglycan	Adjuvant anti-inflammatory in chronic arthritis
Luo et al. [163]	*Aspongopus chinensis*	Oxazole and 3 components N-acetyldopamine derivatives	Anti-tumour
Lee et al. [160]	*Bombyx mori*	E5K6 peptide	Hypoglycemic

12. Concept Clusters Associated with Patent Documents

Figure 11 shows how the clusters related to the topic under discussion were organized. It can be seen, then, the formation of 09 clusters, where concepts are present that are more predominantly associated with each other in the patent documents. Two of these clusters are directly associated with specific insects: silkworm pupa protein and wheat worm. These insects are also cited in other clusters, in the form of silkworm chrysalis, silkworm, silkworm pupa, *Tenebrio molitor*, and *Tenebrio molitor* larva, which would confirm that they have been more extensively inserted in patent documents and, therefore, more explored. This is in line with the scientific literature since it is reported that these are insect species of great utility for food because of their nutritional quality, in addition to the high capacity of breeding on an industrial scale because of the ease of handling [45].

For the silkworm, there is a noticeable emphasis on the use of its protein, especially in powder form, and an association between its pupa and the preparation of products with health benefits and the application of the spray-drying process. Methods of isolation and application of silkworm peptides in food have been patented because of their various functional effects in the human organism, such as modulation of immunity (CN105661554A [172]); antihypertensive (CN107779489A [173]); and hypolipemiant (CN113647637A [174]). The use of spray-drying may be in evidence since there are reports in the literature of its ability to help improve the OH- radical sequestering capacity and the reducing power of silkworm protein hydrolysates, besides having low cost and higher production yield compared to freeze-drying [175].

As for the silkworm, the cluster of the same name presents concepts such as insect powder, edible insect powder, edible insect, and food insect, which may indicate its use in a comprehensive way in order to fortify foods nutritionally. As an example, the patent document KR20190047180A [176] deals with the insertion of mealworm powder in a mix of grains to create a food product rich in nutrients for use in people with nutritional deficiencies. Besides these insects, there is a cluster called royal jelly, which would encompass the concepts of queen bee larva, pupa, bee pupa, honey, and health care food.

The presence of this cluster can be justified by the fact that most patent documents involving bees deal with the combined use of the various products derived from the breeding of this insect, such as royal jelly, pupae, and honey, in the development of foods with enhanced nutritional and functional benefits, such as jams (CN100998382A [177]); creams (CN102334626A [178]) and jellies (CN112089038A [179]).

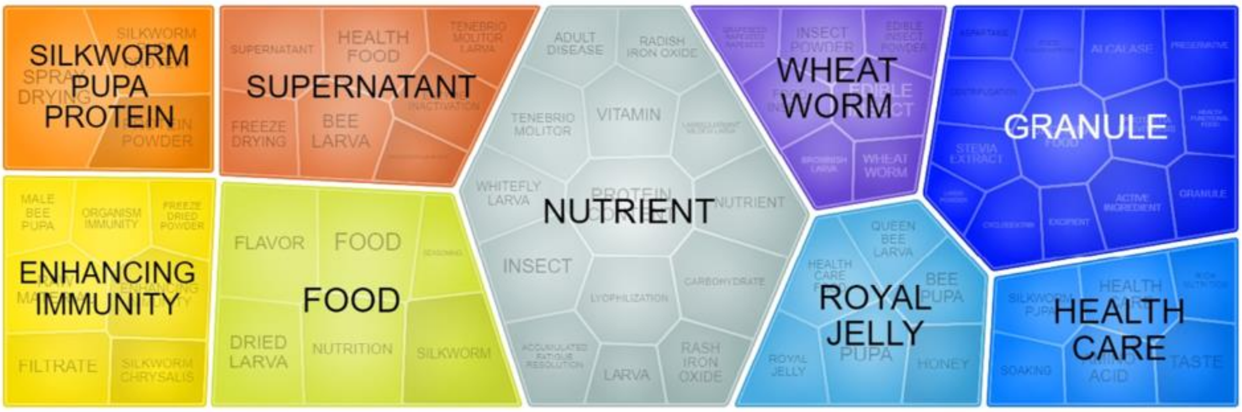

Figure 11. Clusters of concepts related to patent documents involving edible insects and food products. Source: Q. Orbit®.

The cluster called granule is mainly associated with the term functional food. The granulation method is capable of uniting various powdered substances into a single entity or granule. This can occur to prevent the separation process of certain ingredients in the pharmaceutical and food industry in the development of new products [180]. As an example, patent document KR20200019806A [181], deals with the production of *Protaetia brevitarsis* granules with antidiabetic potential to be used in food products, comprising: 55 to 65% by weight of *Protaetia brevitarsis* larval powder, 25 to 35% by weight of lotus root powder and 5 to 15% by weight of red ginseng extract.

The presence of the clusters enhancing immunity, nutrient, and health care confirm the findings described in this study, where one can see the great potential of insect application in food fortification and in conferring functional power and improvement of human health.

13. Economic Projections for the Edible Insects Market

The number of producers and consumers of insects and their products presents the perspective of an increase in the next years, and the forecast is that, by 2023, the market of edible insects will reach, approximately, US$ 1.181 billion (Figure 12a), with a growth of, approximately, 190.80%, in comparison with 2018 (US$ 406.32 million) [182]. Recent data [183,184] indicate that this market will continue to grow significantly in the coming years, potentially reaching US$ 4,630 billion in 2027 and almost US$ 10 billion in 2030. Government and scientific stimuli have resulted in impacts not only in the number of patent deposits or publication of articles in the area, but also in investment in the market. According to Pippinato *et al*. [185], to satisfy emerging trends worldwide, non-traditional sources of protein, such as insects, are gaining increasing importance.

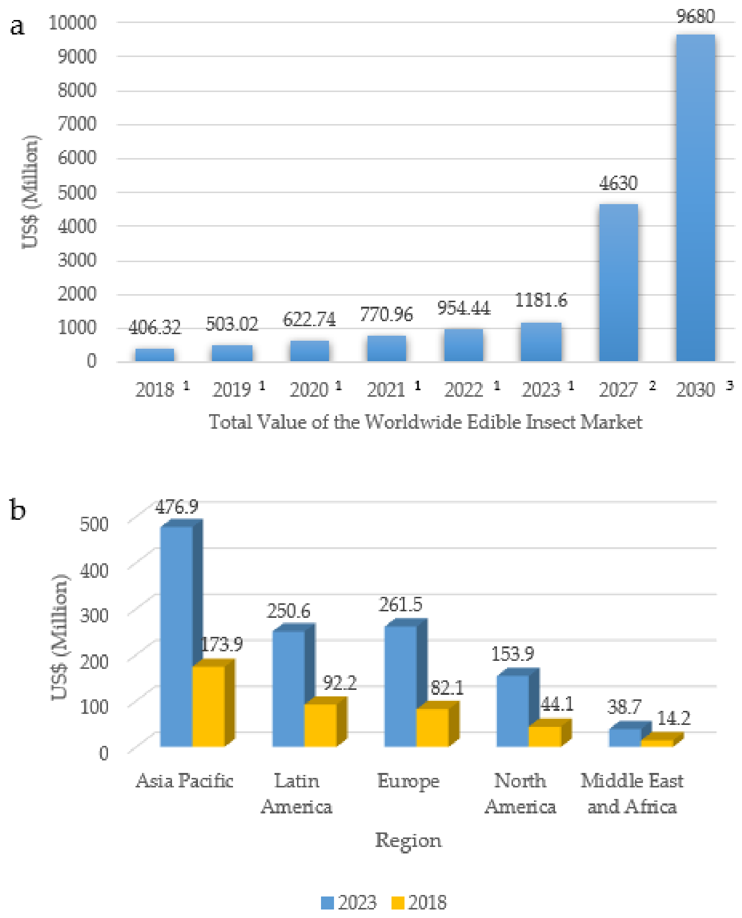

Figure 12. (a) Projection for the World Market Value of Edible Insects. Sources:1[182]; 2[183]; 3[184]. (b) Value of the World Market for Edible Insects by Region. Source: [186]. Values presented in US$ million.

For 2023, the Asia-Pacific region will maintain a larger market size, growing from US$ 173.9 million in 2018 (Figure 12b) to US$ 476.9 million by 2023. However, North America stands out as the region with the highest forecast growth in the edible insects market, with an expected Compound Annual Growth Rate of 28%, followed by Europe at 26%, Middle East & Africa, Latin America, and Asia-Pacific at 22% [186]. Countries like Canada and United States are in the process of the very recent development in edible insects. According to Wilkie [187], until 2012 there were no reports of insect farms for human consumption in North America, however, from 2012 to 2018, approximately 18 farms were built to meet the demand of this new food sector. Apart from this, the North American edible insect market is forecast to increase from US$ 44.1 million in 2018 to US$ 153.9 million by 2023, which is a total growth of 248.97% [186]. In Europe, regulation of the use of edible insects has created a stimulus for this market, which is forecast to grow from US$ 82.1 million in 2018 to US$ 261.5 million by

2023 [186]. According to Pippinato *et al*. [185], the inclusion of insects and their derivatives in the European food industry can be foreseen in the coming years, mainly in the development of protein-source products, to ensure greater diversity in the market.

Entomophagy is quite present in Latin America, where countries such as Brazil, Ecuador Peru, Colombia, and Venezuela stand out in entomophagic habits, due to cultural issues [188]. The edible insects' market in Latin America, in 2018, presented o second largest economic value, at US$ 92.2 million, with a forecast to increase to US$ 250.6 million by 2023, being surpassed by Asia-Pacific and Europe [186]. According to Bermúdez-Serrano [189], there is still a shortage of start-ups in Latin America, and this may be associated with the absence of regulations focused on insect production and commercialization; and strategies specifically created to stimulate the edible insect industry. Therefore, government efforts are still needed to change the view of insects as contaminants in food to make them a new potential for economic development in this region.

According to Baiano [190], the rearing of edible insects is of relevance to making alternative food sources available to the market, considering the trend of population increase. Developing countries in regions such as Southern and Central Africa could especially benefit from this. In Africa, however, it is reported that there is a need to improve the organization of food market chains, which would stimulate agribusiness to diversify its production and insert edible insects as a possibility of income generation [191]. Thus, there are two important issues to be considered to stimulate the market of edible insects. The first is the legal aspect, i.e., it is necessary that countries begin to regulate the production and marketing of insects. The other issue is the need to change the mentality of the western population that tends to react negatively to the possibility of insect consumption [190].

14. Conclusions

The present research assessed patent documents related to edible insects in food products from the last 30 years while discussing novel scientific articles from a similar subject by a hybrid review method. Considering that the edible insect market has been expanding at a remarkable speed, the present study manages to fill a knowledge gap in technological prospecting, especially regarding novel food products developed with insects. Technological prospecting is vital in reducing uncertainties by assisting the consolidated private initiative and start-ups with prior knowledge, increasing systemic competitiveness, and improving decision-making by R&D managers in this specific field.

From the analysis performed through the information contained in patent documents, it is possible to conclude that the application of edible insects in the food industry shows a tendency to develop in the coming years, suggesting that there is still great room for investment in research and market. FAO possibly has a key role in stimulating the development of technologies associated with the use of insects in food products because of the release of the document "Edible insects: future prospects for food and feed security" in 2013, the same year when the period of exponential growth of patent applications began. Edible insects are mainly used as a form of protein supplementation in food products and the silkworm is still the insect with the highest number of patents on the subject studied, possibly because of its high production in the Asian market for consumption and silk extraction. Considering the country of origin of the patented technology, it is possible to report a concentration in the East, with a predominance of patent deposits by China and the Republic of Korea, countries that have more consolidated innovation systems in the area, through joint actions of government incentives and national projects.

For the Western world, cultural rejection of entomophagy can be considered a barrier to more inclusion of insects as food in a global panorama, but that can be overcame with educational actions, such as sensory studies aligned with universities, industry, and the population, as well as other projects for the popularization of science and technology in this theme. The development of foods containing whole insect powder or isolated protein as an ingredient may be an interesting initiative to reduce the prejudice towards eating it while it must be reinforced the capability of the insects of enhancing the nutritional properties of a plethora of globally consumed foods such as cookies, pasta, and brownies.

The lack of legislation regarding insect rearing and its human consumption in some countries also needs to be assessed to provide conditions for the development of insect factories and foment more sustainable food production in those regions, mainly in developing countries that can benefit the most from this high-quality food source.

Overall, the use of insects in the food industry seems to represent a great opportunity to develop products with high added value, besides potentially meeting the demand of countries in a situation of food and nutritional insecurity. Considering the unstable Global panorama, the stimulation of edible insect rearing and consumption is of great relevance in order to maintain country sovereignty in those regions that may be most affected by food insecurity from the Covid-19 pandemic the and Russia-Ukraine war.

Considering the study limitations, it must be pointed out that many patent documents did not fully describe their inventions, which could underestimate some of the parameters evaluated. Also, the Espacenet database may not cover all the protected patent documents in the world, in comparison with other databases, which also may be a factor of data underestimation. For future perspectives, the present study may be useful as a starting point to indicate directions toward the development of novel, nutritionally enriched, functional food products with different insect species. It can be pointed that this subject is still in infant research status in many countries that possess an entomophagy culture. Also, few insect species are intensively studied and applied in foods, considering the diversity of insects found worldwide and this must be assessed.

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5.2

Manuscrito: Replacing conventional substrate with linseed cake improves the omega-3 profile of Zophobas atratus Fabricius (Coleoptera: Tenebrionidae) larvae

Replacing conventional substrate with linseed cake improves the omega-3 profile of *Zophobas atratus* Fabricius (Coleoptera: Tenebrionidae) larvae

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Abstract

Edible insects are an alternative source of protein, lipids, and minerals for human consumption. The use of agro-industrial by-products in insect diets makes it possible to transform materials of low commercial value into nutritious biomass. This study assessed the viability of nutritional enrichment for *Zophobas atratus* larvae by replacing their conventional substrate with linseed cake. The larvae were fed with substrates containing 0%, 25%, 50%, 75%, or 100% linseed cake. Proximate composition, energy value, feed conversion efficiency, mortality rate, fatty acid profile, lipid quality, and mineral content were analyzed. Including up to 75% linseed cake kept feed conversion efficiency the same as the control. Larvae fed 100% linseed cake had a higher protein content (46.68% vs. 41.38% - Control). The concentration of alpha-linolenic acid increased to 28.66% (100%) compared with 1.03% (Control), improving the omega-6/omega-3 ratio (23.61 - Control vs 0.58 - 100%). The larvae can be considered rich in iron (Control), phosphorus, manganese, zinc, and magnesium (Control and 100%); and a source of magnesium (25, 50, and 75%) and iron (25, 50, 75, and 100%). The rearing of *Zophobas atratus* can benefit from the use of linseed cake, especially in terms of protein and alpha-linolenic acid content.

Key words: Entomophagy; new ingredient; mealworm; nutritional composition; lipid modulation

1. INTRODUCTION

Concerns about environmental sustainability occupy a central place in global discussions. A current example is the efficiency of livestock production as the main source of meat and protein for the food market. Animal rearing, especially cattle, has high water and land consumption, with considerable greenhouse gas emissions, when aimed at large-scale production (Xu *et al*., 2021). This adds pressure on the environment and raises questions about the sustainability of these production systems. As an alternative, there is a need to explore more sustainable and efficient production methods. One such potential method is the rearing of edible insects for human consumption (Siddiqui *et al*., 2022), which are already part of the diet of at least 2 billion people worldwide, with more than 1,900 edible species identified (Tanga and Ekesi, 2024).

Among edible insects, mealworms stand out, as they are often used as feed for animals such as reptiles, birds, and monkeys and food for humans (Bordiean *et al*., 2020). Representative species are *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) and *Zophobas atratus* Fabricius (Coleoptera: Tenebrionidae) (Peng *et al*., 2020). Insects of this order are distributed worldwide due to their adaptability to different environments and substrates, as well as their rich nutritional composition and resistance to harmful compounds such as mycotoxins and pesticides (Bordiean *et al*., 2020).

Mealworms have a nutritional content considered suitable for human and animal consumption, offering proteins, fats, minerals, and fibers (Nascimento *et al*., 2022). In addition, these larvae have demonstrated the ability to biodegrade different agro-industrial by-products, including rye bran, rapeseed meal, brewery spent grain, bread scraps, rice straw, and grape pomace (Bordiean *et al*., 2022, Montalbán *et al*., 2022, Nascimento *et al*., 2022), transforming them into insect biomass and suggesting highly functional and adaptable digestive systems. The fatty acid profile for these insects shows high levels of monounsaturated fatty acids (34.80% for *Zophobas atratus* and 50.01% for *Tenebrio molitor*) and saturated fatty acids (43.28% for *Zophobas atratus* and 29.60% for *Tenebrio molitor* (Nascimento *et al*., 2022, Dreassi *et al*., 2017). However, a lower level of polyunsaturated fatty acids has been identified (20.80% for *Zophobas atratus* and 19.89% for *Tenebrio molitor*, with a low value of omega-3 (PUFA n-3) in *Zophobas atratus* (1.40%) (Nascimento *et al*., 2022).

Currently, the commercial rearing of these larvae mainly uses wheat bran or oats as their substrate. However, it should be considered that these ingredients are also used for human food, which leads to competition for inputs and increases the cost of rearing insects. Due to the ability of mealworms to metabolize various components, it is important to assess the feasibility of replacing traditional feeds with more sustainable alternatives (Brandon *et al*., 2018).

Linseed, the seed of *Linum usitatissimum*, is known to be an important source of alpha-linolenic acid (C18:3n3), with a high lipid content (30.07-37.37%) and a significant concentration of protein (19.45 - 21.71%) and fiber (10.04 - 12.04%) (Qiu *et al*., 2020, Khare *et al*., 2021). Global linseed production in 2020 reached approximately 3.5 million tons (Yadav *et al*., 2022), and lipid is a component of great commercial interest due to its oil extraction. On the other hand, the by-product of the oil industry, known as linseed cake, is often used as animal feed, as it is still a rich source of protein (27.80 to 39.40%) and fiber (7.60 to 12.80%), as well as containing moderate levels of lipids (up to 16.90%), maintaining the seed's fatty acid profile, rich in alpha-linolenic acid (C18:3n3; 42.90 to 68.60% of total fatty acids) (Kokić *et al*., 2024).

Some studies have investigated the use of linseed in feeding Tenebrionidae larvae. Examples include: the use of its oil as a way of improving the lipid profile of *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) and *Tenebrio molitor*, obtaining a significant reduction in the omega-6/omega-3 ratio (Oonincx *et al*., 2019; Rossi *et al*., 2022); use of its by-product (cake), replacing the conventional feed of *Tenebrio molitor* larvae by 50%, resulting in larvae with 17.00% alpha-linolenic acid (C18:3n3) compared to 1.73% in the Control, and also with a better omega-6/omega-3 ratio, from 1.71 to 20.55 in the Control (Bordiean *et al*., 2022). These studies indicate the potential to use the linseed and its by-products to feed mealworms, intending to enhance its nutritional fatty acid profile.

Most research focuses on *Tenebrio molitor* (Tavares *et al*., 2022). However, *Zophobas atratus* has some distinct and interesting characteristics compared to *Tenebrio molitor*, such as its dependence on isolation during metamorphosis and its ability to reach larger sizes (Kim *et al*., 2015). *Zophobas atratus* has a higher biomass yield, reaching more than twice the weight of *Tenebrio molitor* (Harsányi *et al*., 2020). Therefore, studies evaluating the use of linseed by-products to feed *Zophobas atratus* larvae may be of interest both from an economic point of view and to elucidate scientific hypotheses regarding the possibility of nutritional enrichment and the development of more sustainable rearing methods.

Considering the nutritional richness of linseed cake, the ability of larvae of the Tenebrionidae family to metabolize various nutritional substrates, and the need to evaluate alternative methods of sustainable food production, this study aimed to assess the feasibility of nutritional enrichment of *Zophobas atratus* larvae, with emphasis on the bioaccumulation of omega-3 fatty acids through its feeding with linseed cake.

2. MATERIAL AND METHODS

2.1 Biological material

2.1.1 Larvae

The company SuperBugs - Alimentos Funcionais (Salvador-BA, Brazil) donated 2000 *Z. atratus larvae* of up to 15 days from ecloding (average larval unit weight of 39.58 mg ± 4.50 and length between 0.5 and 1.0 cm). Prior to the experiment, the larvae were exclusively fed wheat bran (Relva Verde, Ibiporã-SC, Brazil).

2.1.2 Material for formulating substrates

The linseed cake from the cold pressing process for oil extraction was donated by the company Vital Âtman Ltda (São Paulo-SP, Brazil).

The Control substrate was developed with a mixture of 70% commercial poultry growth feed (Imbramil, São Paulo-SP, Brazil) purchased from a local business in the city of Salvador-BA, Brazil, consisting of ground whole corn, soybean meal, wheat bran, meat and bone meal and a mineral/vitamin mixture; and 30% wheat bran (Relva Verde, Ibiporã-SC, Brazil), also purchased from a local business in the city of Salvador-BA, Brazil.

2.2 Methods

2.2.1 Substrate formulation

The Control substrate and the linseed cake were ground in a grain grinder (80393 Hamilton Beach, United States of America) until they reached a particle size of less than 0.71mm (25 mesh). After this, 5 substrates were prepared, which made up the distinct treatments in this study: 0 (Control) (70% commercial poultry growth feed + 30% wheat bran); 25 (75% Control + 25% linseed cake); 50 (50% Control + 50% linseed cake); 75 (25% Control + 75% linseed cake); and 100 (100% linseed cake). Table 1 presents the nutritional composition of each substrate used to feed the *Zophobas atratus* larvae.

2.2.2 Larvae rearing conditions

One hundred larvae were placed in a plastic box measuring 25.5 cm x 11.5 cm x 15 cm (length x height x width), with an opening at the top covered by a tulle mesh. Each box contained the respective substrate (Table 1) at a ratio of 2 g of substrate to 1 g of larval weight (Boukid *et al*., 2021). In addition, 0.3 g of fresh potato (*Solanum tuberosum*) per 1 g of larval weight was offered in cotton layers and replaced twice each week as a water source (Ruschioni *et al*., 2020). The experiment was conducted for 90 days with 4 replicates per treatment. The boxes were arranged randomly on a vertical shelf and kept in an air-conditioned room with temperature control (25 °C ± 1) and relative humidity (50% ± 5), using a digital thermo-hygrometer (KR42 Instrubras, Brazil) and a 12h day/night photoperiod.

Table 1. Nutritional composition of substrates containing different percentages of linseed cake used to feed *Zophobas atratus* larvae.

Nutrient	Treatments				
	0 (Control)	25	50	75	100
Proximate composition (%)					
Carbohydrates	46.63^{a} ± 0.36	39.13^{b} ± 0.25	31.63^{c} ± 0.14	24.13^{d} ± 0.04	16.64^{e} ± 0.08
Crude fiber	16.09^{e} ± 0.22	19.48^{d} ± 0.17	22.87^{c} ± 0.13	26.25^{b} ± 0.08	29.64^{a} ± 0.03
Protein	14.55^{e} ± 0.23	18.28^{d} ± 0.17	22.01^{c} ± 0.11	25.74^{b} ± 0.05	29.47^{a} ± 0.01
Moisture	12.38^{a} ± 0.38	11.75^{ab} ± 0.29	11.13^{bc} ± 0.21	10.51^{cd} ± 0.12	9.89^{d} ± 0.04
Lipid	4.01^{e} ± 0.26	5.27^{d} ± 0.19	6.54^{c} ± 0.11	7.80^{b} ± 0.04	9.06^{a} ± 0.03
Ash	6.35^{a} ± 0.11	6.09^{b} ± 0.08	5.83^{c} ± 0.06	5.57^{d} ± 0.04	5.31^{e} ± 0.03
Fatty acid (%)					
Saturated					
C8:0	0.03^{a} ± 0.00	0.02^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	-
C12:0	0.07^{a} ± 0.00	0.05^{b} ± 0.00	0.04^{c} ± 0.00	0.02^{d} ± 0.00	-
C14:0	0.88^{a} ± 0.02	0.71^{b} ± 0.02	0.53^{c} ± 0.01	0.35^{d} ± 0.01	0.17^{e} ± 0.03
C15:0	0.08^{a} ± 0.00	0.06^{b} ± 0.00	0.04^{c} ± 0.00	0.02^{d} ± 0.00	-
C16:0	21.11^{a} ± 1.19	18.35^{b} ± 0.89	15.50^{c} ± 0.50	12.57^{d} ± 0.00	9.53^{e} ± 0.61
C18:0	8.18^{a} ± 0.26	7.35^{b} ± 0.21	6.51^{c} ± 0.13	5.63^{d} ± 0.03	4.73^{e} ± 0.11
Monounsaturated					
C16:1	0.11^{a} ± 0.00	0.08^{b} ± 0.00	0.06^{c} ± 0.00	0.03^{d} ± 0.00	-
C18:1n9 cis	26.11^{a} ± 0.37	25.24^{b} ± 0.24	24.34^{c} ± 0.13	23.42^{d} ± 0.06	22.48^{e} ± 0.00
Polyunsaturated					
C18:2n6 cis	39.19^{a} ± 0.28	33.39^{b} ± 0.09	27.45^{c} ± 0.30	21.33^{d} ± 0.32	15.04^{e} ± 0.13
C18:3n3	2.27^{e} ± 0.05	12.94^{d} ± 0.40	23.89^{c} ± 0.43	35.16^{b} ± 0.10	46.76^{a} ± 0.61
Other fatty acids	1.98^{a} ± 0.76	1.81^{a} ± 0.57	1.64^{a} ± 0.38	1.46^{a} ± 0.18	1.29^{a} ± 0.01
∑ SFA	30.34^{a} ± 1.47	26.54^{b} ± 1.12	22.63^{c} ± 0.64	18.60^{d} ± 0.03	14.43^{e} ± 0.75
∑ MUFA	26.22^{a} ± 0.37	25.32^{a} ± 0.24	24.40^{ab} ± 0.14	23.45^{ab} ± 0.06	22.48^{b} ± 0.00
∑ PUFA	41.46^{e} ± 0.34	46.33^{d} ± 0.31	51.34^{c} ± 0.13	56.49^{b} ± 0.21	61.80^{a} ± 0.74
omega-6/omega-3	17.26^{a} ± 0.29	2.58^{b} ± 0.09	1.15^{c} ± 0.03	0.61^{d} ± 0.01	0.32^{e} ± 0.00
Metal (mg/100 g)					
Potassium	780.81^{e} ± 5.74	840.42^{d} ± 12.38	900.04^{c} ± 1.92	959.66^{b} ± 8.54	1019.28^{a} ± 5.74
Phosphorus	840.77^{c} ± 1.17	876.06^{bc} ± 14.75	911.34^{abc} ± 28.33	946.63^{ab} ± 41.91	981.91^{a} ± 55.50
Calcium	872.33^{a} ± 109.64	727.53^{ab} ± 81.84	582.73^{bc} ± 54.05	437.92^{cd} ± 26.25	293.12^{d} ± 1.54
Magnesium	322.33^{a*} ± 16.20	308.70^{a*} ± 10.88	318.93^{a*} ± 5.68	315.52^{a*} ± 1.93	312.11^{a*} ± 5.74
Sodium	103.97^{a} ± 6.63	85.21^{b} ± 4.94	66.46^{c} ± 3.26	47.70^{d} ± 1.58	28.94^{e} ± 0.10
Iron	13.32^{a} ± 0.44	11.70^{b} ± 0.27	10.09^{c} ± 0.10	8.48^{d} ± 0.08	6.86^{e} ± 0.25
Zinc	9.25^{a} ± 0.04	8.81^{b} ± 0.05	8.38^{c} ± 0.07	7.94^{d} ± 0.08	7.50^{e} ± 0.10
Manganese	9.21^{a} ± 0.04	8.43^{b} ± 0.03	7.65^{c} ± 0.02	6.87^{d} ± 0.01	6.10^{e} ± 0.00
Copper	0.78^{e} ± 0.04	1.06^{d} ± 0.02	1.33^{c} ± 0.01	1.61^{b} ± 0.03	1.89^{a} ± 0.05
Nickel	0.05^{e} ± 0.00	0.10^{d} ± 0.00	0.14^{c} ± 0.01	0.19^{b} ± 0.01	0.24^{a} ± 0.01
Arsenic	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**
Cadmium	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**

Data presented as mean ± standard deviation. Different letters on the same line indicate a statistical difference between substrates ($p \leq 0.05$) according to Tukey's test, except when the line shows *, which indicates the Mann-Whitney U test. ** indicates results below the detection limit. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake. ∑ PUFA: sum of polyunsaturated fatty acids; ∑ MUFA: sum of monounsaturated fatty acids; ∑ SFA: sum of saturated fatty acids.

Every two weeks, fresh substrates were renewed (considering the larval weight per box) and the uneaten substrate and feces were weighed. The larvae were weighed weekly on an analytical balance (AY220 Shimadzu, Japan). At the end of the experiment, the larvae were separated from the substrates to empty the digestive tract for 24 hours (Kulma *et al*., 2020), followed by freezing at -80 °C and freeze-drying (Lyophilizer L101 Liobras, Brazil) for 48 hours. The slaughtered and freeze-dried larvae were ground in a grain grinder (80393 Hamilton Beach, United States of America) until they reached homogeneous granulometry and stored in plastic containers at -80 °C for later analysis.

2.2.3 Proximate composition

The proximate composition was determined according to AOAC standards (2019). Moisture was determined by oven drying (Tecnal, TE-394/I, Brazil) at 105 °C until constant weight was obtained, ash was determined in a muffle furnace (Lavoisier 402-D, Brazil) by incineration at 550 °C, crude fiber was determined by acid and alkaline extraction. The cold extraction method (Bligh and Dyer, 1959) was used to determine total lipids. Carbohydrates were calculated using the difference between the other macronutrients (Bhattacharjee *et al*., 2013). The energy value was calculated considering 4 kcal/g for carbohydrates and proteins and 9 kcal/g for lipids (Bhattacharjee *et al*., 2013). Crude protein was determined using the Kjeldahl method (AOAC, 2019), where the nitrogen conversion factor used was 4.76 for the larvae (Janssen *et al*., 2017).

2.2.4 Fatty acid profile and lipid quality

The identification and quantification of fatty acids was carried out according to the methodology proposed by Souza *et al*. (2017). To this end, an aliquot of the total lipids was subjected to the saponification reaction with NaOH in methanol (0.5 N), followed by methylation with BF_3 (12% in methanol) and extraction with isooctane. The extracted fatty acid methyl esters were stored in an amber vial in an inert atmosphere (N_2). A gas chromatograph (Perkin Elmer Clarus 680) with flame ionization detector and DB - Fast FAME column (30 m

× 0, 25 mm × 0, 25 μm) was used to separate the fatty acid methyl esters. Helium was used as the carrier gas at a flow rate of 1.0 mL/min, and injections of 1 μL were made in split mode (1:50). The fatty acids were identified by comparing the retention times of the peaks in the samples with the retention times of a standard mixture (189-19, Sigma Aldrich, USA). The fatty acids in the samples were quantified by normalizing the peak areas (% area). The sums of total saturated fatty acids (∑SFA), total monounsaturated fatty acids (∑MUFA), and total polyunsaturated fatty acids (∑PUFA) were calculated, as well as the omega-6/omega-3 ratio. The following lipid quality indices were calculated for *Zophobas atratus* larvae: Atherogenicity Index (AI, Eq. 1), Thrombogenicity Index (TI, Eq. 2), Hypocholesterolemic/Hypercholesterolemic Ratio (H/H, Eq. 3), Health Promotion Index (HPI, Eq. 4), Unsaturation Index (UI, Eq. 5), Oxidizability Index (COX, Eq. 6) and Peroxidizability Index (PI, Eq. 7). Their respective equations are presented below (Chen and Liu, 2020; Duarte *et al*., 2022):

Eq.1 $\mathrm{AI} = (\mathrm{C12{:}0} + 4 \times \mathrm{C14{:}0} + \mathrm{C16{:}0}) / (\Sigma\mathrm{MUFA} + \mathrm{omega}-6 + \mathrm{omega}-3)$

Eq.2 $\mathrm{TI} = (\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0})/((0.5 \times \Sigma\mathrm{MUFA}) + (0.5 \times \mathrm{omega}-6) + (3 \times \mathrm{omega}-3) + \mathrm{omega}-3/\mathrm{omega}-6)$

Eq.3 $\mathrm{H/H} = (\mathrm{cis}-\mathrm{C18{:}1} + \Sigma\mathrm{PUFA})/(\mathrm{C12{:}0} + \mathrm{C14{:}0} + \mathrm{C16{:}0})$

Eq.4 $\mathrm{HPI} = \Sigma\mathrm{UFA}/(\mathrm{C12{:}0} + (4 \times \mathrm{C14{:}0}) + \mathrm{C16{:}0})$

Eq.5 $\mathrm{UI} = (1 \times \%\,\mathrm{monoenoic}) + (2 \times \%\,\mathrm{dienoic}) + (3 \times \%\,\mathrm{trienoic}) + (4 \times \%\,\mathrm{tetraenoic}) + (5 \times \%\,\mathrm{pentaenoic}) + (6 \times \%\,\mathrm{hexaenoic})$

Eq.6 $\mathrm{COX} = ((\mathrm{C18{:}1}) + (10.3 \times \mathrm{C18{:}2}) + (21.6 \times \mathrm{C18{:}3}))/100$

Eq.7 $\mathrm{PI} = (0{,}025 \times \mathrm{C18{:}1}) + (\mathrm{C18:2}) + (2 \times \mathrm{C18{:}3})$

2.2.5 Feed conversion efficiency

To assess the adaptation of the larvae to the substrates and the efficiency of converting food into larval biomass, the following parameters were calculated: Conversion Efficiency of Ingested Feed (ECI (%), Eq. 8 - dry basis), Feed Conversion Ratio (FCR, Eq. 9 - wet basis) and Mortality Rate (MR (%) Eq. 10) (Oonincx *et al*., 2015; Zhang *et al*., 2019) according to the following equations:

Eq. 8 $\mathrm{ECI}\,(\%) = (\mathrm{weight\ gained})/(\mathrm{weight\ of\ ingested\ feed}) \times 100$

Eq. 9 $FCR = \text{(weight of ingested feed)}/\text{(weight gained)}$

Eq. 10 $MR\ (\%) = \text{(number of dead insects)}/\text{(number of initial insects)} \times 100$

2.2.6 Analysis of metals

Metals were determined using inductively coupled plasma optical emission spectrometry (ICP OES; Agilent Technologies, series 720, United States of America). The analyzed metals were copper, sodium, manganese, magnesium, selenium, iron, calcium, zinc, potassium, phosphorus, cobalt, arsenic, cadmium, and nickel. The accuracy of the method was established by analyzing reference material and certified apple leaves (NIST 1515) under the same analysis conditions as the treatments.

2.2.7 Statistical analysis

To assess statistical differences, One-way ANOVA and Tukey's test were applied to results with a normal distribution. The Kruskall-Wallis and Mann-Whitney U tests were conducted for results with a non-normal distribution. Spearman's correlation was carried out between the data from the larvae and the substrates. For all tests, a significance level of 5% was considered. The unit larval mass throughout the experiment period was adjusted in the JMP pro12 program by applying the three-parameter Gompertz growth model.

3. RESULTS

Figure 1 shows the larval mass curve over the 90-day experiment. At the end of the period, there was no statistical difference between the average mass of the Control larvae (0.57 g), with treatments 25 (0.59 g) and 50 (0.60 g) ($p > 0.05$). On the other hand, treatments 75 and 100 resulted in curves with lower mass gain efficiency (0.43 g and 0.27 g respectively) ($p \leq 0.05$).

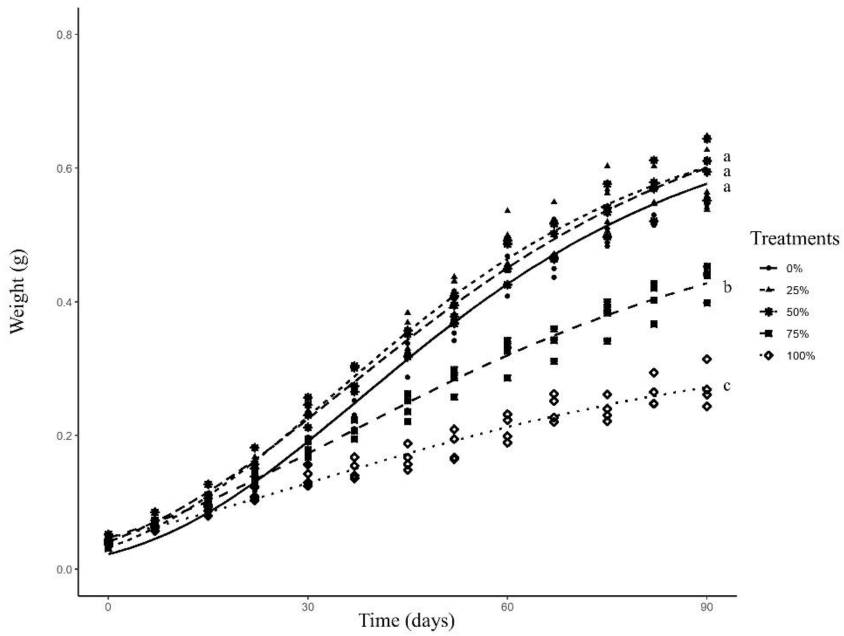

Figure 1. Curve fitted by the Gompertz model with three parameters for the average mass of *Zophobas atratus* larvae fed substrates containing different percentages of linseed cake for 90 days. Different letters between treatments indicate statistical differences at the end of the experiment ($p \leq 0.05$) according to Tukey's test. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake.

Furthermore, according to Table 2, the highest growth rates were found for treatments 25 (0.0336), Control (0.0326), and 50 (0.0296). On the other hand, treatment 100 showed the lowest growth rate (0.0234). The asymptote, or maximum larval mass, was statistically higher for treatments 50 (0.733 g), 25 (0.698 g), and Control (0.692 g). Treatment 75 showed an intermediate value (0.554 g), followed by treatment 100 with the lowest value for this parameter (0.347 g).

Table 2. Parameters estimated according to the 3P Gompertz model for the growth rate of *Zophobas atratus* larvae fed substrates containing different percentages of linseed cake.

Treatments	a	b	c	SEM.a	SEM.b	SEM.c
100	0.347^{d}	0.0234^{c}	29.50	0.041053	0.004379	5.902931
75	0.554^{c}	0.0250^{bc}	36.00	0.041799	0.002747	3.625286
0 (Control)	0.692^{b}	0.0326^{ab}	37.70	0.028494	0.002246	1.645937
50	0.733^{a}	0.0296^{abc}	35.65	0.031231	0.002039	1.824583
25	0.698^{b}	0.0336^{a}	33.30	0.023940	0.002122	1.365813

Equation = $a * e^{e^{-b*(Tempo-c)}}$ where a = asymptote, b = growth rate, c = inflection point, e = exponent, time = breeding days. SEM = Standard deviation. Different superscript letters in the same column indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake.

Table 3 shows the parameters for the efficiency of converting feed into larval mass (ECI and FCR) and the mortality rate. The mortality rate showed lower results for the Control (18.50%) and 25 (20.00%) treatments, with no significant differences ($p \leq 0.05$). Similar results were found for treatments 50 (28.00%) and 75 (29.75%), ($p \leq 0.05$). Treatment 100 (36.00%), on the other hand, showed statistically higher mortality.

The ECI is a percentage indicator of how much of the feed ingested is transformed into mass. In this way, the Control (26.59%), 25 (24.03%), 50 (24.26%) and 75 (22.22%) treatments had the highest percentages of conversion efficiency of ingested feed and were statistically equal ($p > 0.05$). Treatment 100 showed the lowest efficiency, with a statistically lower result (16.58%).

FCR is an indicator that shows the amount of food to be ingested in g by the animal to obtain one g of mass. As they had the lowest values, the Control (3.78), 25 (4.08), 50 (4.07), and 75 (4.54) treatments resulted in better efficiency in the consumption and transformation of the substrate into larval mass. Treatment 100 (6.96) resulted in the statistically highest feed conversion ratio.

Table 3. Mortality Rate (MR (%)), Conversion Efficiency of Ingested Feed (ECI), and Feed Conversion Ratio (FCR) of *Zophobas atratus* larvae fed substrates containing different percentages of linseed cake.

Parameters	Treatments				
	Control	25	50	75	100
MR (%)	$18.50^{b} \pm 6.76$	$20.00^{b} \pm 5.23$	$28.00^{ab} \pm 3.37$	$29.75^{ab} \pm 5.25$	$36.00^{a} \pm 6.22$
ECI (%)	$26.59^{a} \pm 3.73$	$24.03^{a} \pm 0.54$	$24.26^{a} \pm 1.88$	$22.22^{a} \pm 1.81$	$16.58^{b} \pm 0.85$
FCR	$3.78^{a} \pm 0.45$	$4.08^{a} \pm 0.19$	$4.07^{a} \pm 0.36$	$4.54^{a} \pm 0.35$	$6.96^{b} \pm 0.46$

Results presented as mean ± standard deviation. Different letters on the same line indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake. ECI (%) (dry basis) = (weight gained)/(weight of feed ingested) × 100. FCR (wet basis) = (weight of feed ingested)/(weight gained). MR (%) = (number of dead insects)/(number of initial insects) × 100.

Table 4 shows the proximate composition of the larvae fed with substrates containing different percentages of linseed cake. As for moisture, the lowest value was found for the Control (61.05%) and the highest for the 100 treatment (66.01%). In general, the higher the percentage of linseed cake in the larvae's substrate, the greater the increase in moisture. The larvae in the Control treatment had the lowest protein content (41.38%). The percentage of protein increased the higher the proportion of linseed cake in the substrate, reaching a maximum value of 46.68% for treatment 100 ($p<0.05$). The lipid content varied between 32.12% (100) and 34.74% (Control). Intermediate values were found for treatments 25 (33.38%) and 50 (33.78%), ($p>0.05$). The value for carbohydrates ranged from 7.67% for treatment 75 to 13.07% in the Control. Crude fiber was lowest for the Control (7.70%) and highest for treatment 75 (14.25%). Treatments 25 (10.06%) and 50 (10.05%) showed intermediate and statistically equal results. As for the ash content, the Control showed the highest value (3.11%), with a decrease for treatments 25 (2.85%), 50 (2.64%), 75 (2.50%), and 100 (2.51%), with a statistical difference only between the Control and 75 treatments. Finally, the energy value was a minimum of 494.82 kcal in treatment 75 and a maximum of 530.46 kcal in the Control. On the other hand, treatments 25 (515.22 kcal), 50 (518.22 kcal), and 100 (516.01 kcal) were statistically the same.

Table 4. Proximate composition of *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Parameters	Treatments				
	Control	**25**	**50**	**75**	**100**
Moisture (%)	$61.05^{c} \pm 0.74$	$60.59^{c} \pm 0.58$	$62.26^{bc} \pm 0.61$	$63.12^{b} \pm 0.87$	$66.01^{a} \pm 0.40$
Proteins (%)	$41.38^{d*} \pm 0.30$	$42.36^{c*} \pm 0.27$	$42.15^{c*} \pm 0.08$	$43.21^{b*} \pm 0.03$	$46.68^{a*} \pm 0.04$
Lipids (%)	$34.74^{a} \pm 0.32$	$33.38^{a} \pm 0.15$	$33.79^{a} \pm 0.95$	$32.37^{a} \pm 0.76$	$32.12^{a} \pm 2.15$
Carbohydrates (%)	$13.07^{a} \pm 0.92$	$11.35^{ab} \pm 0.31$	$11.37^{ab} \pm 0.91$	$7.67^{c} \pm 0.68$	$10.04^{bc} \pm 1.81$
Crude fiber (%)	$7.70^{d*} \pm 0.42$	$10.06^{bc*} \pm 0.75$	$10.05^{b*} \pm 0.04$	$14.25^{a*} \pm 0.55$	$8.65^{cd*} \pm 0.39$
Ash (%)	$3.11^{a*} \pm 0.07$	$2.85^{b*} \pm 0.02$	$2.64^{c*} \pm 0.03$	$2.50^{d*} \pm 0.02$	$2.51^{d*} \pm 0.02$
Energy (Kcal)	$530.46^{a} \pm 1.78$	$515.22^{a} \pm 3.68$	$518.22^{a} \pm 4.64$	$494.82^{b} \pm 5.22$	$516.01^{a} \pm 12.31$

Results presented as mean ± standard deviation. Different letters in the same column indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test, except when the column shows *, which indicates the Mann-Whitney U test. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake.

Table 5 shows the fatty acid profile of *Zophobas atratus* larvae. Thirteen fatty acids were identified for all the treatments, showing that there were no changes in the profile, although there were variations in their percentages.

As for the main variations, the treatments showed increasing values of alpha-linolenic acid (C18:3n3), the higher the percentage of linseed cake in the substrate. The highest value was found for treatment 100 (28.66%), which was statistically higher than the Control (1.03%). As for linoleic acid (C18:2n6 cis), the maximum value was found for the Control (24.22%) and the minimum for treatment 100 (16.62%). Finally, palmitic acid showed a statistically significant reduction from 30.56% in the Control to 17.03% in treatment 100.

These changes in fatty acid content had an impact mainly on the sum of polyunsaturated fatty acids, which increased from 25.25% for the Control to 45.28% in treatment 100; and on the sum of saturated fatty acids, which showed a maximum value in the Control (41.71%) and a minimum in treatment 100 (24.92%). In addition, the differences in linoleic (C18:2n6 cis) and alpha-linolenic (C18:3n3) acids were able to alter the omega-6/omega-3 ratio, which varied from 23.61 in the Control to 0.58 in treatment 100.

Table 5. Fatty acid profile of *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Fatty acids (%)	Treatments				
	0 (Control)	25	50	75	100
Saturated					
C8:0	$0.75^{a} \pm 0.09$	$0.88^{a} \pm 0.16$	$0.66^{a} \pm 0.04$	$0.84^{a} \pm 0.02$	$0.79^{a} \pm 0.09$
C10:0	$0.11^{a*} \pm 0.01$	$0.12^{a*} \pm 0.01$	$0.09^{a*} \pm 0.00$	$0.09^{a*} \pm 0.00$	$0.09^{a*} \pm 0.00$
C12:0	$0.07^{ab*} \pm 0.02$	$0.34^{a*} \pm 0.00$	$0.06^{ab*} \pm 0.00$	$0.05^{ab*} \pm 0.00$	$0.03^{b*} \pm 0.00$
C14:0	$1.14^{b} \pm 0.03$	$1.28^{a} \pm 0.01$	$1.04^{c} \pm 0.00$	$0.95^{d} \pm 0.04$	$0.73^{e} \pm 0.04$
C15:0	$0.23^{c} \pm 0.00$	$0.27^{a} \pm 0.00$	$0.25^{b} \pm 0.00$	$0.21^{d} \pm 0.00$	$0.17^{e} \pm 0.01$
C16:0	$30.56^{a} \pm 0.29$	$27.19^{b} \pm 0.08$	$24.89^{c} \pm 0.33$	$22.20^{d} \pm 0.09$	$17.03^{e} \pm 0.17$
C17:0	$0.45^{b*} \pm 0.01$	$0.52^{ab*} \pm 0.01$	$0.79^{a*} \pm 0.02$	$0.48^{ab*} \pm 0.00$	$0.58^{ab*} \pm 0.15$
C18:0	$8.39^{a*} \pm 0.21$	$6.67^{ab*} \pm 0.04$	$6.08^{ab*} \pm 0.23$	$5.83^{ab*} \pm 0.12$	$5.51^{b*} \pm 0.26$
Monounsaturated					
C16:1	$0.85^{a*} \pm 0.01$	$0.83^{ab*} \pm 0.00$	$0.79^{ab*} \pm 0.02$	$0.69^{ab*} \pm 0.00$	$0.63^{b*} \pm 0.01$
C17:1	$0.68^{ab*} \pm 0.03$	$0.70^{a*} \pm 0.00$	$0.51^{ab*} \pm 0.01$	$0.50^{ab*} \pm 0.00$	$0.38^{b*} \pm 0.01$
C18:1n9 cis	$29.99^{a*} \pm 0.22$	$27.98^{ab*} \pm 0.12$	$27.59^{ab*} \pm 0.17$	$27.36^{ab*} \pm 0.15$	$27.04^{b*} \pm 0.15$
Polyunsaturated					
C18:2n6 cis	$24.22^{a} \pm 0.33$	$22.11^{b} \pm 0.26$	$21.14^{c} \pm 0.16$	$18.86^{d} \pm 0.03$	$16.62^{e} \pm 0.24$
C18:3n3	$1.03^{e} \pm 0.09$	$9.77^{d} \pm 0.15$	$14.43^{c} \pm 0.33$	$19.67^{b} \pm 0.07$	$28.66^{a} \pm 0.45$
Other Fatty Acids	$1.50^{ab} \pm 0.27$	$1.34^{b} \pm 0.23$	$1.82^{ab} \pm 0.14$	$2.26^{a} \pm 0.51$	$1.75^{ab} \pm 0.15$
∑ SFA	$41.71^{a} \pm 0.45$	$37.26^{b} \pm 0.31$	$33.58^{c} \pm 0.51$	$30.65^{d} \pm 0.27$	$24.92^{e} \pm 0.60$
∑ MUFA	$31.52^{a*} \pm 0.18$	$29.52^{ab*} \pm 0.13$	$29.03^{ab*} \pm 0.13$	$28.56^{ab*} \pm 0.15$	$28.05^{b*} \pm 0.15$
∑ PUFA	$25.25^{e} \pm 0.27$	$31.88^{d} \pm 0.41$	$35.57^{c} \pm 0.49$	$38.53^{b} \pm 0.10$	$45.28^{a} \pm 0.67$
omega-6/omega-3	$23.61^{a*} \pm 2.11$	$2.26^{b*} \pm 0.01$	$1.47^{b*} \pm 0.02$	$0.96^{b*} \pm 0.00$	$0.58^{b*} \pm 0.01$

Results presented as mean ± standard deviation. Different letters on the same line indicate a statistical difference between treatments ($p \leq 0.05$) according to the Tukey test, except when the line shows *, which indicates the Mann-Whitney U test. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake. ∑ PUFA: sum of polyunsaturated fatty acids; ∑ MUFA: sum of monounsaturated fatty acids; ∑ SFA: sum of saturated fatty acids.

Table 6 shows the lipid quality indices analyzed for *Zophobas atratus* larvae. In general, there was a significant increase between treatments for almost all parameters as the presence of linseed cake in the larvae's substrate increased. Thus, the values for UI, PI, COX, H/H and HPI were minimum in the Control treatment (83.05, 27.02, 3.01, 1.73 and 1.61, respectively) and maximum in the 100 treatment (147.27, 74.61, 8.17, 4.06, 3.68, respectively).

The exception was TI and AI, where there was a statistically significant reduction as the linseed cake content in the substrate increased. The minimum values for these parameters were found in treatment 100 (0.21 and 0.27, respectively) and the maximum in Control (1.29 and 0.62, respectively).

Table 6. Lipid quality indices of *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Lipid Quality Parameters	Treatments				
	Control	25	50	75	100
UI	$83.05^{e} \pm 0.65$	$103.03^{d} \pm 0.84$	$114.46^{c} \pm 1.49$	$125.27^{b} \pm 0.01$	$147.27^{a} \pm 1.98$
PI	$27.02^{e} \pm 0.15$	$42.34^{d} \pm 0.55$	$50.68^{c} \pm 0.82$	$58.88^{b} \pm 0.10$	$74.61^{a} \pm 1.14$
COX	$3.01^{e} \pm 0.01$	$4.56^{d} \pm 0.05$	$5.57^{c} \pm 0.08$	$6.46^{b} \pm 0.01$	$8.17^{a} \pm 0.12$
H/H	$1.73^{e} \pm 0.01$	$2.07^{d} \pm 0.01$	$2.43^{c} \pm 0.04$	$2.84^{b} \pm 0.02$	$4.06^{a} \pm 0.01$
HPI	$1.61^{e} \pm 0.01$	$1.88^{d} \pm 0.01$	$2.22^{c} \pm 0.03$	$2.57^{b} \pm 0.03$	$3.68^{a} \pm 0.02$
TI	$1.29^{a*} \pm 0.02$	$0.63^{b*} \pm 0.01$	$0.46^{c*} \pm 0.02$	$0.35^{d*} \pm 0.01$	$0.21^{e*} \pm 0.01$
AI	$0.62^{a} \pm 0.01$	$0.53^{b} \pm 0.01$	$0.45^{c} \pm 0.01$	$0.39^{d} \pm 0.01$	$0.27^{e} \pm 0.01$

Results presented as mean ± standard deviation. Different letters in the same row indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test, except when the column shows *, which indicates the Mann-Whitney U test. H/H: Hypocholesterolemic/hypercholesterolemic ratio; HPI: Health promotion index; UI: Unsaturation index; COX: Oxidizability index; PI: Peroxidizability index; AI: Atherogenicity index; TI: Thrombogenicity index. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake.

The metal profile of the *Zophobas atratus* larvae is shown in Table 7. The metals phosphorus, sodium, zinc and potassium increased as the linseed cake content in the substrates increased. Potassium was present in higher concentrations in the 100 larvae (594.90 mg/100g) and lower in the control (526.14 mg/100g). Phosphorus varied from 527.67 mg/100g in the control to 589.75 mg/100g in the larvae fed 100. Sodium varied from 65.99 mg/100g (Control) to 78.28 mg/100g (100). For zinc, the Control larvae had 7.91 mg/100g and the treatment 100 had 8.84 mg/100g.

Magnesium, calcium, copper, iron and manganese also varied between treatments. The magnesium content showed higher values ($p<0.05$) for treatment 100 (124.84 mg/100g) and Control (123.00 mg/100g) and lower results for treatment 75 (112.95 mg/100g). Calcium varied between 34.17 mg/100g (50) and 39.81 mg/100g (Control). Copper was present in all treatments and ranged from 0.90 mg/100g (75) to 1.01 mg/100g (100). Iron ranged from 1.24 mg/100g in the 75 treatment to 2.60 mg/100g in the Control. Manganese ranged from 1.06 mg/100g (75) to 1.21 mg/100g in the Control and 50 treatments.

On the other hand, arsenic, cadmium and selenium were not found in any of the treatments and nickel was only found in treatments 50 (0.05 mg/100g), 75 (0.03 mg/100g) and 100 (0.05 mg/100g).

Table 7. Metal composition in *Zophobas atratus* larvae fed with substrates containing different percentages of linseed cake.

Metals (mg/100 g)	Treatments				
	Control	25	50	75	100
Potassium	$526.14^{c} \pm 9.27$	$531.85^{c} \pm 7.83$	$552.64^{b} \pm 9.90$	$533.94^{bc} \pm 15.33$	$594.90^{a} \pm 1.25$
Phosphorus	$527.67^{b} \pm 13.78$	$530.38^{ab} \pm 19.10$	$542.90^{ab} \pm 23.60$	$561.35^{ab} \pm 16.39$	$589.75^{a} \pm 33.55$
Magnesium	$123.00^{ab} \pm 3.00$	$118.82^{bc} \pm 0.37$	$117.29^{cd} \pm 0.36$	$112.95^{d} \pm 1.95$	$124.84^{a} \pm 0.29$
Sodium	$65.99^{d} \pm 0.96$	$68.32^{c} \pm 0.10$	$73.99^{b} \pm 1.01$	$69.81^{c} \pm 0.07$	$78.28^{a} \pm 1.24$
Calcium	$39.81^{a} \pm 0.60$	$36.30^{ab} \pm 2.45$	$34.17^{c} \pm 1.12$	$34.41^{c} \pm 3.27$	$39.10^{ab} \pm 0.32$
Zinc	$7.91^{c} \pm 0.19$	$8.21^{bc} \pm 0.12$	$8.78^{ab} \pm 0.03$	$7.89^{c} \pm 0.44$	$8.84^{a} \pm 0.19$
Iron	$2.60^{a*} \pm 0.69$	$1.32^{b*} \pm 0.03$	$1.70^{ab*} \pm 0.56$	$1.24^{b*} \pm 0.20$	$1.80^{a*} \pm 0.36$
Manganese	$1.21^{a*} \pm 0.01$	$1.20^{a*} \pm 0.00$	$1.21^{a*} \pm 0.01$	$1.06^{b*} \pm 0.04$	$1.15^{ab*} \pm 0.05$
Copper	$0.92^{ab} \pm 0.01$	$0.91^{b} \pm 0.01$	$0.98^{ab} \pm 0.01$	$0.90^{b} \pm 0.07$	$1.01^{a} \pm 0.01$
Nickel	< 0,025**	< 0,025**	$0.05^{a*} \pm 0.01$	$0.03^{a*} \pm 0.01$	$0.05^{a*} \pm 0.01$
Arsenic	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**
Cadmium	< 0,50**	< 0,50**	< 0,50**	< 0,50**	< 0,50**
Cobalt	< 0,025**	< 0,025**	< 0,025**	< 0,025**	< 0,025**
Selenium	< 0,025**	< 0,025**	< 0,025**	< 0,025**	< 0,025**

Results presented as mean ± standard deviation. Different letters on the same line indicate a statistical difference between treatments ($p \leq 0.05$) according to Tukey's test, except when the line shows *, which indicates the Mann-Whitney U test. ** indicates results below the detection limit. Treatments: 0 (Control) = 70% poultry growth feed + 30% wheat bran; 25 = 75% Control + 25% linseed cake; 50 = 50% Control + 50% linseed cake; 75 = 25% Control + 75% linseed cake; 100 = 100% linseed cake.

Supplementary Table 1 shows the correlations established between the parameters of the *Zophobas atratus* larvae and of the substrates. The results indicate that the substrate parameters of protein, lipid, fiber, C18:3n3, and PUFA showed a strong and positive correlation with the larvae parameters of protein content, moisture, FCR, mortality, C18:3n3, and PUFA. This was also seen between the substrate parameters of carbohydrate, moisture, ash, energy value, omega-6/omega-3, C18:2n6, sum of MUFA and SFA, and the larvae parameters of lipid content, carbohydrate, ash, ECI, mass gain, omega-6/omega-3, C18:2n6, sums of MUFA and SFA.

On the other hand, substrate parameters of protein, lipid, fiber, C18:3n3, and the sum of PUFA showed a strong negative correlation with larvae parameters of lipid content, carbohydrate, ash, ECI, mass gain, omega-6/omega-3, C18:2n6, sums of MUFA and SFA. The same was found between substrate parameters of carbohydrate, moisture, ash, Kcal, omega-6/omega-3, C18:2n6, sum of MUFA and SFA and the larvae parameters of protein, FCR, moisture, mortality, C18:3n3, and sum of PUFA.

Supplementary Table 1. Results for Spearman's correlation between parameters of *Zophobas atratus* larvae and substrates containing different percentages of linseed cake.

Larval parameters	Substrate parameters											
	Carbohydrates	Crude fiber	Proteins	Moisture	Lipids	Ash	omega-6/omega-3	C18:2n6	C18:3n3	∑ PUFA	∑ MUFA	∑ SFA
Moisture	**-0,84**	**0,86**	**0,88**	**-0,87**	**0,85**	**-0,82**	**-0,89**	**-0,79**	**0,81**	**0,85**	**-0,74**	**0,84**
Proteins	**-0,89**	**0,88**	**0,84**	**-0,86**	**0,88**	**-0,88**	**-0,85**	**-0,91**	**0,92**	**0,86**	**-0,80**	**0,90**
Lipids	**0,72**	**-0,63**	**-0,71**	**0,61**	**-0,69**	**0,66**	**0,65**	**0,74**	**-0,67**	**-0,69**	0,42	**0,65**
Carbohydrates	**0,72**	**-0,61**	**-0,67**	**0,58**	**-0,65**	**0,67**	**0,62**	**0,72**	**-0,67**	**-0,70**	0,40	**0,65**
Crude fiber	-0,33	0,33	0,31	-0,31	0,34	-0,31	-0,29	-0,35	0,33	0,29	-0,19	0,34
Ash	**0,89**	**-0,91**	**-0,86**	**0,90**	**-0,90**	**0,90**	**0,89**	**0,87**	**-0,90**	**-0,87**	**0,76**	**0,92**
Energy value	**0,61**	**-0,53**	**-0,59**	0,50	**-0,56**	**0,57**	**0,54**	**0,59**	**-0,53**	**-0,60**	0,29	**0,55**
MR	**-0,75**	**0,79**	**0,70**	**-0,81**	**0,79**	**-0,72**	**-0,73**	**-0,73**	**0,76**	**0,65**	**-0,82**	**0,75**
ECI	**0,75**	**-0,72**	**-0,73**	**0,71**	**-0,71**	**0,78**	**0,75**	**0,75**	**-0,77**	**-0,80**	**0,57**	**0,76**
FCR	**-0,70**	**0,81**	**0,79**	**-0,82**	**0,80**	**-0,70**	**-0,80**	**-0,70**	**0,71**	**0,72**	**-0,60**	**0,76**
Unit Larval Mass	**0,86**	**-0,85**	**-0,89**	**0,84**	**-0,84**	**0,85**	**0,88**	**0,89**	**-0,89**	**-0,89**	**0,69**	**0,90**
omega-6/omega-3	**0,98**	**-0,97**	**-0,97**	**0,96**	**-0,97**	**0,97**	**0,96**	**0,96**	**-0,95**	**-0,99**	**0,81**	**0,99**
C18:2n6	**0,99**	**-0,95**	**-0,97**	**0,93**	**-0,95**	**0,97**	**0,96**	**0,99**	**-0,97**	**-0,99**	**0,78**	**0,96**
C18:3n3	**-0,94**	**0,97**	**0,96**	**-0,95**	**0,96**	**-0,97**	**-0,97**	**-0,97**	**0,99**	**0,97**	**-0,79**	**0,97**
∑ PUFA	**-0,96**	**0,98**	**0,94**	**-0,97**	**0,97**	**-0,95**	**-0,96**	**-0,96**	**0,97**	**0,93**	**-0,85**	**0,96**
∑ MUFA	**0,92**	**-0,90**	**-0,90**	**0,89**	**-0,91**	**0,93**	**0,93**	**0,92**	**-0,95**	**-0,92**	**0,72**	**0,89**
∑ SFA	**0,96**	**-0,96**	**-0,97**	**0,95**	**-0,95**	**0,97**	**0,98**	**0,96**	**-0,97**	**-0,99**	**0,77**	**0,96**

Results in bold indicate a significant correlation ($p < 0.05$). MR: Mortality Rate; ECI: Conversion Efficiency of Ingested Feed; FCR: Feed Conversion Ratio; ∑ PUFA: sum of polyunsaturated fatty acids; ∑ MUFA: sum of monounsaturated fatty acids; ∑ SFA: sum of saturated fatty acids.

4. DISCUSSION

4.1 Larval efficiency in mass gain and feed conversion

Feeding *Zophobas atratus* larvae by partially replacing conventional feed with linseed cake is a topic with great potential in the context of insect production as a food source for humans, as it allows for a reduction in costs without compromising production efficiency. The results indicate a good yield in terms of total mass gain, Conversion Efficiency of Ingested Feed (ECI) and Feed Conversion Ratio (FCR), as well as a Mortality Rate (MR%) close to the Control. In terms of ECI, the Control larvae and treatments 25, 50 and 75 showed statistically similar results, suggesting that partially replacing the Control substrate with up to 75% linseed cake does not compromise the efficiency of the larvae in converting such by-product. A study conducted by Nascimento *et al*. (2022) found an ECI for *Z. atratus* larvae fed a control substrate (70% poultry growth feed + 30% ground corn) of approximately 25%, a result close to that of the present study. As for FCR, a substrate obtained from a commercial *Zophobas atratus* breeding company was used in a study by van Broekhoven *et al*. (2015) and the FCR found was 3.64, close to the Control, 25, 50 and 75 treatments, which corroborates the commercial applicability of the substrates developed in this study.

On the other hand, the exclusive use of linseed cake as a substrate proved to be insufficient to provide adequate rearing for *Zophobas atratus* larvae. Substrates with a high protein content and low carbohydrate content showed lower feed conversion efficiency for *Tenebrio molitor*, in line with the results described by Zhang *et al*. (2019) and with the findings of the present study, as the linseed cake had a significantly higher protein content (102.54% higher) and a 64.31% lower carbohydrate content than the control feed. This may indicate that the larvae tend to grow more adequately on substrates with a balance of nutrients, which may justify the better results on substrates with intermediate proportions of linseed cake.

Kröncke and Benning (2022) report that *Tenebrio molitor* larvae tend to develop better when consuming substrates high in carbohydrates and proteins and low in lipids (around 67.3 to 71.5% for carbohydrates, 19.9 to 22.8% for proteins and 8.6 to 10.0% for lipids). This corresponds to a carbohydrate:protein ratio of between 3.13:1 and 3.38:1. For the present study, the minimum ratio found was 0.56:1 (100), far below the recommendation proposed by Kröncke and Benning (2022). On the other hand, substrates 25 and 50 showed good production yields and intermediate carbohydrate:protein ratios of 2.14:1 and 1.43:1, respectively, indicating flexibility for this parameter.

The larvae are also able to select and consume parts of the substrate that are sources of that specific nutrient that is most needed at the time. In this way, lower concentrations of linseed cake in the substrates of *Zophobas atratus* larvae can promote the supply of nutrients in sufficient quantities to be self-selected by the larvae, generating larval production close to Control (Choi and Lee, 2022).

Most of the correlations between the larval rearing efficiency variables and the nutritional composition of the substrates were moderate, close to 0.70 (or -0.70), which reinforces the importance of a balance of nutrients in the substrate of *Zophobas atratus* larvae. In addition, linseed cake contains some anti-nutritional factors, such as linatin, which can compromise the larval development of insects, depending on the amount present (Oonincx *et* *al*., 2019; Gai *et al*., 2023) and may have been a determining factor in the higher mortality rate in this study for treatment 100. Linatin, for example, is an antagonist of vitamin B6 and reduces its bioavailability. Compromising the availability of this nutrient can cause delayed mass gain and increased mortality in insects (Abbas, 2020).

Linseed cake contains various polyphenols (20.8 mg GAE/g, (Ho *et al*., 2007)), substances that have a protective function in vegetables against insects. Niveyro *et al*. (2023) described that the presence of polyphenols in the substrate can be a factor in inhibiting their consumption by insects. Furthermore, Beninger *et al*. (2004) found that the consumption of chlorogenic acid by the species *Lymantria dispar* Linnaeus (Lepidoptera: Lymantriidae) and *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) was capable of significantly impairing larval growth. Roth *et al*. (1997) found that phenolic glycosides impaired larval growth of the species *Lymantria dispar*.

Therefore, the presence of these anti-nutritional factors in greater quantities in the substrate composed exclusively of linseed cake may have compromised the mass gain and increased the mortality of *Zophobas atratus* larvae.

4.2 Nutritional characterization of the larvae

In general, the composition of the larvae fed with different substrates showed a high protein content (between 41.38% and 46.68%) and lipid content (between 32.12% and 34.74%), as well as moderate values of crude fiber (between 7.70% and 14.25%), carbohydrates (between 7.67% and 13.07%) and ash (between 2.50% and 3.11%). The results found are in line with the literature described by Harsányi *et al*. (2020) and Nascimento *et al*. (2022), when they studied the nutritional composition of the same insect species fed with vegetable waste, garden waste, manure, cereal straw and grape waste.

4.2.1 Proteins

The protein content showed a significant increase in the larvae fed exclusively on linseed cake compared to the Control. This represents an important ability to convert organic material, originally disposable, into biomass of high nutritional value, as noted by Ruschioni *et al*. (2020), when feeding *Tenebrio molitor,* an insect from the same family as *Zophobas atratus*, with a substrate consisting of 25% olive oil cake and 75% wheat bran and verifying a percentage of 47.58% protein. The protein content of *Z. atratus* is close to that found for boneless beef loin (approximately 47%, dry basis), but is below that of boneless pork loin (approximately 67%, dry basis) (USDA, 2023). Soybeans, on the other hand, have between 40 and 42% protein on a dry basis, similar to that found for *Z. atratus* (USDA, 2023). This means that *Z. atratus* biomass is a rich source of protein, even compared to conventional foods.

In addition, the larval protein content showed strong correlations with all the nutritional characteristics of the substrate: protein (0.84), lipid (0.88), carbohydrate (-0.89), moisture (-0.86), crude fiber (0.88), ash (-0.88), omega-6/omega-3 (-0.85), C18:2n6 (-0.91), C18:3n3 (0.92), ∑ PUFA (0.86), ∑ MUFA (-0.80) and ∑SFA (-0.90). Paying attention to these parameters can help rear insects with a biomass richer in protein.

Some studies have reported the influence of the protein content of the substrate on larval protein. Adámková *et al*. (2020) carried out a study with *Tenebrio molitor*, feeding their larvae wheat bran and lentil flour. As a result, it was found that increasing the protein content of the substrate led to an increase in larval protein, ranging from approximately 54% for larvae fed a low-protein substrate (16.20% protein content in the substrate) to up to 65% for larvae fed a high-protein substrate (24.10% protein content in the substrate) (dry basis). Van Broekhoven *et al*. (2015) found similar results with *Zophobas atratus* larvae fed various wastes, including cookies, bread crumbles and discarded grains. As a result, a substrate rich (39.10% protein content in the substrate) in protein enabled a maximum larval protein value of 42.50% to be reached, compared to a substrate poor (11.90% protein content in the substrate) in protein (34.20%). This trend was also seen in this study, and may be associated with the fact that, due to the higher protein content, the larvae are more easily able to ingest, metabolize and transform the amino acids into larval protein.

Considering the Recommended Daily Intake (RDA) of protein for a healthy adult with a minimum level of physical activity (0.8 g of protein per kg of body weight/day), a 75 kg adult would need 60 g of protein (Wu, 2016). This value can be achieved by consuming between 128.53 g (100) and 144.99 g (Control) of *Zophobas atratus* larvae throughout the day (dry basis). In comparison, approximately 127.65 g of boneless beef loin (dry basis) would be required (USDA, 2023).

The whole *Zophobas atratus* larva or its isolated protein have great potential as ingredients in the protein enrichment of processed foods, such as animal feed, food supplements, protein bars and products with high global consumption. As examples, cookies fortified with 30% *Zophobas atratus* flour showed a 63% increase in their protein content (from 9.40% to 14.92%) (Sriprablom *et al*., 2022); snacks developed with *Alphitobius diaperinus flour,* where fortification in the proportion of 30% insect flour could result in a 99.30% increase in protein for the snack (from 12.53% to 24.98%) (Roncolini *et al*., 2020).

4.2.2 Lipids and fatty acids

The lipid content remained statistically constant, regardless of the food source of the *Z. atratus larvae*. Kulma *et al*. (2020) found a range of 31.30 to 36.00% lipids in *Z. atratus larvae* fed wheat bran between 60 and 120 days old, close to the values found in this study. Arrese and Soulages (2010) argue that the lipid content of the substrate has a positive correlation with the concentration of lipid in the larval biomass, which is the opposite of what was found in this study.

Nascimento *et al*. (2022) found that *Zophobas atratus* larvae fed substrates with higher concentrations of carbohydrates had a higher lipid content (ranging from 40.50% to 45.58%), which would be in line with the correlation found in this study. Lipid synthesis in insects can occur from the conversion of carbohydrates into triglycerides (Arrese and Soulages, 2010). In this way, it could be assumed that, for the present study, the lower carbohydrate content of the substrates containing linseed cake, combined with the higher lipid content of these substrates, may have been a balancing factor in the total lipids found in the larval biomasses. To modulate the lipid content of the larvae, the following strong correlations with substrate parameters should be considered: protein (-0.71); carbohydrate (0.72), and C18:2n6 (0.74).

As for the percentages of fatty acids, significant changes were observed as the content of linseed cake incorporated into the substrates of *Z. atratus* larvae increased. For the Control larvae, the predominant fatty acids were palmitic (C16:0), oleic (C18:1n9 cis), linoleic (C18:2n6 cis) and stearic (C18:0). These data are in agreement with the study carried out by Kulma *et al*. (2020), who fed wheat bran to *Zophobas atratus* larvae and found values for palmitic acid (C16:0) between 25.60% and 28.34%; oleic acid (C18:1n9 cis) between 27.75% and 29.67%; linoleic acid (C18:2n6 cis) between 19.91% and 21.33%; and stearic acid (C18:0) between 11.93% and 12.93%, depending on the period of larval development. The high presence of alpha-linolenic acid (C18:3n3) in linseed cake (Table 1) may have been able to modulate the lipid profile of the larvae, to the extent that, for treatment 100, the predominant fatty acid was alpha-linolenic (C18:3n3) (28.66%), which showed an increase of almost 28 times in relation to the value of the Control larvae (1.03%). The ∑ PUFA increased from 25.25% in the Control to 45.28% (100). This increase can be explained by the larvae's ability to consume and accumulate alpha-linolenic acid (C18:3n3). Similar results were verified by Lawal *et al*. (2021), when using varying proportions of linseed in the substrate of *Tenebrio molitor*. They found that the content of alpha-linolenic acid (C18:3n3) increased from 0.36% in the Control larvae to 6.40% in the larvae fed 20% linseed as a substitute for wheat bran.

In this study, there was also a significant reduction in ∑ SFA, from 41.71% in the control to 24.92% in larva 100. A greater and more significant reduction was seen in palmitic acid, from 30.56% to 17.03%, which may be associated with the type of substrate offered. Linseed cake had little SFA (14.43% - Table 1) and only 9.53% palmitic acid. Even so, the larvae still had levels of SFA and palmitic acid, which may be justified by the fact that fatty acids of 12 to 18 carbons can be biosynthesized by some insect species to fulfil important functions in their organisms (Hoc *et al*., 2020, Lawal *et al*., 2021).

The increase in alpha-linolenic acid (C18:3n3) was able to modify the omega-6/omega-3 ratio. The omega-6/omega-3 ratios found in this study ranged from 23.61 in the Control larva to 0.58 in the 100 larvae, indicating an ability to invert this ratio depending on the type of substrate offered to the larvae. In general, insects of the Coleoptera order have a high omega-6/omega-3 ratio. As an example, *Tenebrio molitor* larvae studied by Dreassi *et al*. (2017) had a ratio between 21.55 and 34.27, well above the values for beef tenderloin (2.67) (Orkusz, 2021) and the values recommended for human consumption in the literature (not higher than 5) (Kulma *et al*., 2020, Mukhametov *et al*., 2022). The present study, however, was able to verify that modifications to the substrate of *Z. atratus* larvae can alter the omega-6/omega-3 ratio to values closer to the human consumption recommendation.

Polyunsaturated fatty acids play an important role in regulating inflammatory processes in the body, where omega-3, in general terms, can play an anti-inflammatory role and omega-6, a pro-inflammatory role. Excessive consumption of omega-6 can cause a physiological imbalance and generate a series of adverse effects, including a chronic inflammatory state and, in the long term, neoplastic processes (Orkusz, 2021). In this way, we can consider the importance of this study in the sense of modulating the levels of omega-6 and omega-3 in *Z. atratus* so that its consumption provides a balance in the omega-6/omega-3 ratio in the human body. (2020), omega-3 fatty acids are capable of significantly reducing major adverse cardiovascular events, myocardial infarction and mortality from heart problems. The European Food Safety Authority (EFSA, 2010) proposes an adequate intake of alpha-linolenic acid (C18:3n3) of 0.5% of daily energy intake (or approximately 1.1g for a 2000 kcal diet). The consumption of 100 g of *Zophobas atratus* larvae in this study can provide 0.34 g (Control), 3.12 g (25), 4.68 g (50), 6.09 g (75) or 8.80 g (100) of C18:3n3 (dry basis) (Weihrauch *et al*., 1977), depending on the substrate offered. Thus, helping to reach the recommended daily intake of omega-3 for humans. Considering the consumption of oil extracted from larvae, the values can reach 0.97 g/100 g (Control), 9.33 g/100 g (25), 13.84 g/100 g (50), 18.80 g/100 g (75) and 27.39 g/100 g (100) (Weihrauch *et al*., 1977), demonstrating the richness in alpha-linolenic fatty acid (C18:3n3) in the larvae fed linseed cake and making it possible to use the insect in its whole form or the extracted oil as a source of this polyunsaturated fatty acid. As an example, Tzompa-Sosa *et al*. (2019) extracted oil from *Tenebrio molitor,* which was liquid at room temperature and had compounds related to pleasant aromas, suggesting that it could be used as a table oil as an alternative to olive oil and as a food ingredient in various preparations, such as cakes, pies and cookies.

Regarding other lipid quality parameters evaluated, the UI is an index that assesses the degree of unsaturation of the lipids present in a food source. In its formula, fatty acids with a higher number of double chains have a greater impact on its result, but it still considers fatty acids with a low degree of unsaturation. Ghassemi-Golezani and Farhangi-Abriz (2018) observed a maximum value of 155 for the UI of soybeans, while Realini *et al*. (2013) found a value of 111 in porcine *longissimus thoracis* muscle. For the present study, it was found that the UI values for *Zophobas atratus* larvae Control (83.05), 25 (103.03) and 50 (114.46) were close to the result found for pigs, but lower than soybeans. On the other hand, as the linseed content in the substrate increased, the larvae showed an increase in this content, probably due to the accumulation of omega-3. Thus, treatment 100 showed the highest value (147.27), close to that found for soybeans and above that found for porcine *longissimus thoracis*. This corroborates the possibility of modulating the fatty acid profile of *Zophobas atratus* larvae by feeding substrates rich in fatty acids with a higher degree of unsaturation.

The peroxidizability index (PI) is used to assess the susceptibility of a lipid source to undergo peroxidation reactions. The peroxidation process can lead to rancidity or deterioration of the food and oxidative stress in biological systems. The lower the index, the less susceptible the food is to peroxidation (Gharibzahedi and Altintas, 2023). On the other hand, Wołoszyn *et al*. (2020) report that higher PI values indicate a food's greater protective potential against coronary artery disease. For the present study as the polyunsaturated fatty acid content of the larvae increases, their PI becomes higher, a fact confirmed by the variation in treatments: 27.02 (Control), 42.34 (25), 50.68 (50), 58.88 (75) and 74.61 (100). Zula and Desta (2021) found a PI of 38.27 for raw Nile tilapia, close to the values found for Control (27.02) and 25 (42.34) larvae. Gruffat *et al*. (2020) found the PI for the *longissimus thoracis* of lambs to be between 23.40 and 38.20, close to the Control (27.02) and 25 (42.34) treatments in this study. Gharibzahedi and Altintas (2023) analyzed the oil from the larvae of *Alphitobius diaperinus* (Tenebrionidae) and found variations in PI between 34.99 and 41.69, depending on the extraction method. Thus although the increase in alpha-linolenic acid (C18:3n3) in larvae fed linseed cake is potentially beneficial from a nutritional point of view, some undesirable effects may also become more present, such as greater susceptibility to oxidation and peroxidation. It is important to note that the oxidative stability of an oil can be modulated through the incorporation of antioxidant agents such as vitamin E, plant extracts and the formation of blends with oils with a lower content of polyunsaturated fatty acids (Fadda *et al*., 2022).

The Oxidizability Index (Cox) analyzes the effect of fatty acid composition on the oxidative stability of lipids, with an emphasis on unsaturated fatty acids. Thus, lower Cox values are desirable to obtain a food with greater oxidative stability (Kotsou *et al*., 2023). The Cox value of the Control larva (3.01) is higher than that of virgin palm oil (1.29 (Mba *et al*., 2017)), is close to that found for olive oil (approximately 2.25 (Khaleghi *et al*., 2023)) and is lower than that found for canola oil (4.46 (Mba *et al*., 2017)). On the other hand, the Cox of larva 25 (4.56) is close to that of canola oil. This would indicate an oxidative stability similar to that of commonly marketed oils. Larvae 50 (5.57), 75 (6.46) and 100 (8.17) have higher values than the other three oils presented above. This means that the greater presence of polyunsaturated fatty acids in larvae 25, 50, 75 and 100, especially alpha-linolenic acid (C18:3n3), may be a determining factor in the increased oxidative susceptibility of the lipids. Kotsou *et al*. (2023) found a similar trend. In their study, *Tenebrio molitor* larvae were fed varying concentrations of wheat bran and *Moringa oleifera* leaves. The Cox values of the larvae ranged from 4.76 to 5.29, where a reduction in this index was noticed as the polyunsaturated fatty acid content of the substrate decreased.

The H/H ratio is associated with the ability of a given fatty acid source to influence lipoprotein metabolism and with the risk of developing cardiovascular diseases. Therefore, higher values are of greater interest, as they indicate a higher proportion of lipid-lowering fatty acids and a lower risk of developing cardiovascular events when consuming the food (Santos-Silva *et al*., 2002; Paszczyk and Łuczyńska, 2020). Paszczyk and Łuczyńska (2020) found considerably lower values than the present study (between 1.73 - Control and 4.06 - 100) for cow (0.55), sheep (0.55) and goat (0.52) cheeses. Hanula *et al*. (2022) found an H/H of 1.30 for beef burgers. *Zophobas atratus* larvae fed wheat bran and brewery grains showed values (1.74) close to those found for the Control in the present study (1.73), but lower compared to the other treatments (Da Silva *et al*., 2024). In this way, the values in this study are higher than other animal sources, reflecting greater safety in their consumption regarding the development of cardiovascular diseases.

The HPI is an index used to assess the nutritional quality of the fatty acids present in a food source and is associated with the safety of its consumption in terms of the risk of atherogenesis (Chen and Liu, 2020). The higher its value, the greater its benefit for protecting cardiovascular health. Fadıloğlu *et al*. (2023) found a value of 1.20 for the HPI of beef burgers and Alabiso *et al*., (2021) 1.83 for beef salami. On the other hand, Kotsou *et al*. (2023) found values of 2.82, 2.57 and 2.49 for *Tenebrio molitor* larvae fed wheat bran and spent coffee beans, where the HPI decreased as the coffee bean content in the substrate increased. For the present study, the results of the Control (1.61), 25 (1.88) and 50 (2.22) treatments were above those found for beef hamburger (Fadıloğlu *et al*., 2023), close to beef salami (Alabiso *et al*., 2021) and below those found by Kotsou *et al*. (2023) for *Tenebrio molitor*. On the other hand, the *Zophobas atratus* larvae in the present study fed with higher linseed contents (75 and 100) showed substantially higher values (2.57 and 3.68 respectively) than those found by Fadıloğlu *et al*. (2023) and Alabiso (2021). This indicates that, in general, the larvae of insects from the Tenebrionidae family may have an HPI with values of great nutritional interest.

The AI and TI indices are used to assess the risks of consuming a particular source of lipids for human health. The AI is established by the ratio between saturated fatty acids and unsaturated fatty acids. The TI, on the other hand, can express the predisposition to clot formation, but it is also calculated considering saturated and unsaturated fatty acids (Orkusz, 2021). Mlček *et al*. (2019) found an AI of 0.70 and a TI of 1.40 for *Z. atratus* larvae fed wheat bran, close to the Control larvae in this study (0.62 and 1.29, respectively) and Orkusz's (2021) findings for sirloin (0.81 and 1.27, respectively). However, due to the modification of the fatty acid profile in the *Z. atratus larvae* studied, it was possible to see a significant reduction in both parameters to much lower values, of up to 0.27 for AI and 0.21 for TI, indicating greater safety in the consumption of this insect regarding arterial and thrombogenic risks, even when compared to traditional foods.

4.2.3 Fibers

The crude fibers found in insects are in their exoskeleton in the form of chitin. For the present study, the results ranged from 7.70% in the Control larva to 14.25% in larva 75. Dragojlović *et al*. (2022) found values between 5.73% and 9.12% for *Z. atratus larvae* fed various substrates containing cabbage, carrots and linseed. This may strengthen the idea that chitin production in larvae is dependent on the type of substrate consumed. Chitin can be partially metabolized by chitinases. In addition, it has been found that oligosaccharides derived from the breakdown of chitin can promote beneficial effects in rats in the control of glucose metabolic disorders and in the suppression of regulators of lipogenesis, gluconeogenesis, adipocyte differentiation and inflammation in adipose tissues (Zheng *et al*., 2018).

4.2.4 Ash and metal profile

In this study, changes in the substrates of *Z. atratus* larvae were not able to significantly alter the ash content, which was between 2.50% and 3.11%. The values were close to those described by Kuntadi *et al*. (2018), of 3.41% for larvae of the same species. Insects contain a diversity of micronutrients, which play important roles in maintaining their biological processes. According to van Huis *et al*. (2013), iron, zinc, copper, calcium, potassium and magnesium can be considered the main minerals present in insects. In this study, phosphorus, potassium, magnesium, sodium, calcium, zinc, iron, manganese and copper were present in all *Z. atratus* larvae. Selenium, arsenic, cadmium and cobalt were not identified; and nickel was only present in larvae 50, 75 and 100. Similar results were found by Nascimento *et al*. (2022), with the exception that sodium, iron and potassium were more present in *Z. atratus larvae* fed different concentrations of grape residue.

Considering the reference values for daily mineral intake (INSTITUTE OF MEDICINE, 1997; INSTITUTE OF MEDICINE, 2001; INSTITUTE OF MEDICINE, 2004) (700 mg/day of phosphorus; 400 mg/day of magnesium; 11 mg/day of zinc; 8mg/day of iron and 2.3 mg/day of manganese for a young adult male between 19 and 30 years old); and the food labeling rules defined by FAO/WHO (2007), the Z. *atratus* larvae studied can be considered high in phosphorus (>210 mg/100g), magnesium (>120 mg/100g for the Control and 100 larvae), zinc (>3.3 mg/100g), iron (>2.4 mg/100g for the Control larva) and manganese (>0.69 mg/100g); and a source of magnesium (>60 mg/100g for larvae 25, 50 and 75) and iron (>1.2 mg/100g for larvae 25, 50, 75 and 100). This implies a high mineral richness of *Z. atratus*.

Furthermore, considering the metal contents of the substrates (Table 1), and the correlations between larval and substrate metals (Supplementary Table 2), it is possible to verify the correlation only for phosphorus (significant correlation of 0.55, $p<0.05$). According to Oonincx and Finke (2021), because they don't have a mineralized exoskeleton, mealworms aren't able to bioaccumulate minerals such as copper, zinc, lead, or cadmium. These elements can be present in greater quantities in the larvae due to their accumulation in their guts and not necessarily due to their absorption by the insects. This may explain the lack of correlation between the magnesium, iron, manganese, sodium, and calcium levels in the substrates and the larvae in this study and may indicate the need for a longer fasting period so that the larvae can empty the digestive tract more completely and the metals results are more reliable.

Supplementary Table 2. Results for Spearman's correlation between metal parameters of *Zophobas atratus* larvae and substrates containing different percentages of linseed cake.

Larval parameters	**Diet parameters**							
	Calcium	**Potassium**	**Phosphorus**	**Magnesium**	**Iron**	**Sodium**	**Zinc**	**Copper**
Calcium	0,14	-	-	-	-	-	-	-
Potassium	-	0,22	-	-	-	-	-	-
Phosphorus	-	-	**0,55**	-	-	-	-	-
Magnesium	-	-	-	-0,27	-	-	-	-
Iron	-	-	-	-	0,44	-	-	-
Sodium	-	-	-	-	-	-0,30	-	-
Zinc	-	-	-	-	-	-	-0,27	-
Copper	-	-	-	-	-	-	-	-0,06

Results in bold indicate a significant correlation ($p < 0.05$).

5. CONCLUSION

The results of this study suggest that the rearing of *Zophobas atratus* larvae could benefit from the application of linseed cake as a nutrient source. The larval rearing indicators showed that replacing conventional commercial substrate with up to 75% linseed cake could statistically maintain feed conversion efficiency. On the other hand, total replacement would not be recommended, as rearing was not efficient in this way.

In addition, this study also indicates that the consumption of linseed cake by the larvae can favor an increase in the protein content of their biomass and, above all, can modulate the fatty acid profile, with emphasis on the bioaccumulation of alpha-linolenic acid (C18:3n3) and the improvement of the omega-6/omega-3 ratio and lipid quality indicators. This could make the consumption of *Zophobas atratus* by humans nutritionally healthier.

The production and consumption of insects has become more popular in recent years. Thus, the use of an agro-industrial by-product, such as linseed cake, as a substitute for conventional substrates in their rearing is a way of reducing costs, as well as promoting more sustainable rearing, contributing to the circular economy.

As a limitation, the linseed cake used was not supplemented to the same extent as the conventional commercial substrate, which may have been a factor compromising the rearing efficiency of the larvae fed only on the by-product. Further studies are therefore suggested, focusing on the ideal conditions for supplementing agro-industrial by-products so that they are nutritionally equal to commercial substrates, as well as assessing the safety of human consumption of insects fed linseed cake.

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6 CONCLUSÃO GERAL

Este estudo fortalece a hipótese de que a substituição do substrato convencional por torta de linhaça na alimentação de larvas de *Zophobas atratus* pode ser uma estratégia viável para o enriquecimento nutricional dessas larvas. A inclusão de até 75% de torta de linhaça manteve a eficiência de conversão alimentar semelhante ao controle, sugerindo que essa proporção pode ser considerada uma prática sustentável e economicamente viável na criação desta espécie.

Contudo, a substituição total do substrato convencional pela torta de linhaça mostrou-se inadequada, uma vez que comprometeu a eficiência da criação das larvas. Esse achado destaca a necessidade de moderação no uso desse coproduto agroindustrial e sugere que um equilíbrio entre substratos comerciais e alternativos pode ser o caminho mais promissor para a criação sustentável de *Zophobas atratus*.

Além disso, a bioacumulação de ácido alfa-linolênico e a melhoria na relação ômega-6/ômega-3 nas larvas alimentadas com 100% de torta de linhaça indicam que esse método pode não apenas enriquecer nutricionalmente a biomassa das larvas, mas também oferecer um alimento mais saudável para o consumo humano. Isso aponta para um potencial de aplicação significativo, especialmente no contexto da economia circular e da produção sustentável de alimentos.

No entanto, é importante ressaltar que a torta de linhaça utilizada neste estudo não foi suplementada de forma a se equiparar nutricionalmente ao substrato convencional, o que pode ter impactado a eficiência da criação. Por isso, recomenda-se que estudos futuros explorem as condições ideais para a suplementação de coprodutos agroindustriais, visando torná-los equivalentes aos substratos comerciais. Além disso, é fundamental avaliar a segurança do consumo humano de insetos alimentados com torta de linhaça, garantindo que essa prática seja segura e benéfica.

Em síntese, este estudo abre novas perspectivas para a criação sustentável de *Zophobas atratus*, mas também aponta para a necessidade de um aprofundamento nas pesquisas, de modo a otimizar as condições de criação e garantir a segurança alimentar. A continuidade dessa linha de investigação pode contribuir para um avanço significativo na produção sustentável de insetos.

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10. II Congresso Virtual UFBA 2021. 2021. (Congresso).
11. VIII Encontro Paranaense de Engenharia de Alimentos (VIII EPEA) e VII Semana Acadêmica de Engenharia de Alimentos (VII SEMEA) Online. 2021. (Outra).
12. VI SEMINÁRIO DE AVALIAÇÃO DE PESQUISA CIENTÍFICA E TECNOLÓGICA (SAPCT) E V WORKSHOP DE INTEGRAÇÃO E CAPACITAÇÃO EM PROCESSAMENTO DE ALTO DESEMPENHO (ICPAD). 2021. (Seminário).
13. Webinário Internacional de Ciência e Tecnologia em Alimentos. 2021. (Outra).
14. Congresso Brasileiro de Inovação e Tecnologia na Gastronomia e Ciência de Alimentos. 2020. (Congresso).
15. Congresso Virtual UFBA 2020. 2020. (Congresso).