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REVIEW ARTICLE

Edible black ant Smith (*Carebara vidua*) as human food – A systematic review

S.A. Siddiqui[1,2]*, *L.-H. Ho*[3], *S.C. Adimulam*[4], *A. Nagdalian*[5], *B. Yudhistira*[6], *R. Castro-Muñoz*[7] and *S.A. Ibrahim*[8]*

[1]Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Essigberg 3, 94315 Straubing, Germany; [2]German Institute of Food Technologies (DIL e.V.), Prof.-von-Klitzing Str. 7, 49610 D-Quakenbrück, Germany; [3]School of Food Industry, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia; [4]Adimulam Biotech LLP, H No-130 Sri Sai Surya Bhavan, 515134 Puttaparthi, Andhra Pradesh, India; [5]Laboratory of Food and Industrial Biotechnology, North Caucasus Federal University, 355017, Stavropol, Russia; [6]Department of Food Science and Technology, Sebelas Maret University, Surakarta, 57126, Indonesia; [7]Faculty of Civil and Environmental Engineering, Department of Sanitary Engineering, Gdansk University of Technology, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland; [8]Food and Nutritional Sciences Program, North Carolina Agricultural and Technical State University, Greensboro, NC 27401, USA; *s.siddiqui@dil-ev.de; ibrah001@ncat.edu

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Abstract

Meeting food security is one of the major global challenges to ensure sufficient supply of food for current and future generations, considering increasing population growth and climate change issues. Consequently, the consumption of edible insects as an alternative food source has recently gained global attention for combating global food insecurity. The present review aims to provide information on the recent progress in consumption of edible black ant Smith, particularly *Carebara vidua*, as the main focus. The global consumption record of edible black ant Smith and consumer acceptance as well as the strategies used to increase consumer acceptance of eating edible black ant smith were proposed. In addition, the bioecology of black ant Smith was covered in this review. Further, details are provided in this review on the benefits to health, economy, and environment of practicing eating edible insects such as black ant Smith. Focus on the potential uses of *Carebara vidua* as a food ingredient in culinary cuisine and their safety concerns from rearing until processing were highlighted. The SCOPUS database was analysed using bibliometric software to understand the connections between recent scientific outputs and ant as human food thoroughly. BioRender software was used to create scientific figures. It is noteworthy to highlight that black ant smith contains high protein and micronutrient, especially iron and zinc are higher than that of plant-, animal-based food, and seafood that contribute significantly to meeting the daily protein and mineral intake amount for human. Moreover, the exhibition of antimicrobial and antioxidant properties of edible black ant smith suggests that it can be used as a future functional ingredients for food, pharmaceutical, and cosmetics purposes. Hence, edible black ant smith is promising as an alternative and potential source of food or medicine for sustainable food security.

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Keywords

alternative food source – climate change – edible insects – food security

1 Introduction

The United Nations (UN) predicts the world population reaching nearly 9.8 billion in 2050 and expected to increase to 11.2 billion in 2100 (UN Department of Economic and Social Affairs, 2017; Khan *et al.*, 2020; Naseem *et al.*, 2021) and therefore, the demand for food will correspondingly increase (Kim *et al.*, 2019; Papastavropoulou *et al.*, 2022; Garza-Cadena *et al.*, 2023). It is expected that food requirements will increase to about 85% in 2050 (Raza *et al.*, 2019; Castro-Munoz *et al.*, 2022). Furthermore, natural resources such as water and land will become proportionally scarce as the population increases (FAO, 2021; Aguirre-Unceta, 2023; Lange and Nakamura, 2023). In addition, Defrance *et al.* (2020) have forecasted a reduction in the adverse effects of climate change on agricultural production per capital by the year 2050. Under these circumstances, limited space for rearing animals and aquatics will result in an increase in food prices with concomitant increase in food demand by 70% (Hlongwane *et al.*, 2020; Lee *et al.*, 2021; van Huis, 2023). A high demand for livestock and seafood has contributed to shortage in the market and may cause future food security issues (Legendre and Baker, 2020; Verneau *et al.*, 2021). Therefore, there is an urge to explore new food resources to substitute conventional livestock for the protein source for human consumption.

Edible insects are an excellent healthy alternative natural source of protein, specifically, in essential amino acids (Tang *et al.*, 2019; del hierro *et al.*, 2020; Papastavropoulou *et al.*, 2022; van Huis, 2023). In addition, insects require less resources such as feed, water, and farmland for growing as compared to livestock production systems (Lange and Nakamura, 2021). Thus, contributing to a positive impact to the environment by reducing greenhouse gas emissions (GHGs) (Hermans *et al.*, 2021; Lanng *et al.*, 2021; Ojha *et al.*, 2021a; Dagevos and Taufik, 2023). The risk of disease infection of livestock is also significantly eliminated (Kim *et al.*, 2019; Kwak *et al.*, 2020).

Entomophagy is the technical term used to describe the practice of eating the insects at different growing stages (i.e. the eggs, larvae, pupae or adults) (Pali-Schöll *et al.*, 2019; Lee *et al.*, 2020; Ho *et al.*, 2022a; Aung *et al.*, 2023). Earlier, European Food Safety Authority (EFSA) Scientific Committee has recorded 12 insect species as potential a food source; house cricket (*Acheta domesticus*), American grasshopper (*Schistocerca americana*), banded cricket (*Gryllodes sigillatus*), black soldier fly (*Hermetia illucens*), African migratory locust (*Locusta migratoria migratorioides*), housefly (*Musca domestica*), honeycomb moth (*Galleria mellonella*), silkworm (*Bombyx mori*), lesser wax moth (*Achroia grisella*), superworm (*Zophobas atratus*), lesser mealworm (*Alphitobius diaperinus*), and mealworm (*Tenebrio molitor*) (EFSA, 2015). According to Siddiqui *et al.* (2023a), there are more than 2000 species of insects that have been identified as edible, but only 80% of these are confirmed suitable for human consumption, and 4% have been recognised to have medicinal value (van Itterbeeck and Pelozuelo, 2022). In developing nations, notably in regions namely Sub-Saharan Africa and South Asia are incorporating edible insects as a dietary food source (Tao and Li, 2018; Hlongwane *et al.*, 2020). According to Raheem *et al.* (2019a), such as, beetles, bees, ants, locusts, termites, true bugs, grasshoppers, cicadas, scale insects, flies, crickets, dragonflies, caterpillars, leaf and plant hoppers are the edible insects that are prevalently used for human consumption. Accordingly, the Hymenoptera order including bees, wasps, and ants are globally ranked at the third place (14%) of most consumed insects after beetles from Coleoptera order (31%), and caterpillars from Lepidoptera order (18%) (Raheem *et al.*, 2019a).

Ants are one of the major insect groups and they are the biggest family of insects that consists of various species. Ants belong to the order of Hymenoptera, family of Formicidae in the subfamily of Formicinae (Jongema, 2017; Ramalho *et al.*, 2017). Although ants have been regarded as pests, numerous edible ant species offer a novel protein source and essential nutrients for global communities (Ondede *et al.*, 2022). The ingestion of the black ants, for example *Carebara vidua* in some regions of countries such as Kenya, Uganda, Zambia, Zimbabwe, Congo, South Africa, Botswana, Malawi, Sudan, South Sudan, Namibia, and Mozambique is driven by the high nutritional value (i.e. protein, lipid, minerals, and vitamins) of this species of black ants (Raheem *et al.*, 2019a; van Huis, 2021; Hlongwane, 2021). Moreover, edible black ants are well endowed with bioactive compounds that are shown to have

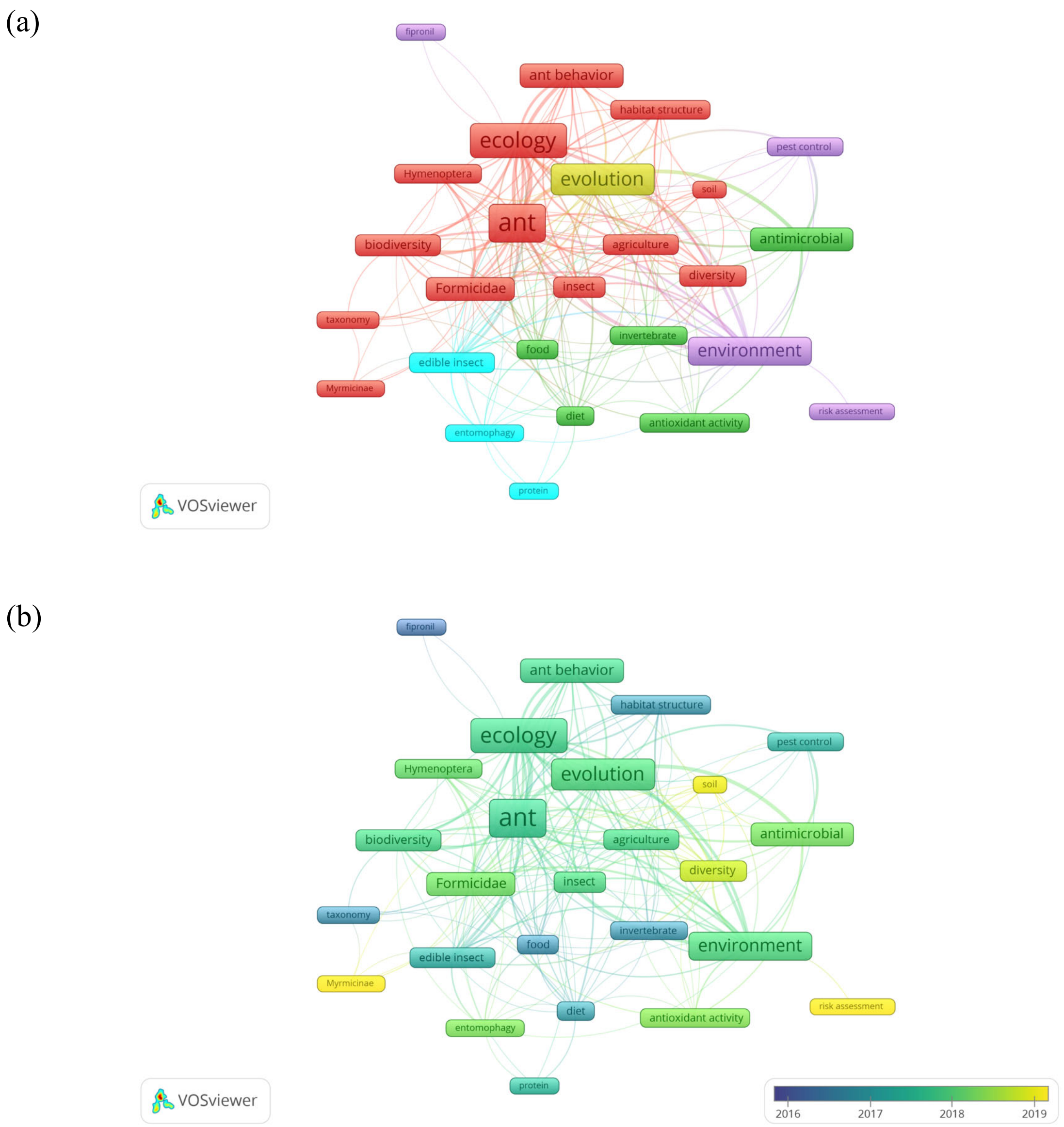

FIGURE 1 A bibliometric network map of scientific output on ants as human food https://bit.ly/3Lh1lRM. The network based on five coloured clusters (a) represents the relatedness of the scientific journal articles and conference papers and (b) an overlay visualization at a specific period for the occurrence of the keyword from 2016 (blue) to 2019 (yellow). The data was collected from SCOPUS database using keywords "ant" AND "species" AND "human" AND "food" from time range of 2012-2022 and figures were created using VOSviewer software.

health promoting factors for general well-being of the human body and potential to be developed as new healthcare promoting food products (Mattia *et al.*, 2019; Ramakrishnan and Selvaraju, 2020; Zhang *et al.*, 2022).

It is noteworthy that protein content in ants has the potential to be used as a future alternative protein source befitting a human balanced and healthy diet. Surprisingly, ants also possess antimicrobial and antioxidant properties that might have the potential to be used as a nutraceutical agent in the development of functional food and healthcare products (Figure 1a-b). The current review aims to record complete published scientific research on ants, particularly *Carebara vidua* as human food. It covers starting from the records of world consumption of edible black ant smith (*Carebara vidua*) until the processing and packaging of edible black ant-based food products.

2 Methodology

Searching strategy and data selection

A bibliometric analysis was performed for better understanding the connections between recent scientific outputs and ant as human food and to categorise potential research gaps of a current reviewed topic. This review article employed the list of publications from SCOPUS database between the years 2012 to 2022. The database of SCOPUS was obtained in September 25, 2023 to retrieve the relevant documents. This review retrieved and downloaded the associated publication using the search Boolean operators that has been appertained to the TITLE-ABS ("food*" OR "human food*") AND (edible) AND (insect*) AND ("ant*") AND ("Carebara vidua" OR smith OR "black ant*"). The database identified the word or term existence in any section of the search category or the articles. The downloaded publication lists returned 3,135 document results. Subsequently, the search string was then refined to searching on the source type merely published in the form of journal article and conference proceeding. The duplicate and irrelevant to topic files were subsequently eliminated. The review journal article was sorted during this stage based on their titles and abstract to avoid redundancy documents. The final list of publications was 187.

Data analysis and visualization

The bibliometric evaluation was carried out using VOSviewer Sofware Version 1.6.18 programme to visualizing and creating bibliometric networks based on the collected data. The Data was harmonized in Thesaurus file to avoid duplicate and variation of keyword. The networks consist of parameters related to the inserted keywords by categorised the keywords into five clusters group that indicates with five different colours. The red grouping represents keywords used to describe ant habitats and species. The green cluster grouped terms referred to the potential of ant as a nutraceutical agent, meanwhile blue grouping emphasizes keywords that are associated with acceptance of ants as food. The yellow and purple clusters grouped keywords focused on the ant's life cycle, and ants' environmental management, respectively.

Scientific figure preparation

BioRender software was employed to create scientific figures from an image library. Drag- and drop- the selected relevant icons from icon library to the template enable to draw the scientific image.

3 Records of consumption and consumer acceptance of edible black ant Smith (*Carebara vidua*)

Ants have been consumed as human food in various regions around the world, especially in poverty-stricken and developing countries (Dube *et al.*, 2013). Earlier, Ayieko *et al.* (2012) reported that over 200 species of black ants including *Carebara vidua* Smith are identified as edible in the community of Lake Victoria basin. However, there is no detailed taxonomy or specific species of edible black ants were further reported. Jongema (2017) identified 19 genus (65 species) of edible ants including black ant smith that are suitable for human consumption, that is, *Carebara* (3 spp.), *Polyrhachis* (5 spp.), *Componotus* (9 spp.), *Atta* (8 spp.), *Oecophylla* (3 spp.), *Lasius* (2 spp.), *Monomorium* (1 sp.), *Dorylus* (1 sp.), *Melophorus* (3 spp.), *Myrmecia* (2 spp.), *Formica* (10 Spp.), *Myrmecocystus* (2 spp.), *Pogonomyrmex* (6 spp.), *Acromyrmex* (2 spp.), *Cephalotes* (1 sp.), *Eciton* (1 sp.), *Liometopum* (2 spp.), *Crematogaster* (3 spp.), and *Tetramorium* (1 sp.) (Supplementary Table S1). The number of countries by biogeographic regions practicing ant-eating in Afrotropical region is 14 countries, in Neotropical region is 14 countries, in Palaearctic region is 12 countries, in Oriental region is 7 countries, in Indo-Australian region is 5 countries, and in Australasian region is 1 country (Figure 2).

In general, black ants are collected and consumed primarily during their larval or pupal phases (Raheem *et al.*, 2019a). China recorded the highest consumption amount of ant species among the countries, that is, 21 species (Figure 2). Black ant species are widely consumed at all stages in China as they believe in the beneficial medicinal effects of black ants (specifically, *Polyrhachis dives* and *Polyrhachis vicina* Roger) (Zhang *et al.*, 2022; Shakur, 2023). Black ants are widely used as functional ingredients in preparation of various health foods and tonics for medication and healthcare, functions such as reduced hyperuricemia, anti-aging, boost immune system, anti-tumour, and anti-inflammatory (Sun *et al.*, 2013; Yu *et al.*, 2013; van Huis, 2018; Patel, 2019). Minority group of tribes in Guangxi Zhuang, China cooked black ants with bitter melon as their food consumed during summer, while in Northeast China, black ants are often used to braise with meatballs and bean curd (Tang *et al.*, 2015).

On the other hand, many species of ants, particularly *Carebara vidua* are gaining momentum as a food source across Afrotropical region (i.e. Kenya, Uganda, Zambia, Zimbabwe, Congo, South Africa, Botswana, Malawi, Sudan, South Sudan, Namibia, and Mozambique) due to their rich nutritional profile and high medicinal value to treat various diseases (Ayieko *et al.*, 2012). In this region of countries, food security is a major issue, whereby nutrient deficiency in daily diets has caused malnutrition among children and women (Giller, 2020; Acosta-

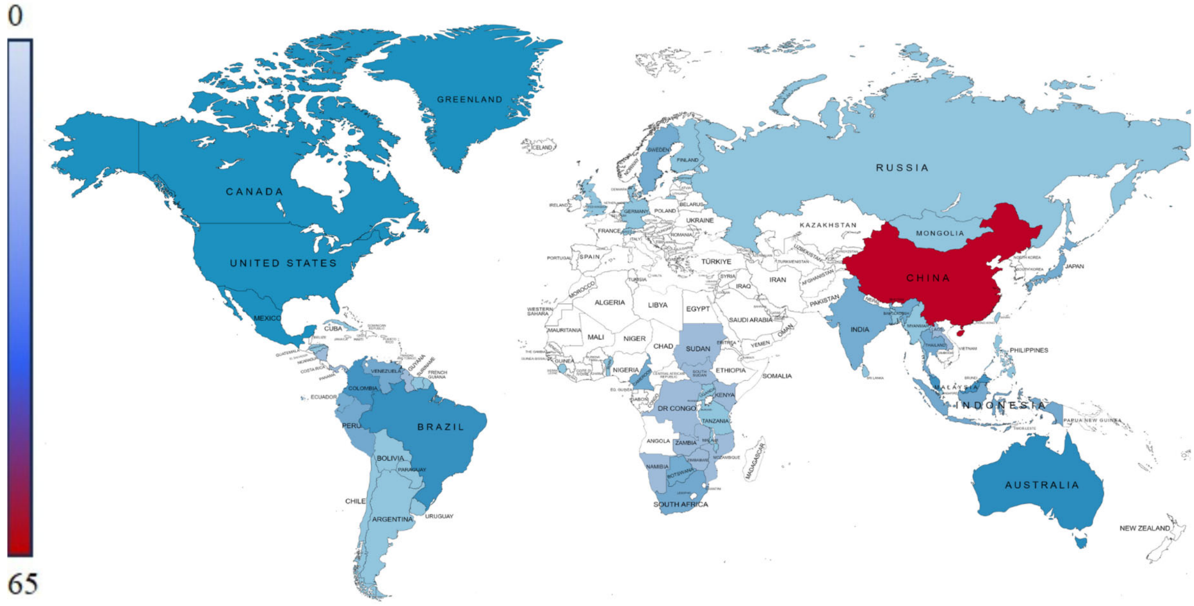

FIGURE 2 Value indication of edible ant species through heat map. Low to high intensity of blue color represent least to high number of species consumption, respectively, in each country.

Estrada *et al.*, 2021; Siddiqui *et al.*, 2023a). Hence, it is necessary to search for alternative natural resources to combat this situation. For example, by eating edible insects including black ant smith. In Africa, *Carebara vidua* are consumed in their adult stage (Hlongwane *et al.*, 2021). The whole body of black ants is valuable (as discussed in the following section) to be eaten (Ayieko *et al.*, 2012). According to Ayieko *et al.* (2012), *Carebara vidua* is traditionally consumed (either raw, roasted, or fried) by Luo and the Abaluhya community of Kenya as a part of their diet, where the oily abdomen of *Carebara vidua* is responsible for the desirable flavour of dish (uncooked, roasted, or fried). The higher composition of fat and certain fatty acids in the abdomen of black ants Smith may serve as flavour precursor during cooking or food processing and ultimately enhancing the taste, thereby increasing consumer acceptance (Cheseto *et al.*, 2020; Shahidi and Hossain, 2022). Recently, Kolobe *et al.* (2023) reported that high proportion of polyunsaturated fatty acid (PUFA) in *Carebara vidua* is directly influence the flavour of food materials, enhancing the palatability by facilitating their flavour retention. A recent survey finding revealed that 70% of the respondents from Western cultures express a preference to consume edible insects-based food products when the natural appearance is concealed (Ros-Baró *et al.*, 2022). Consequently, the high surface area to volume ratio of black ant Smith allows to rapidly be processed and incorporated into multiple recipes such as enriched flour, breads, cookies, noodles, chocolates and confectionaries. These factors play a crucial role in improving the consumer acceptance of ant smith (*Carebara vidua*) as a viable human food.

In Zimbabwe, a survey involving 200 urban and 175 rural respondents highlighted that a larger percentage of individuals in rural areas (89.7%) were consuming insects, whereas in urban areas, the edible insect consumption rate is lower at 80.0% (Manditsera *et al.*, 2018). The survey findings suggest that, delicious taste of edible insects serves as a motivator for insect consumption in both rural and urban regions of Zimbabwe with respective respondent frequencies 89.2% and 74.4% for rural and urban areas, respectively (Manditsera *et al.*, 2018). The taste and texture (dry and crunchy texture with salty taste) of edible insects are considered as crucial sensory attributes that influence the consumption of edible insects as reviewed by the respondents (Manditsera *et al.*, 2018). Correspondingly, a questionnaire finding focused that in Nigeria out of 120 respondents, 81.67% of them preferred to include the edible insects as part of their diet. Among them, 33.2% specifically cited the insects' taste as the primary reason for their consumption (Ancha *et al.*, 2021). In Botswana, a research by Morêki (2014) showed that approximately 53% of Mogonono villagers in Kweneng district used *Carebara vidua* for consumption which played an essential role in food and nutrition security. Moreover, the questionnaire findings highlighted that *Carebara vidua* is the most preferable edible insect among the local population in Burundi and it ranks second in terms of taste. Conversely, in Uganda, *Carebara vidua* is still recognised as a preferred edible insect but lowest rank with a score of 10 (Okia *et al.*, 2017). Thus, it can be concluded that black ants from different regions may also influence the sensory attributes, especially the taste of edible insects.

In some parts of Afrotropical countries, tribal people eat the whole black ant (*Carebara vidua*) at adult stage, either in raw form or cooked by frying or roasting (Morêki, 2014; Hlongwane, 2021; van Huis, 2021). While, roasted adults of the African weaver ant, tailor ant, or sewing ant of *Oecophylla longinoda* are delicacies in Sub-Saharan Africa, Cango, Cameroon, and Tanzania. They eat the whole body of adult ants by grilling them by mixing the ants with local herbs or fruits (Wetterer, 2017; Félix, 2019; Vayssières *et al.*, 2022; Omonmhenle and Iyekowa, 2023). In Myanmar, Thailand, India, Loas, Malaysia, and China, the *Oecophylla smaragdina* is either eaten raw as chutney or cooked by boiling or processed into various food products such as salad, roasted snack food, fish soup, chili paste or curried lightly with vegetable (van Itterbeeck, 2014; van Itterbeeck *et al.*, 2014). In Thailand, the processed adult ants are packed and sold in cans (Seni, 2017; Melgar-Lalanne *et al.*, 2019; Mozhui *et al.*, 2020). Interestingly, the larva, pupae, and adult of ants (*Lasius* sp.) is cooked with botan-prawns in Tokyo, Japan (Belluco *et al.*, 2018). *Camponotus* sp. is commonly eaten raw or cooked by roasting, toasting, boiling, or frying. It is also used as flavouring or seasoning for preparation of soups and salads (Varelas and Langton, 2017; Ronque *et al.*, 2018; van Huis, 2018; Ellison and Gotelli, 2021).

It is remarkable that several species of *Camponotus* such as *Camponotus maculatus* and *Camponotus pennsylvanicus* have been employed as traditional remedy in Burkina Faso, West Africa and India (Mazurkiewicz *et al.*, 2016; Schrader *et al.*, 2016; Salyer, 2018; Buczkowski, 2019; Ouango *et al.*, 2022; Siddiqui *et al.*, 2023b), whereas, *Camponotus inflatus* is consumed by Aboriginal Australian in Australia as part of their diet for the source of natural sugar. They mix the ants with salads which is responsible for sweet sour flavour in their dish (Faast and Weinstein, 2020; Islam *et al.*, 2022; Dong *et al.*, 2023). In Neotropical countries (i.e. Colombia, Brazil, and America), the alate female, soldier, and winged adult of ants (*Atta laevigata*) are used as roasted food while the reproductive adult of ants (*Atta Mexicana*) is eaten raw with salt in Mexico (Chowdhury *et al.*, 2017; Manno *et al.*, 2018). In addition, the edible ants are toasted, ground, and mixed with tomato, chili pepper, garlic, clove, and salt to prepare chicatanas sauce in San Lorenzo and Oaxaca regions of Mexico (Melo-Ruiz *et al.*, 2018; Solís and Casas, 2019). Specifically, in Mexico, the Mexicans consume the queen of *Atta cephalotes* and *Atta Mexicana* cooked in chili hot sauce (Melgar-Lalanne *et al.*, 2019). In some parts of Mexico, the black ants (*Monomorium minimum*) are used to prepare seafood cocktails with shrimp or served with rice by sprinkling them on top of rice (Woolf *et al.*, 2021). Summarised information towards the frequency of consumption of edible ants in different countries is shown in Figure 3.

Edible insects are commonly consumed as part of the human diets, particularly consumed by communities in Africa, South America, and Asia, but not common in North America and Europe (Ho *et al.*, 2022a) and has created a 'disgust' feeling and hence unwillingness or rejection to consume in industrialised nations (Mlcek *et al.*, 2014; Olivadese and Dindo, 2023). A person with a high level of food neophobia, even if he/she is a professional cook will be refused to taste and also cook novel foods. Conversely, the consumer with low food neophobia, typically keen to try novel foods with enjoyment (Alhujaili *et al.*, 2023; Zamfirache, 2023). For Examples, in Italy the edible insects received the lowest acceptance level compared with seaweed and jellyfish (Palmieri *et al.*, 2023). According to Orsi *et al.* (2019), the consumers in Germany exhibited a limited interest or unwillingness in consumption of edible insects due to the food neophobia and disgust factor. In Japan, the acceptance level for insect as food stood at 20% comparable to other meat alternatives (van Huis and Rumpold, 2023). Nonetheless, the potential acceptance and culinary uses of *Carebara vidua* in more developed countries remain largely unexplored. People in West think insects are dirty, not safe for consumption, and disgusting (Costa-Neto, 2014; Tan *et al.*, 2017).

Prevalently, the nutritional value and minimal environmental impact globally are often used as advertising aid for the consumption of edible insects. But it is insufficient to overcome consumer skepticism towards consuming edible insects. Therefore, food technologists, marketers and entrepreneurs are playing a crucial role in implementing diverse strategies to enhance the gastronomic experience and improve the marketing of edible insects including products made from black ant Smith. Many entrepreneurs and manufacturers participated in the insect market by developing new and commercially accessible insect-based products to meet the market demands and market profit. According to Aydoğan (2021), anthropo-entomophagy has become globally known among the rising food industries, whereby, the nutritional value and incorporation of arthropods into food have become their new interestFor instance, Raubenheimer and Rothman

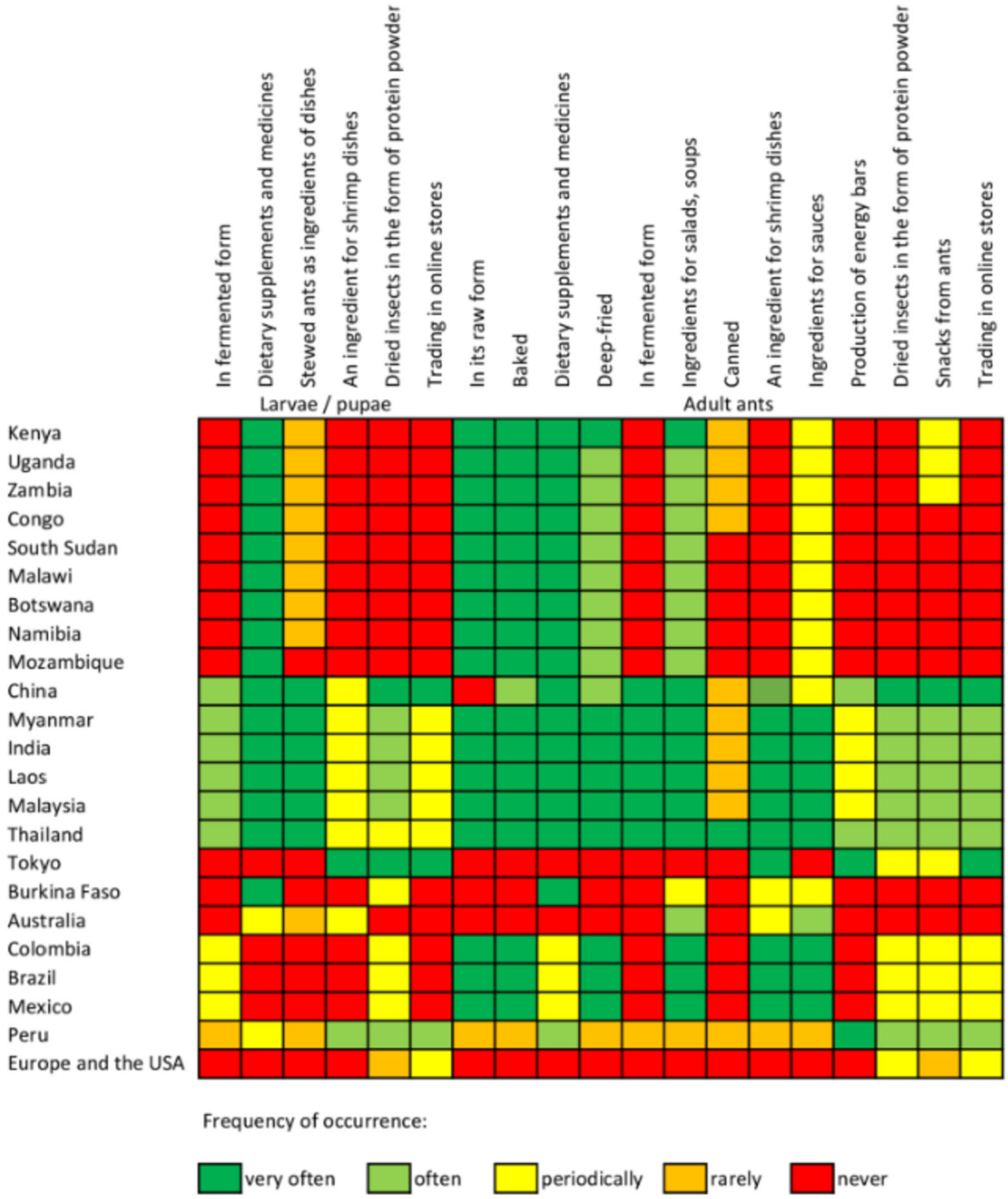

FIGURE 3 Color-coded matrix of consumption of edible ants in different countries.

(2013) reported that the adults, eggs, larvae, and pupae of edible insects were creatively utilised in food product development in order to achieve consumer acceptance. Furthermore, utilization of processed edible insects in food products is a beneficial strategy rather than using whole insects which will be prevented from the disgusting factor (Baiano, 2020; van Huis and Rumpold, 2023). Processing the insects into more appropriate and convenient forms (such as extracts, flour or powder, and paste) instead of presented as raw or directly visible is necessary to boost the acceptance level among the consumers (van Huis, 2013; Ojha *et al.*, 2021b). Recently, edible insects have received worldwide interest to be used as a substitute for plant- and animal-based proteins in the development of different forms of high-protein food (Williams *et al.*, 2016; Khampakool *et al.*, 2020; Lee *et al.*, 2021; Qian *et al.*, 2022). For instance, the processed cricket, mealworms, cochineals, and silkworm are the most used edible insects in food product development (Table 1) to enhance the nutritional value and improve consumer acceptance of the insects-based products. However, there is still a gap between the utilization of edible black ant Smith and food product development. They are merely eaten raw or cooked after frying or roasting.

In addition, the organoleptic attributes of the insect-based products are also vital to boost consumer acceptance. According to Deroy *et al.* (2015), commercialization of insect-based products is dominantly based on their sensory characteristics such as taste, appearance,

TABLE 1 Edible insect-based ingredients in food product development

Types of insects	Food products derived from edible insects	References
Black ant	In China, the black ants are processed into powder form and also as fermented foods in the form of wine which possess higher antioxidant activity.	Rastogi, 2011
Cochineals	Carmine powder extracted from cochineals (*Dactylopus coccus*) was widely used in the production of cupcakes, yogurt, toffees, and coffee as a natural colouring agent.	Voltolini *et al.*, 2014
Silkworm	The finding of the internal preference map demonstrated that the vectors of most consumer panels are directed towards pasta products formulated with 10 g of silkworm powder which indicates strong preference, while the control paste (0 g of insect powder) received moderate preference.	Biró *et al.*, 2019
Cochineals	Brazilian sausage with a formula of 0.0075% nitrite and 0.02% cochineal carmine (CC75) was discovered as a best formula due to its high acceptability among consumer panels. Specifically, the finding from 9-point hedonic scale evaluation revealed that CC75 sausages achieved a high score (indicating moderately) for colour and overall acceptability, with ratings of 7.26 and 7.55, respectively.	Bellucci *et al.*, 2020
Cricket	A chocolate bar formulated with 5.5% of cricket flour showed highest acceptance among the Italian consumer (score 7: like very much).	Cicatiello *et al.*, 2020
Cricket and mealworms	Protein powder and bars derived from cricket and mealworms are readily available as commercial products in varied colourful packaging.	Naseem *et al.*, 2021
Leafcutter ant	Utilization of *Atta sexdens* in development of cereal energy bars has also been recorded in Peru	Lozada-urbano *et al.*, 2023
Cricket	Inclusions or toppings namely sunflower seeds, dates, almonds, honey, and chocolates in food bars will attract the consumers' attention, evoke a sense of temptation and make the product even more appealing.	van Huis and Rumpold, 2023

and flavour could be a promising marketing strategy. In addition, educating the consumers with comprehensive information like nutritional benefits, taste experience, and food safety of insect-based products has the potential to enhance the willingness to give it a try (Alhujaili *et al.*, 2023; van Huis and Rumpold, 2023). It also certainly minimised the food neophobia among the consumers. For instance, Park *et al.* (2022) discovered that in the United States, famous actors, actresses, and athletes were employed as part of advertising campaigns in order to promote the insect-based products which significantly impact the preferences of targeting males. Furthermore, marketing strategies are essential in introducing edible insect products to consumers and fostering their acceptance. According to Phonthanukitithaworn *et al.* (2021), several effective marketing strategies such as various packaging designs with clear product labelling, attractive advertising, and accessible distribution channels were employed in Thailand to promote the insect-based food products. In addition, innovative promotions like buy one, free one offers can motivate consumers to try insect-based products. According to Aydoğan (2021), various types of edible insects, including black ants (particularly *Polyrhachis* sp.) are sold worldwide and can be procured from online shops. Most edible insect-based foods are sold in mini size packaged snacks. They are often packed in sophisticated and creative packaging so as to attract consumer acceptance and hence increase the selling unit of insect products (Collins *et al.*, 2019). Food products made of edible insects are highly sustainable and healthy. Unfortunately, the current situation of limited legal regulation on the safe use of edible insects for human consumption has resulted in selling more slowly (Reverberi, 2021). Therefore, innovative insect products with excellent organoleptic qualities, attractive visual presentation, ensuring safety and compatible with local cuisine is significantly achieved the acceptance of consumer.

4 Bioecology of black ant Smith (*Carebara vidua*)

Ants are one of the most ubiquitous and diverse groups of insects that live in colonies in their earthbound or subterranean habitats (Ronque *et al.*, 2018). It is the largest family of social insects under the order Hymenoptera and generally comprises ~80% of arthropods in the tropical rainforest (Goropashnaya *et al.*, 2012; Yusah and Fayle, 2014; Raj *et al.*, 2017; Sano *et al.*, 2018; Buxton *et al.*, 2021). Tang *et al.* (2015) reported that over 20,000 species of ants are widely distributed on earth. Interestingly, the number of species of ants has shown to be significantly higher than the termites species (~3,000 species), social bees (~2,000 species), and social wasp species (~1,100 species) (Guénard, 2013). The abundance of the ant species is due to their systematic organization. Ants play an important role in balancing the whole ecosystem (Cerdá *et al.*, 2013; Siqueira *et al.*, 2017), whereby it acts as an engineer in the ecosystem responsible to modify the physical and chemical properties of soil, facilitates decomposing of organic matters, and contributes to nutrient cycling change (Bierbaß *et al.*, 2015; Lopes *et al.*, 2016; Schowalter and Ring, 2017; Farder-Gomes *et al.*, 2019; Svanberg and Berggren, 2019; Reeves *et al.*, 2020; Buxton *et al.*, 2021; Pimentel *et al.*, 2022).

Ants are diverse and abundant in terrestrial ecosystems, especially in tropical and subtropical regions (Subedi *et al.*, 2021), whereby the humid and warm environment is preferable (Garcia *et al.*, 2013; Ruiz and Ahrendts, 2020). Most of the ant species are thermophilic organisms that are able to live at high temperature (Sommer and Wehner, 2012). A total of 65 ant species are recorded as edible and distributed in 6 ecozones (Supplementary Table S1). Neotropical is the zone with the highest number of distributions (29 species), followed by Palaearctic zone (21 species), Australasian zone (12 species), Afrotropical zone (8 species), Indo-Australian zone (8 species), and Oriental zone (6 species). Some of the edible ant species are recorded to distribute in more than one country in different zones, thus, resulting in a higher total number of distribution species zones than the aforementioned number. For example, *Carebara castanea* Smith is distributed in Indo-Australian (Singapore), Oriental (Thailand and Laos), Palaearctic (China), and Afrotropical (South Africa) zones (Kelemu *et al.*, 2015; Naukkarinen, 2016; Jongema, 2017; Belluco *et al.*, 2018).

The species of *Carebara vidua*, a type of edible black ants Smith belongs to Hymenoptera order, which shares the same order with bees, and wasps, and Formicidae family (Ayieko *et al.*, 2012; Morêki, 2014). The black ant Smith is classified under the family Formicidae as the Formicidae originates from the Latin word "formica" meaning "ant" in English (Figure 4). The idea is that particular ants release formic acid from their body (Aydoğan, 2021). This type of edible black ant Smith lives in holes with well-constructed tunnels (subterranean) or complex and systematic chambers. These tunnels are very important for the living of the colony members to store their food and to protect their larvae from predators (Ayieko *et al.*, 2012). The edible black ant Smith is predominantly distributed in Africa, Asia, and South America, and less present in North America. Among the edible black ant Smith, the species of *Carebara vidua* has the highest record of distribution in Africa (Jongema, 2017). The number of countries in the Afrotropical realm distributed with edible black ants Smith (*Carebara vidua*) is 11 countries. South Africa is the region with the highest number of distribution records (5 countries), followed by East Africa (4 countries), North Africa (1 country), and Central Africa (1 country) (Table 2).

Black ant Smith is one of the eusocial insects (Lewis *et al.*, 2014) that consist of four major castes in a colony; queens, males, soldiers and workers (Figure 5), with respective roles (Ligon *et al.*, 2014). The queen of this social insect is responsible for reproduction (Barkdull and Moreau, 2023), while the male ant (drones) is responsible to mate with the queen ant for dispersal and reproduction. Other female ants (worker) are sterile and cannot mate with male ants (Schultner *et al.*, 2017). The soldier is responsible for hunting and guarding the colony. The worker ants are involved in activities associated with defense, building the nest, food supply, and care taking of the offspring as well as in charge of the maintenance of the nest for the whole member of the colony (Yan *et al.*, 2014; Verza *et al.*, 2017). Thus, the worker ants are the major group in the colony. Adult social insects including ants possess behavioral changes according to their age, the older worker ants are responsible to work outside their nest, like foraging, while the younger worker ants are committed to work inside or tunnels of the nest (Ronque *et al.*, 2018). In addition, *Carebara vidua* is an omnivores organism, they consume

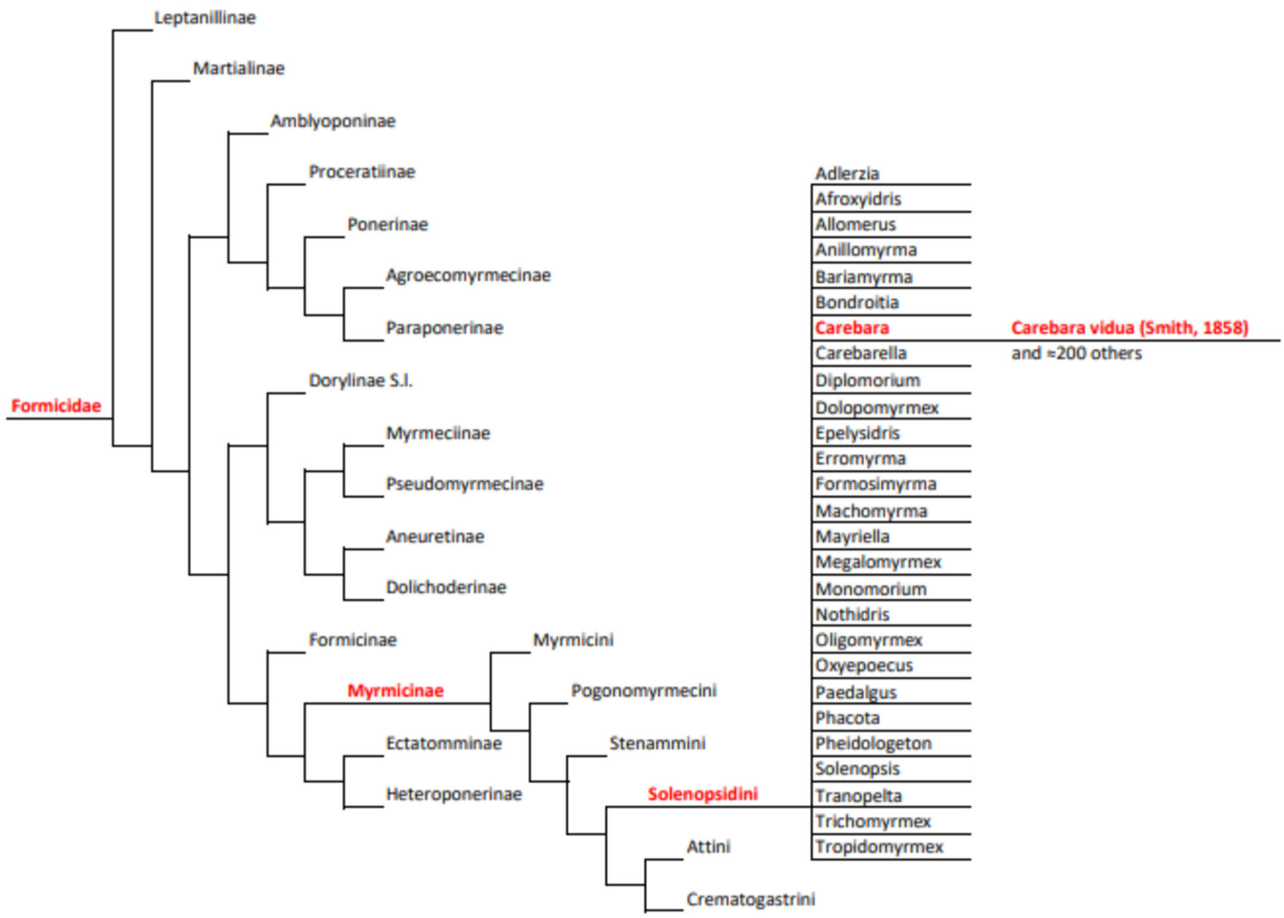

FIGURE 4 Cladogram of black ant Smith (*Carebara vidua*) based on data of Johnson *et al.* (2013).

TABLE 2 The country of distribution of black ant (*carebara vidua*) Smith

Country of distribution	References				
	Ayieko *et al.*, 2012	Jongema, 2017	Raheem *et al.*, 2019a	van Huis, 2021	Hlongwane, 2021
Kenya	✓		✓	✓	
Zambia		✓	✓	✓	✓
Malawi			✓		✓
Sudan			✓		✓
Namibia					✓
Mozambique			✓		
South Sudan			✓		
Democratic Republic of Congo (DRC)			✓		
Botswana			✓		
Uganda				✓	
Zimbabwe		✓		✓	

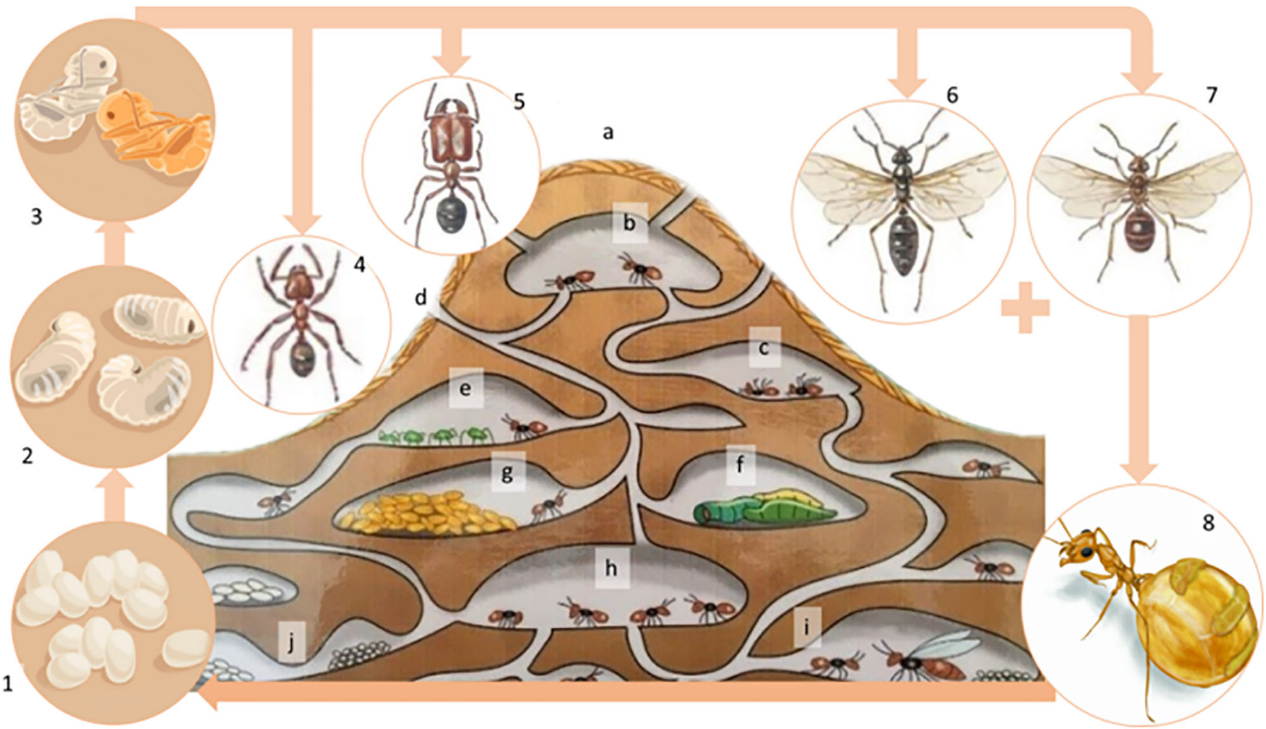

FIGURE 5 Biocenosis of black Ant Smith: (1) eggs, (2) larvae, (3) pupae, (4) worker, (5) soldier, (6) male ant (drone), (7) female ant (princess), (8) the wingless female ant (queen). Structure of colony: coating of the colony (a), the uppest or "warm" chamber (b), cemetery or waste chamber (c), entrance or ventilation (d), the aphid chamber (e), mining chamber (f), chamber for food storage (g), wintering chamber (h), a queen ant chamber (i).

both living and dead organisms including leafy matter, nectar, sugary substance, glandular, plant secretion, and other insects (i.e. aphids, scales, worms, and larvae of lepidoptera) (Ayieko *et al.*, 2012).

The reproductive queen and male of *Carebara vidua* undertake once in a year during the nuptial flight phase during the long rainy season. During the season, they often appear in a group from their subterranean nest (van Huis, 2021). The queen and male of *Carebara vidua* often emerge from the nest to the "silent place" or undisturbed environment at around 11:00-15:00 after the rain to pair up, mate, and then shade their wings to begin a new colony (Ayieko *et al.*, 2012; Ondede, 2023) in the month May to June (Okia *et al.*, 2017).

After mating between the reproductive queen and male of *Carebara vidua*, the queen seeks for a suitable nesting site to form a chamber in the ground and lays her first brood eggs in a shallow chamber. The eggs hatched after 5-7 days in the warm chamber (Ayieko *et al.*, 2012). Whereas the male ant dies soon after completing their task. According to Verza *et al.* (2017), the eggs, larvae, and pupae of most social insects from Hymenoptera are immobile, and very dependent on adult worker ants to supply for their needs such as feeding, displacement, and cleaning. The adult worker ants also participated in the molting process, whereby they are responsible to help the larvae and pupae to hatch from their eggs and emerge from the ecdyses (Giehr *et al.*, 2020). Each of the reproductive adults (males) of *Carebara vidua* is chosen as it emerges from the hole for production of the next colony (Ayieko *et al.*, 2012). Accordingly, the narrow tunnels of the nest become slightly bigger and also the number of chambers and tunnels increased as the colony size had increased (Cardoso *et al.*, 2014; Gautrais *et al.*, 2014; Farias *et al.*, 2020) and hence, more space is required. According to Ayieko *et al.* (2012), the lifespan of *Carebara vidua* can survive for many years, assuming that there is fewer or no interruption in their habitat. To date, the life cycle of black ant Smith on the development of larvae, pupae, and adults is not clear due to scarcity in the literature.

The whole-body size of *Carebara vidua* is different among groups, specifically, the size of queen ants is bigger than the female (workers) and male ants. The queen of *Carebara vidua* generally has a black body and wings. The size of queen *Carebara vidua* is approximately 20 to 25 mm long. They have a large abdomen in round shape, size ranges from 6 to 6.2 mm in diameter with a thin gaster of one nodule (Ayieko *et al.*, 2012). The large abdomen contained eggs and other fatty substances as

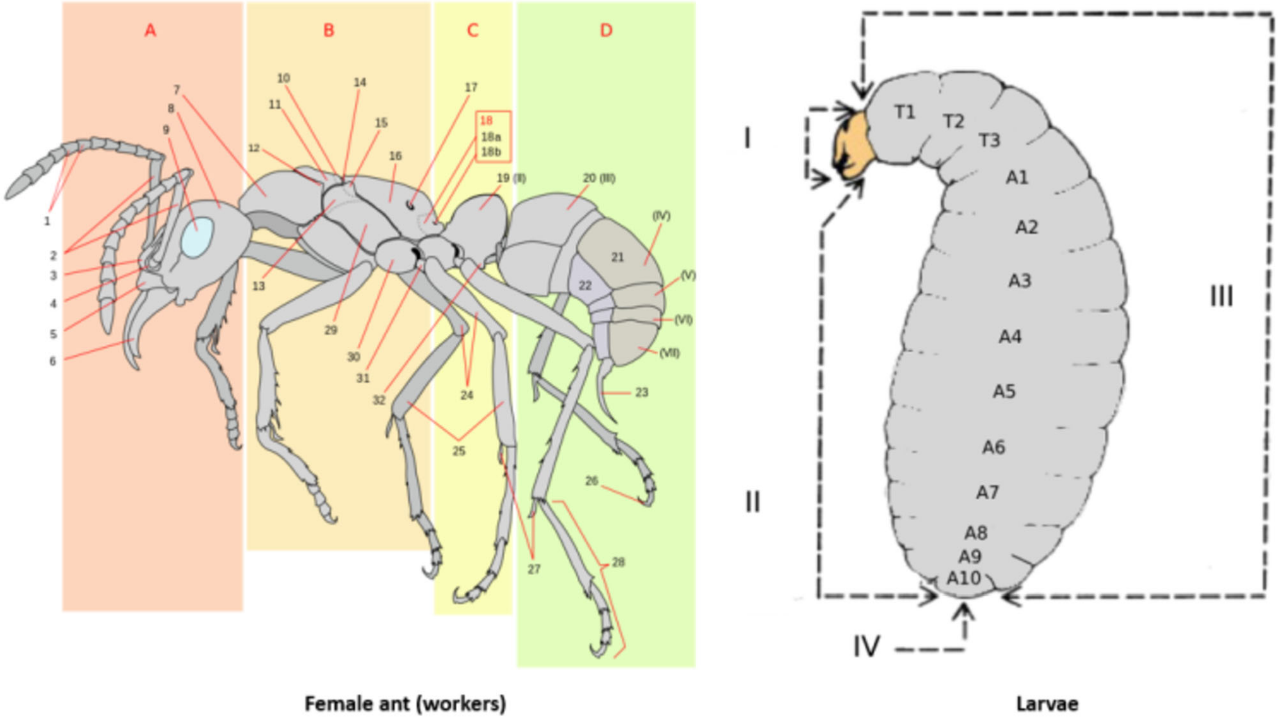

FIGURE 6 Anatomical features of black ant Smith: Female ant (workers): head (A), alitrunk or thorax (B), petiole (C), gaster (D), abdomen (C+D), funiculus (1), scape (2), frontal lobes (3), antennal fossa (4), clypeus (5), mandibles (6), pronotum (7), occiput (8), compound eye (9), scutellum (10), mesonotum (11), mesothoracic spiracle (12), anepisternum (13), metanotum (14), metathoracic spiracle (15), propodeum (16), propodeal spiracle (17), metapleural gland (18), bulta (18a), orifice (18b), petiole (19), postpetiole (20), tergite (21), sternite (22), sting (23), femur (24), tibia (25), tarsal claw (26), tibial spurs (27), tarsus (28), katepisternum (29), coxa (30), trochanter (31), ventral process (32); Larvae: head (I), ventral surface of body (II), dorsal surface of body (III), posterior end of body (IV), thoracic spiracles (T1-T3), abdominal spiracles (A1-A10).

a supplement and nourishment for the development of eggs during the nuptial flight, oviposition and thus, beginning of the next colony (Abril and Gómez, 2020). Similarly, the queen of *Carebara vidua*, the male ant also winged but with white abdomen and the body size is smaller than the queen, approximately 10 mm long. The size of the female ant (workers) of *Carebara vidua* is small, approximately 3 to 5 mm long, wingless (Ayieko *et al.*, 2012) but with a sting for defense against predators and competitors (Aili *et al.*, 2014). Anatomical features of female ant (workers) and larvae are presented in Figure 6.

The body structure of black ant Smith is divided into three different parts: head, thorax, and abdomen. Like other insects of Hymenoptera, *Carebara vidua* has orthognathous head with a pair of compound eyes, and three simple eyes or ocelli also developed at an equilateral triangular formation on the dorsal surface of the head (Narendra *et al.*, 2016). The *Carebara vidua* has a pair of antennae consisting of scape and pedicel as well as multi-segmented flagellum (Ayieko *et al.*, 2012) with the first segment of the antennae; scape is attached to the head. The antennae of ants function to sense the physical and chemical characteristics of the environment (Wang *et al.*, 2016). The mouth of *Carebara vidua* is projected downward with mandibulate biting type (Ayieko *et al.*, 2012). According to Angelini *et al.* (2012), insects with mandibulate mouthpart like ants typically has a labrum (upper lip), a strong pair of unmodified or unsegmented mandibles (jaws) with present of two pairs of multibranched appendages (i.e. maxillae and labium or lower lip) as sensory segment. The mandibles of ants are functions to perform various tasks such as manipulating the objects, defense purpose, food processing as well as a communication tool among them (Zhang *et al.*, 2020).

The thorax of this black ant Smith is short and consists of three segments: prostenum (first segment), mesothorax (middle segment), and propodeum (last segment). These segments are closely adhered to each other, and a pair of legs is present in each segment (Ayieko *et al.*, 2012). The prostenum of *Carebara vidua* is literally unnoticeable. However, in the middle segment, which is the largest segment of thorax, contributes to major flight muscles. In the segment of mesothorax, dorsally, it is covered by two parts of mesonotum; sculum and scutellum (Ayieko *et al.*, 2012).

The abdomen of *Carebara vidua* comprises two segments; the first segment of abdomen is tightly attached to the mesothoracic segment to form a petiole or the gaster or stalk with absence of node or dorsal projection. There is a transverse flexible suture along the scutum of mesothoracic gaster. Furthermore, in this segment, it forms a strong constriction between the segments of first and second abdominal both ventrally and dorsally. In the queen and male of *Carebara vidua*, they bear two pairs of translucent wings without scales: a pair of fore-wings and a pair of hind wings. The fore-wings are closely attached to the anal cells. Moreover, the front wings usually are bigger than the behind wings. Tegulae or a small oval sclerite often present at the bottom part of the wings (Ayieko *et al.*, 2012).

5 Economic importance of black ant Smith (*Carebara vidua*)

Food security is a critical global issue that continues to be debated day by day with increasing population (van Huis, 2021). As the human population grows, the requirement for food, land and water resources for agricultural purposes will also increase significantly (Y. Khan *et al.*, 2020; Naseem *et al.*, 2021). Naseem *et al.* (2021) revealed that when the sustainable ecosystem's threshold is exceeded, it not only causes food security crisis but also results in increment of food prices, disease outbreaks, degradation of agricultural land, energy shortages and imbalance distribution of resources. Consequently, this has a higher potential to jeopardize the stability of the global economy. Undoubtably, entomophagy practice is a most sustainable, practical, and feasible solution for global food security crises. In the evolving landscape of entomophagy, black ant Smith are receiving focal points of attention from researchers due to their excellent nutritional composition and its therapeutic effects (Ayieko *et al.*, 2012).

Global Market Insight (2020) recorded that global market size for edible insects including beetle, locusts, cricket, and ants achieved a value exceeding US $112 million in 2019. The revenue is anticipated to increase from 2019 to 2026 with a Compound Annual Growth Rate (CAGR) of 47% due to the high-nutritional profile, easy availability and economical processing techniques. Furthermore, the edible insect market between 2018 and 2023, is set to grow from about US $400 million to nearly US $1.2 billion (Tanga and Kababu, 2023). Further forecasting revealed that this market could be worth about US $8 billion (Guiné *et al.*, 2021). With the growing interest in the edible insect market, *Carebara vidua* emerges as a promising candidate which possesses impressive nutritional qualities. It is poised to contribute not only to global nutritional needs but also to economic advancement of the local communities that can be involved in their foraging and rearing (Hlongwane *et al.*, 2020; Papastavropoulou *et al.*, 2022).

Black ant Smith as an effective economic tool

The existence of modern approach through edible insects such as *Carebara vidua* in playing a dominant role to develop a sustainable economy by efficiently converting feed to meat, development of farming, traditional market, and potential food industry of edible insects, are discussed in the following sections.

Feed – meat conversion efficiency

Feed conversion factor (FCR) quantifies the amount of feed ingested by an individual animal or insects to generate a 1 kg of body mass. Rational, FCR is directly related to the body weight of an animal (Davison *et al.*, 2023). For instance, crickets able to gain 1 kg of weight with less than 2 kg of food, whereas chickens, pigs, and cattle required approximately 2.5 kg, 5 kg and 10 kg of food, respectively, to gain the similar weight (Lange and Nakamura, 2021; Naseem *et al.*, 2021). In addition, insects have a higher rate of FCR than other livestock which contributed to shorter production duration (Caparros Megido *et al.*, 2014; Fernandez-Cassi *et al.*, 2019). This is because insects are poikilothermic in which energy is not required to maintain the body temperature. Thus, the abundance of energy obtained from their feed will be utilised in their reproduction and growth which efficiently impacts on FCR (Abdullahi *et al.*, 2022; Lange and Nakamura, 2023). Therefore, black ants Smith, require less feed than crickets and are considered to have a higher feed efficiency. As a result, it will be responsible for the reduction of insects' feed cost and enable the insects to develop farming for long term economical sustainability (van Huis, 2013; Naseem *et al.*, 2021; Gomes *et al.*, 2023).

Edible insects' farming

Farming is a practice can also be considered as an approach that promotes the knowledge, growth, availability, predictability and productivity of sustainable nutritious sources of edible insects for human consumption. According to Naseem *et al.* (2021), cultivation of edible insects holds significant economic importance, notably in developing regions namely Southeast Asia and South Africa, as it serves as a lucrative source of revenue. Furthermore, edible insect farming (particularly crickets) is presently practiced in Korea, Thailand, Vietnam, and Laos to boost the local economy (Hazarika *et al*., 2020).

In addition, edible insects farming is considered more economical than conventional livestock farming. This is attributed to the fact that insect farming only requires significantly less space, land, and water resources than livestock farming (Testa *et al*., 2017; Abdullahi *et al*., 2022). Doreau *et al*. (2012) demonstrated that approximately 70% of water is utilised in agricultural practices including livestock farming. For instance, 2,200 L/kg of large amounts of water is required for livestock rearing whereas only less amount of water (below 2 L) is needed for insect rearing (for example, mealworms) (Naseem *et al*., 2021). In addition, certain edible insects can be cultivated using organic by-products like biological waste (manure and compost) (Lange and Nakamura, 2021). Moreover, the incorporation of biological waste into the diet of edible insects may enhance the profitability of insects farming (Lange and Nakamura, 2021). Besides, insect farming also entails minimal capital investment for rearing, technical support, and equipment for harvesting than livestock farming (Testa *et al*., 2017). Additionally, insect farming also required minimal infrastructures including nests, containers, and plastic sheets with a limited investment in order to reduce economic risk (Naseem *et al*., 2021). Hence, farming holds the potential to significantly contribute to conservation of edible insect habitats and global food security which is highlighting the economic importance.

Furthermore, In Thailand the application of Good Agricultural Practices (GAP) especially in cricket faming, mark a crucial milestone for advancing the insect farming industry and accessing global markets (Krongdang *et al*., 2023). The edible insects' market in Thailand also emphasizes both importing and exporting flows which highlight the productivity of the edible insects. For instance, between the year 2014-2022, certain edible insects namely crickets, grasshoppers, and bamboo caterpillars were traded internationally which imports to China and Myanmar exceeded US $800,000, whereas exports to the United States, Japan, and England totaled over US $200,000 in 2022 (Krongdang *et al*., 2023). Gahukar (2016) demonstrated that Thailand's silkworm farming involves 137,000 households which responsible for 80% of the country's total silkworm production. In addition, the processed product of silkworm pupae generating exports valued at US $50.8 million for the Thai government in 2004. In India, farmers have been consistently earning a net profit of from US $1,843 to US $1,905 by cultivating the domesticated mulberry silkworms which achieved by planting mulberry crops on just 0.4 hectares of land. According to Hanboonsong *et al*. (2013), cricket farmers in Thailand follow a 45-day cycle to harvest insects from the farm, yielding approximately 750 kg of insects with market prices ranging from 15 to 300 Thai Baht per kg. This generates an annual income of around US $468-1,093 (150,000-350,000 Thai Baht).

According to Krongdang *et al*. (2023), farmer in Thailand earn over 7,000 million Baht through economic insect production with the assistance of advancing technologies and contributes to enhancing the efficiency of farmers' insect production. For instance, a wholesaler in Talad Thai market (Bangkok), sells approximately 30-40 kg of waver ants per day and make a daily profit of around THB 2,000-2,500 (Hanboonsong *et al*., 2013). Moreover, in Nakhon Rachasima, Thailand, the annual value of weaver ant harvest is about US $620,000, which also exported the frozen ant brood to Asian stores, including European and Japanese markets. In Laos, weaver ant and its larvae and pupae are sold for approximately US $12 per kilogram which generated 30% of the annual household income in rural Thailand (Payne and Van Itterbeeck, 2017). Edible insect farming, therefore, holds the potential to significantly contribute to the conservation of edible insect habitats and global food security, underscoring its productivity and economic importance. However, the sustainable farming of black ant Smith, specifically *Carebara vidua* and its mass production growth is still in the early stages.

Traditional market of edible insects

Traditionally, the harvesting and ingestion of edible insects relied on indigenous knowledge and cultural aspects that were performed by local communities. According to Okia *et al*. (2017), the above-mentioned practices including marketing have remained traditional and undeveloped. Ayieko *et al*. (2012) demonstrated that Luos community collects the *Carebara vidua* for their own consumption and seldom sells at local markets due to the minimal collection of the ants. Besides, they also collect other edible insects such as lake flies and elate termites in large quantities which they prefer to consume rather than selling it for a small profit at market (Ayieko *et al*., 2012). For a sustainable growth and productivity of edible insects' market, it is essential to gain a comprehensive understanding of the widespread utilization of edible insects. Thus, a conceptual model of edible black ant Smith utilization is

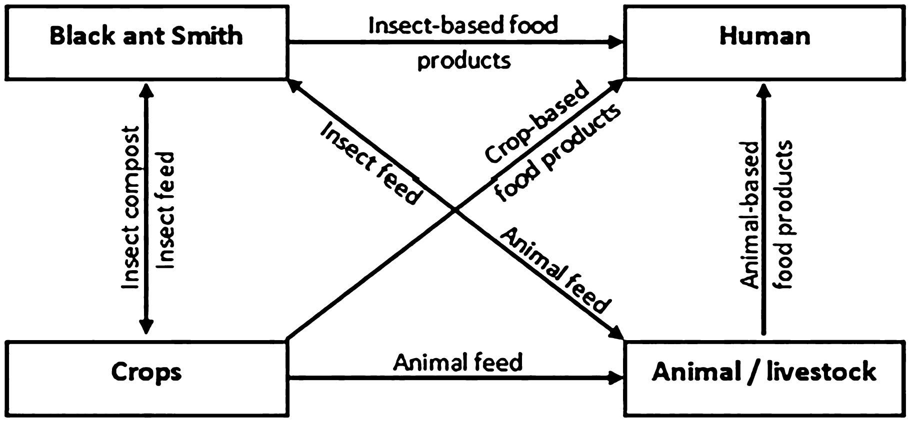

FIGURE 7 Conceptual model of edible black ant Smith utilization. The figure was modified from Kinyuru and Ndung'u (2020).

illustrated in Figure 7. Figure 7 highlights the versatility of edible black ant utilization as food and feed. By demonstrating numerous ways to incorporate edible insects into the human diet and animal feed, this model assists the growth of the edible insect market and generates economic advantages.

Edible insects can be used directly as animal feed. van Itterbeeck (2014) revealed that *Oecophylla smaragdina* (weaver ant) commonly used as feed for songbirds in Indonesia. In addition, livestock waste also can be utilised as insect feed. For instance, fish waste used as insect feed to develop omega-3 rich black soldier fly larvae (Barroso *et al.*, 2019). Surendra *et al.* (2020) reported that by feeding animal waste to insects, it efficiently converts the waste into a high protein source as same as fish and soy meal. Moreover, insect waste serves as a bio-fertilizer for crops, which, in a cyclical manner, can be utilised for animal feed and human food production (Kinyuru and Ndung'u, 2020). Hence, the versatile involvement of edible insects not only enriches the human diet and also contributes to diverse market sector development, fostering income generation as well as a pivotal driver of economic growth.

Supply chain of edible insect markets plays a pivotal role in generating employment chances at different phases of the supply chain including farming, processing, marketing and distribution. This contributes to local and regional economic development. Generally, food processors, collectors, wholesalers, retailers, and consumers are the main participants involved in the supply chain of edible insects (Figure 8). Food processors received the edible insects from the farm for production of edible insects-based food products and also for product development purposes. Furthermore, collectors including commercial and subsistence collectors will collect both edible insects as well as products and sold to wholesalers, retailers and final consumers. Odongo *et al.* (2018) revealed that approximate gross revenue of *Ruspolia differens* (edible grasshopper) commercial collectors in Uganda is US $2,696.00 per swarming season. In addition, the retailers' gross avenue from the sales of fried *Ruspolia differens* is approximately US $690.80 per season (Odongo *et al.*, 2018). Thus, trade in *Ruspolia differens* has created profitable opportunities for households, collectors, sellers, and street vendors in the edible insect value chain. Besides, a study by van Huis *et al.* (2013) revealed that edible insect harvesting, and collection can provide job opportunities and generate income at both household and industrial scale of processing.

Guiné *et al.* (2022) reported that the edible insect market in the Asia-Pacific region is forecasted to surpass US $270 million by 2024. Even though more than half of the market is currently dominated by Asia-Pacific and Latin America, the most significant growth is anticipated in North America and Europe due to the increasing market demand of edible insects for human consumption. As the demand for edibles insects increases, may resulting in a rise in insect prices as demand outpaces supply. For example, the income from weaver ants' brood starts at US $1.2-1.4 per kg at the collector level, increases to US$1.6-1.7 per kg at the middlemen level, and is ultimately sold to consumers for US $3.5-5 per kg in high-demand urban areas (Mozhui *et al.*, 2023). In western countries, edible insects sell as exotic food which driven by the demand from immigrant communities or the emergence of niche markets (van Huis *et al.*, 2013). In Western Kenya, the community is able to harvest approximately 1 to 1.5 kg of *Carebara vidua* during the peak season of the black ant Smith emerging from the nest, where such amount of harvest would be enough to supply a daily meal for five people in a household (Ondede, 2023). However, detailed information about the price and market demand for black ant smith is not well-commercialized.

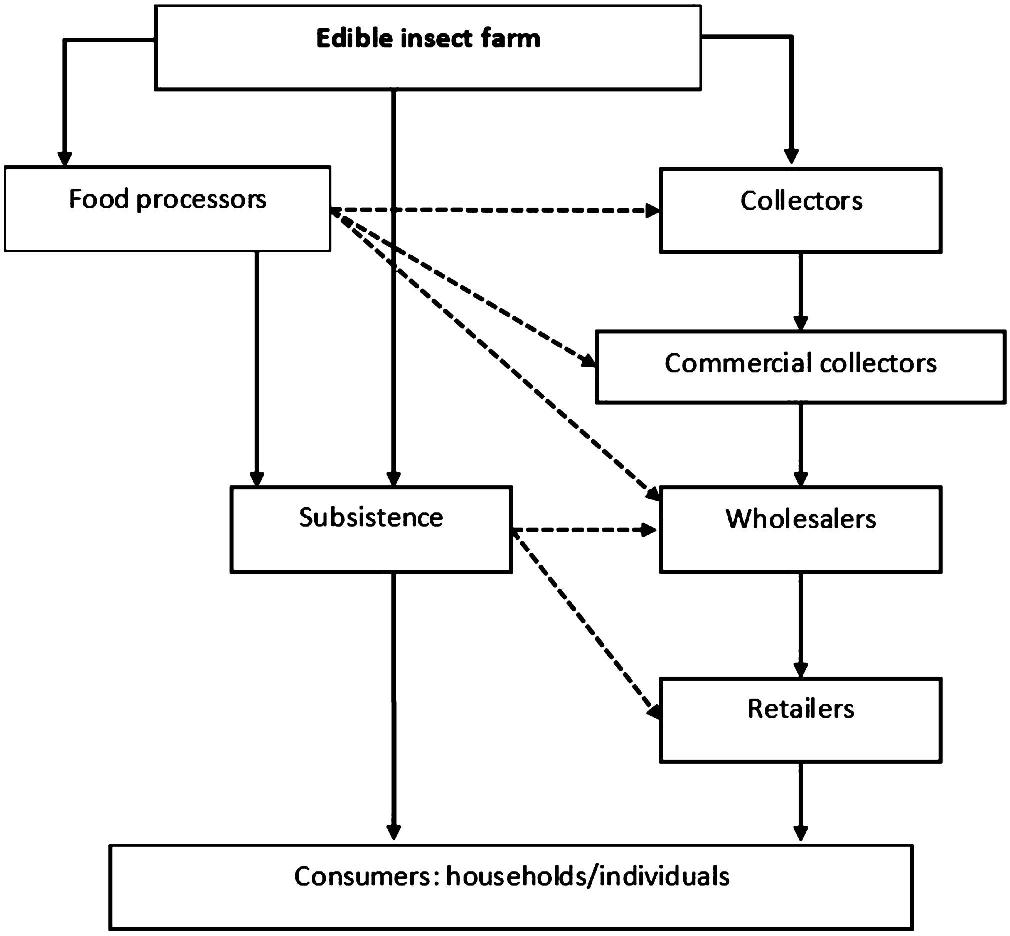

FIGURE 8 Flow diagram of edible insect's supply chain. The figure was modified from Odongo *et al.* (2018).

In Thailand, both weaver ant sellers and collectors can obtain decent income. However, this practice is considered as sideline occupation due to the seasonal harvesting of weaver ants (Hanboonsong *et al.*, 2013; van Itterbeeck *et al.*, 2014). For example, weaver ant sellers in the north region are able to sell around 5 to 10 kg of weaver ants which are approximately able to earn within THB 1,200 to THB 3,000 per day (Hanboonsong *et al.*, 2013). Gahukar (2020) demonstrated that edible insects play an essential economic role, especially for those communities with low-income, who rely on insects' seasonal availability and sale in local markets. For example, a family in Tanzania is able to earn up to 50% of the total family income by selling *Ruspolia differens* which are affordable to cover basic needs including education fees (Mmari *et al.*, 2017). Furthermore, in Botswana, a family is capable of earning nearly US $122 per month by collecting and selling mopane worms from the mopane trees located around their village huts (Gahukar, 2020). Additionally, Melgar-Lalanne *et al.* (2019) reported that over the past decade, numerous companies, and startups in the worldwide, particularly in South Asia, Europe and North America, have initiated efforts to commercialize the edible ants-based food product for human consumption (Table 3).

Potential food industry of edible insects

The consumption style of edible insects in the rural market is dominantly in the form of raw, fried, roasted, smoked, and salted. However, in recent times, edible insects, particularly black ants have evolved to shelf stable edible insect-based products such as canned edible insects, flour products, snacks, confectioneries, alcoholic beverages, fat-based products and many more (Tao and Li, 2018; van Huis, 2018; Islam *et al.*, 2022; van Huis and Rumpold, 2023). Moreover, Pali-Schöll *et al.* (2019) reported that edible insects and its products are recognised as novel food and also a promising alternative food source which gained substantial global attention due to its excellent nutritional and therapeutic qualities. Besides, the commercialization and promotion of edible insects are anticipated to create profitable opportunities as well as introducing the essential nutrients into the diets of vulnerable populations (Krongdang *et al.*, 2023). Raheem *et al.* (2019a) revealed that in Thailand, several insect food manufacturers play a crucial role

TABLE 3 Commercialized edible ant and its food products sold in global market

Company	Products	Price (US $)	Country	Source
AntOnTop	Black ant Smith (*Carebara vidua*) colonies	14,099	Poland	AntOnTop, 2024; https://antontop.com/carebara-vidua/
Circle Harvest	Real ant candy (*Iridomyrmex*.) (10 g) Real ant lollipops (*Iridomyrmex*.)	4.21 6.02	Australia	Circle Harvest, 2024 https://circleharvest.com.au/
Eat Crawlers	Dried black ants (*Polyrhachis*.) (10 g)	8	New Zealand	Eat Crawlers, 2024) https://eatcrawlers.co.nz/
Entosense, LLC	Gourmet black ants (*Lasius niger*) (15 g) Flavoured chicatana (*Atta Mexicana*) (15 g)	29.95 29.95	United States	Entosense, 2019 https://www.entosense.com/
Europe-entomophagie	Dried black ants (kilo packs)	53.89	France	Europe-entomophagie, 2014; https://www.europe-entomophagie.com/en/
JR Unique Foods Ltd.	Canned weaver ant (larvae & pupae) Dried black ant eggs (5 g) Dried weaver ant eggs (5 g) Salted dried weaver ants (*Oecophylla*.) (10 g) Salted dried black ants (*Polyrhachis*.) (5 g) Brined weaver ant eggs (70 g)	12.804.80 5.50 5.60 5.60 $0.99	Thailand	Thailand Unique, 2024; https://www.thailandunique.com/
Next Food FZE	Flavoured black ants (spicy) (10 g)	6.00	Thailand	Next Food, 2024; https://www.next-food.net/

as supply chain by supplying essential ingredients like insect flour to their business collaborators in order to yield food products including pasta, ramen noodle, bakery products, and protein supplements. These products are marketed to both domestic and international markets to meet consumer demands. Over the past decade, a multitude of companies and startups, especially South Asia, Europe, and North America have been established to commercialise the insect-based food products for human consumption (Melgar-Lalanne *et al.*, 2019).

In addition, E-shopping platforms are also utilised as a commercialization medium for edible insect products. For instance, over 100 gourmet food products made from insects are currently being sold on Amazon.com platform (Melgar-Lalanne *et al.*, 2019). Driven by increasing demand, several companies in Thailand have expanded the insect products into a wide range of packages which are available in frozen, dried, and deep-fried forms (Raheem *et al.*, 2019a). As a commercialization strategy, insect-based products are marketed and can be purchased in a wide range of market channels including retail supermarkets, convenience stores, and E-shopping platforms (Krongdang *et al.*, 2023). As per Thailand's national strategy within 2018 to 2037, edible insects are being positioned as a sustainable solution to address the growing demand for wholesome and nutritious food in both local and international markets (Krongdang *et al.*, 2023). Thus, edible insect products show significant potential in the future food industry. As consumer preferences continue to evolve and the demand for edible insect products grows, it will create new opportunities for other economically valuable edible insects, such as the black ant Smith to be commercialised in both domestic and global markets.

6 Nutritional value of black ant Smith (*Carebara vidua*)

In general, insects are identified as a good source of macro- and micro-nutrients (van Huis and Oonincx, 2017; kim *et al.*, 2019). Edible black ant Smith has generous sources of proteins and other nutrients (i.e. fat, minerals, and vitamins) (Figure 9) that may be enough to supply daily nutrient requirements for the human body as equivalent to nutrients obtained from typical animal sources (Bernard and Womeni, 2017).

Macronutrient content

Earlier, Ayieko *et al.* (2012) had proven that the black ant Smith collected from the Lake Victoria region in Kenya have high nutritional value, particularly protein, fat, and minerals. This edible ant holds promise as a nutritional resource for human consumption. Ayieko *et al.* (2012) reported that black ant Smith has protein content of 40.83 g/100 g of dry weight, 39.79 g/100 g of dry weight, and 44.64 g/100 g of dry weight for female black ant Smith (whole body), thorax, and abdomen parts, respectively. Accordingly, Jose *et al.* (2022) reported that black ant Smith contains 53.84 g/100 g of protein (by dry weight). In another report by Rumpold and Schlűter (2013a), the edible black ant Smith contains 42.50 g/100 g (dry weight) of protein (Table 4). According to Ojha *et al.* (2021a), the nutritional compositions vary depending on several factors, for example diet, habitat, season, sex, insect reproduction stage, as well different parts of the body. In comparison with other species of black ants, it could be noted that the *Carebara vidua* is subject to a slightly higher protein content than the other species of black ants such as *Polyrhachis vicina* Roger (from Zhejiang) (36.12 g/100 g of dry weight) and *Camponotus pennsylvanicus* (22.50 g/100 g of dry weight). In addition, black ant Smith also presented higher amount of protein content than Escamol ant (*Liometopum apiculatum*) (37.33 g/100 g of dry weight) and sugar ant (31.86 g/100 g of dry weight) (Hoey-Chamberlain *et al.*, 2013; Virginia *et al.*, 2013; Mathew *et al.*, 2014; Escamilla and Ariza, 2021).

For fat content, it could be observed that the composition of fat in an edible black ant is generally subject to a slight variation. For example, the *Carebara vidua* has a mean value fat content of 33.38-49.77 g/100 of dry weight, which is about nearly 3-fold and 50-fold higher amounts of fat than the *Polyrhachis vicina Roger* and *Camponotus pennsylvanicus*, receptively (Table 4). Moreover, the amount of fat in black ant Smith is higher than other edible ants as reported by Rumpold and Schlűter (2013a), Mathew *et al.* (2014), and Alagappan *et al.* (2021) for leafcutter ant, escamol ant, black ant, sugar ant, and Australian green ant, respectively.

Apart from fat, the edible black ant species showed a significant amount in fiber ranging from 1.46 to 29.13 g/100 g of dry weight, depending on the species. Whereby, the *Polyrhachis vicina* Roger demonstrates to contain the highest amount of fiber, followed by *Carebara vidua* and the species with the lowest fiber contents was recorded in *Camponotus pennsylvanicus* (Table 4). Insects are known to have fiber in the form of chitin, an important constituent of the cell wall in building the structure of insect exoskeletons (Abidin *et al.*, 2020). According to Kipkoech (2023), the characteristics (i.e. low water holding capacity) of the fiber of insects plays an important role in binding with cholesterol at the lower part of the digestive tract, hence could help in decreasing the cholesterol absorption by excretion of excess cholesterol from human body. Besides, the fiber in the form of chitin has similar structure to indigestible cellulose, an insoluble dietary fiber with potential prebiotic properties that could be served as a good food for the growth of beneficial bacteria in the human body (Carlson *et al.*, 2018). Thus, insects, especially the edible black ants can be processed as functional food ingredients to benefit human health.

On average, the ash content of edible black ants ranges 0.95-4.16 g/100 g of dry weight. The highest ash contents are found in *Polyrhachis vicina* Roger (from Guizhou) with 4.16 g/100 g of dry weight. Besides, different parts of insects, particularly *Carebara vidua* have been shown to contain different amounts of ash (Table 4). The mean value of total carbohydrate content of black ant Smith ranges 0.28-12.43 g/100 g of dry weight. A low amount of total carbohydrate is also can be observed in *Polyrhachis vicina* Roger (Ayieko *et al.*, 2012; Rumpold and Schlűter, 2013a) (Table 4), which attributed to a high amount of protein and fat.

For fatty acids, palmitic, Linoleic, and Oleic acids are the major fatty acid presence in this edible insect, for all the body segments of both males and females, but linolenic fatty acid is not detected both in female and male of blank ant Smith (Ayieko *et al.*, 2012) (Table 5). Both males and females of the edible black ant Smith have demonstrated similarities in most of the evaluated fatty acids. However, arachidic acid was found only presence in females black ant Smith (Ayieko *et al.*, 2012). In comparison with other edible black ants, the black ant Smith showed a higher in most of the saturated fatty acid (i.e. palmitic, myristic, stearic, lauric, and arachidic) than *Polyrhachis vicina* Roger (from Zhejiang

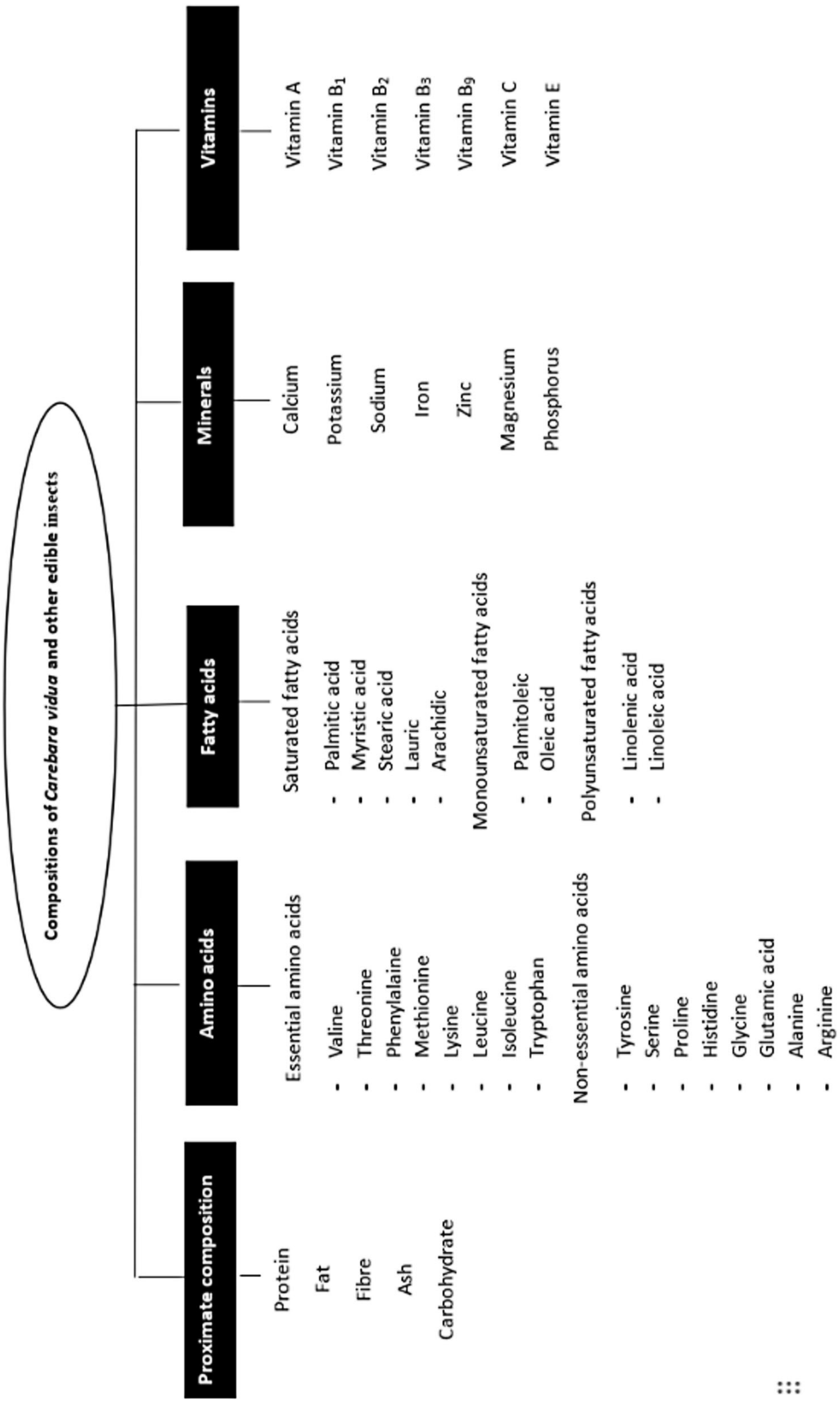

FIGURE 9 The nutritional compositions in *Carebara vidua* and other edible insects (by Order).

TABLE 4 Comparative mean value of macro-nutrient compositions (g/100 g of dry weight basis) of different edible black ant species

Black ant species	Macro-nutrient composition					References
	Protein	Fat	Fiber	Ash	Total carbohydrate*	
Carebara vidua[a] (Black ant Smith)	40.83	47.51	6.91	1.55	6.18	Ayieko *et al.*, 2012
Carebara vidua[b] (Black ant Smith)	39.79	42.07	10.09	2.07	12.43	Ayieko *et al.*, 2012
Carebara vidua[c] (Black ant Smith)	44.64	49.77	4.31	0.95	0.28	Ayieko *et al.*, 2012
Carebara vidua[d] (Black ant Smith)	42.50	49.45	7.19	1.61	–	Rumpold and Schlűter, 2013a
Carebara vidua[e] (Black ant Smith)	53.84	33.38	9.49	2.20	–	Jose *et al.*, 2022
Polyrhachis vicina Roger (from Zhejiang) (Black ant)	36.12	18.00	29.13	2.52	14.22	Rumpold and Schlűter, 2013a
Polyrhachis vicina Roger (from Guizhou) (Black ant)	45.40	17.40	28.88	4.16	4.16	Rumpold and Schlűter, 2013a
Camponotus pennsylvanicus (Black carpenter ant)	22.50	1.00	1.46	1.66	32.32	Abulude and Fagbayide, 2017

[a]Results recorded from whole body of female *carebara vidua* Smith; [b]Results recorded from thorax of female *carebara vidua* Smith; [c]Results recorded from abdomen of female *carebara vidua* Smith; [d]Results recorded from unknown parts of female *carebara vidua* Smith; [e]Results recorded from *carebara vidua* Smith after removing wings during processing; *Value obtained via calculation; "–" = not reported

and Guizhou) (Rumpold and Schlűter, 2013a). Furthermore, the unsaturated fatty acids, particularly monounsaturated fatty acid, are recorded to be higher than saturated fatty acid. However, the high amount of unsaturated fatty acid may accelerate the rate of spoilage via oxidation during the handling and processing of insect-based products (Kipkoech, 2023), hence could affect the quality of food during storage.

Considering the mean values of the different orders of insects, the protein content ranges 35-61 g/100 g (based on dry weight basis) (Ayieko *et al.*, 2012; Rumpold and Schlűter, 2013a; Kim *et al.*, 2019; Jose *et al.*, 2022). The edible black ant Smith presented a lower protein content than the other edible insects from order; Orthoptera (i.e. crickets, grasshoppers, and locusts) and blattodae (i.e. cockroaches) (Table 6). In addition, the amount of protein (per 100 g) in edible black ant Smith (different parts of body) is higher than the protein value of several commonly consumed protein sources from plant and animal origins such as soybean (39.90 g/100 g), mung beans (31.7 g/100 g), bamboo shoot (28.90 g/100 g), cauliflower (24.2 g/100 g), cabbage (16.40 g/100 g), wheat flour (8.09-13.00 g/100 g), beef (19.59-22.97 g/100 g), pork (16.89-27.70 g/100 g), poultry (21.47-23.96 g/100 g), egg (10.94-11.75 g/100 g), and fish (15.00-24.00 g/100 g) (Chakravorty *et al.*, 2016; Rehman *et al.*, 2016; Abraha *et al.*, 2018; Hammuel *et al.*, 2019; Orkusz, 2021; Khalid *et al.*, 2023; Meenakumari *et al.*, 2023) (Figure 10). The excellent protein source of *Carebara vidua* exhibited remarkable ability to replace the plant protein with insect protein than mammals (Deroy *et al.*, 2015). This insect being highly nutritious can contribute to a significant daily dietary need for the local community. The ant being highly protein content can contribute significantly to meeting the daily protein (23-56 g) requirement for human (USA NIH, 2023).

In addition, black ant Smith also presented a higher amount of fat, saturated fatty acids, and monounsatu-

TABLE 5 Comparative mean value of fatty acid composition (% of total fatty acid) of different edible black ant species

Fatty acid composition	Edible black ant species				
	Carebara vidua[a,1,2] (black ant Smith)	*Carebara vidua*[b,1,2] (black ant Smith)	*Carebara vidua*[c,1,2] (black ant Smith)	*Polyrhachis vicina* Roger[2] (from Zhejiang) (black ant)	*Polyrhachis vicina* Roger[2] (from Guizhou) (black ant)
Unsaturated fatty acid (% of total fatty acid)					
Palmitoleic (C16:1, n7)	3.31	2.93	1.86	8.90	8.20
Linoleic (C18:2, n6)	10.61	10.20	12.19	1.70	2.10
Linolenic (C18:3, n3)	nd	nd	nd	0.60	1.0
Oleic (C18:1, n9)	51.26	46.78	51.44	60.50	63.00
MUFA	54.57	49.71	53.30	71.30	72.40
PUFA	10.61	10.20	12.19	3.70	3.10
Saturated fatty acid (% of total fatty acid)					
Palmitic (C16:0)	25.94	28.75	27.06	19.00	17.50
Myristic (C14:0)	1.38	1.83	1.06	0.60	0.60
Stearic (C18:0)	5.78	5.94	4.82	4.30	4.30
Lauric (C12:0)	1.72	2.20	0.48	–	0.70
Arachidic (C20:0)	nd	1.36	1.11	–	0.30
SFA	34.82	40.08	34.53	25.50	23.90

[a]Results recorded from male abdomen of *carebara vidua* Smith; [b]Results recorded from female abdomen of carebara *vidua* Smith; [c]Results recorded from female thorax of *carebara vidua* Smith; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; "nd" = not detected; "–" = not reported ([1]Ayieko *et al.*, 2012; [2]Rumpold and Schlűter, 2013a).

rated fatty acids, but a slight lower in polyunsaturated fatty acids than the other type of edible insects from a variety of insect order such as grasshoppers, locusts, and crickets (Orthoptera), caterpillars (Lepidoptera), cockroaches (Blattodea), termites (Isoptera), and beetles and grubs (Coleoptera) (Rumpold and Schlűter, 2013a) (Table 6). According to Ramos-Bueno *et al.* (2016), the fat content of insects is generally about similar with plant-based oil (vegetable) and fats of animals. Moreover, compared with pork and beef, insects are recorded to be rich in monounsaturated fatty acids and polyunsaturated fatty acids (Kipkoech, 2023). Besides, the amount of fat in the edible black ant Smith is comparatively higher than the fat-rich fruits, such as avocado (29 g/100 g) and olive (23 g/100 g) (Ho *et al.*, 2022b).

Insect protein has been shown to contain a significant amount of amino acids (i.e. essential amino acids and non-essential amino acids). Most edible insects in different orders supply a very good amount of essential amino acids (Table 6) that are able to fulfill human nutrition requirements for amino acids. According to Inje *et al.* (2018), the protein of the insect is commonly high in threonine and lysine and low in cysteine and methionin. Furthermore, the essential amino acids such as lysine, threonine, and tryptophan are commonly present in a very small amount in the protein of cereal (i.e. wheat flour), which is known as staple diets in many countries (Orkusz, 2021; Khalid *et al.*, 2023). The high lysine content of the edible insects indicates that they have a very high potential and may be supplemented for daily diet to enrich diets. Currently, the amino acid

TABLE 6 Comparative mean value of nutritional compositions of edible black ant Smith (*Carebara vidua*) with other edible insects (by order)

Nutrient compositions	Edible black ant Smith (*Carebara vidua*) and edible insects (by order)								
	Carebara vidua	Orthoptera[1]	Lepidotera[2]	Isoptera[3]	Hymenoptera[4]	Hemiptera[5]	Diptera[6]	Coleoptera[7]	Blattodae[8]
Proximate composition (%)									
Protein	39.79-53.84*	61.32	45.38	35.34	46.47	48.33	49.48	40.69	57.30
Fat	42.07-49.77*	13.41	27.66	32.74	25.09	30.26	22.75	33.4	29.30
Fibre	0.95-9.49*	9.55	6.60	5.06	5.71	12.4	13.56	10.74	5.31
Ash	0.95-2.20*	3.85	4.51	5.88	3.51	5.03	10.31	5.07	2.94
Total carbohydrate	0.28-12.43*	12.98	18.76	22.84	20.25	6.08	6.01	13.20	4.53
Amino acids (mg/g protein)									
Histidine (His)	–	21.20	23.70	51.40	27.00	15.70	22.30	26.30	19.40
Isoleucine (lle)	–	39.60	40.40	51.10	47.80	31.50	32.60	45.6	29.20
Leucine (Leu)	–	74.80	62.70	78.30	78.40	49.80	57.40	74.20	56.40
Lysine (Lys)	–	53.90	57.70	54.20	53.80	28.00	62.90	50.60	48.00
Mehionine (Met)	–	19.30	22.10	7.50	23.80	21.70	27.20	16.20	29.80
Cysteine (Cys)	–	12.80	12.20	18.70	12.90	12.90	5.30	14.6	11.60
Phenylalanine (Phe)	–	46.60	46.30	43.80	47.50	34.40	50.60	47.1	30.60
Tyrosine (Tyr)	–	61.50	49.10	30.20	55.30	38.70	56.70	55.7	62.30
Threonine (Thr)	–	35.80	40.00	27.50	41.70	29.90	38.80	35.2	34.60
Tryptophan (Trp)	–	8.10	11.20	14.30	10.30	10.30	28.30	10.1	6.00
Valine (Val)	–	50.30	54.10	73.30	60.50	44.30	46.90	51.9	53.80
Arginine (Arg)	–	53.60	46.90	69.40	43.50	24.90	49.40	53.9	41.50
Serine (Ser)	–	41.90	48.40	–	38.20	10.30	60.00	42.6	41.90
Proline (Pro)	–	53.90	44.90	–	66.70	–	27.80	64.1	65.00
Alanine (Ala)	–	77.40	48.90	–	72.30	26.40	58.90	69.5	56.60
Glycine (Gly)	–	540	43.80	–	81.30	16.40	45.10	55.2	58.70
Glutamic acid (Glu A)	–	94.50	103.40	–	134.30	23.70	98.60	123.7	99.70
Fatty acids (% fatty acid)									
SFA	34.53-40.08*	32.05	37.04	41.97	29.88	43.89	33.02	38.49	–
MUFA	49.71-54.57*	29.37	23.36	22.00	48.76	32.39	47.23	35.72	–
PUFA	10.20-12.19*	37.08	39.76	36.04	21.18	22.89	15.95	27.14	–

Example of edible insects (by order): [1]Crickets, grasshoppers, and locusts; [2]Butterflies and months; [3]Termites; [4]Ants and bees; [5]True bugs; [6]Flies; [7]Beetles and grub; [8]Cockroaches; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids, "–" = not reported; "*" = range based on different parts and sex of black ant Smith (*Carebara vidua*) (Ayieko *et al.*, 2012; Rumpold and Schlűter, 2013a; Jose *et al.*, 2022).

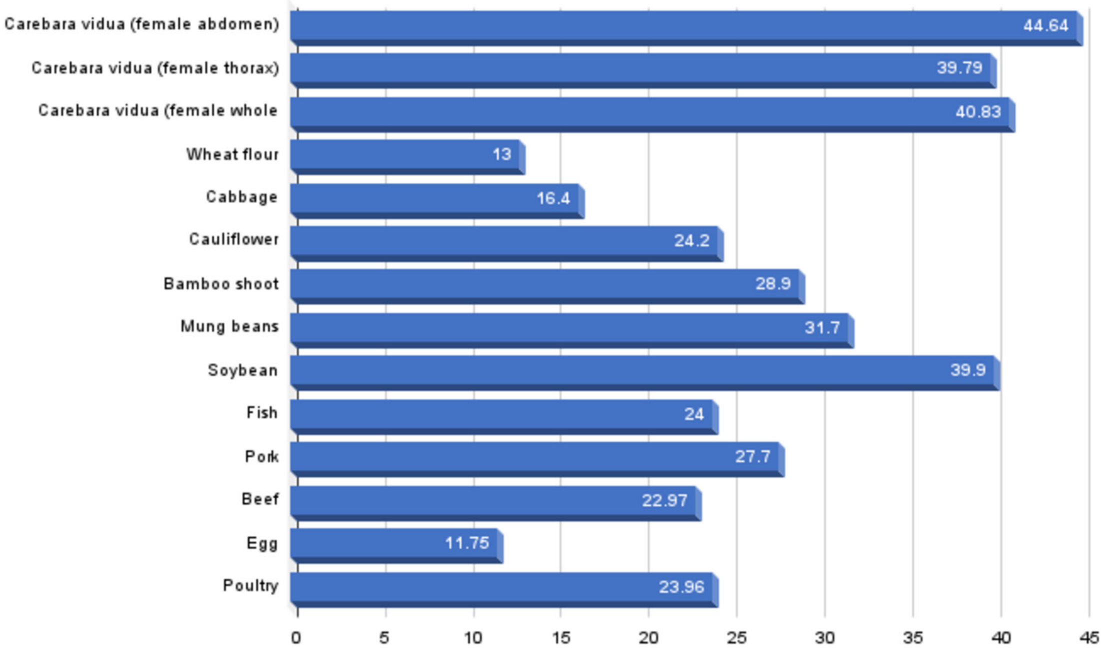

FIGURE 10 Comparison of protein contents of *carebara vidua* and conventional foods of plant and animal origin. Source: Chakravorty *et al.* (2016), Rehman *et al.* (2016), Abraha *et al.* (2018), Hammuel *et al.* (2019), Orkusz (2021), Meenakumari *et al.* (2023), Khalid *et al.* (2023).

compositions of the edible black ant Smith are not yet known as most of the reported studies are focusing on other types of edible black ant that have traditional Chinese medicine value, such as *Polyrhachis vicina* Roger. Hence, analyzing the amino acids of edible black ant Smith is critical to be carried out for better understanding of their functionalities.

Micronutrient content

Insects, including black ant Smith, are reported to be a good source of macro- and micro- minerals that are essential for maintaining overall health of the body. Based on the findings presented by Ayieko *et al.* (2012), the whole body of black ant Smith is high in nutrition, containing ample amounts of calcium (22.23 mg/100 g of dry weight), potassium (51.73 mg/100 g of dry weight), sodium (26.23 mg/100 g of dry weight), iron (10.69 mg/100 g of dry weight), zinc (5.69 mg/100 g of dry weight), magnesium (10.41 mg/100 g of dry weight), and phosphorus (106.04 mg/100 g of dry weight) (Table 7). In another study reported by Jose *et al.* (2022), *Carebara vidua* obtained from the market in Lilongwe city, Malawi has higher amount of phosphorus (224.61 mg/100 g) than *Carebara vidua* collected from the Lake Victoria, Kenya (105.85-106.91 mg/100 g of dry weight) (Ayieko *et al.*, 2012) (Table 7). Remarkably, edible black ant species *Polyrhachis vicina* Roger has higher concentration in all of the evaluated mineral elements than *Carebara vidua*. Adámková *et al.* (2020) have summarised the macro- and micro- minerals of insects can be influenced by the rearing conditions such as the temperature of the environment and feed compositions.

It can be noted that all the edible black ants evaluated are low in calcium (13.17-108.00 mg/100 g dry weight) and do not meet daily required intake amount for adults (1,000-1,200 mg/day for adults aged 19-70 years old) (USA NIH, 2023). Besides, for potassium, consuming dried edible black ants (100 g per day) is also inadequate for the daily uptake (recommended dietary allowance: potassium is 2,600-3,400 mg/day for aged between 19 to 70 years) (USA NIH, 2023). On the other hand, black ant Smith shows a very low concentration of sodium (21.29-30.28 g/100 g, depending on the parts of the body). This amount is below the recommended dietary allowance for adults (1,500 mg/day) (USA NIH, 2023). Intake of a low sodium diet is very important to prevent the overload of fluid volume in the body of patients with heart failure problems (Lee and Tseng, 2022). In future, the edible black ants, especially *Carebara vidua* (the abdomen part) could be utilised in the development of low-sodium food.

It is noteworthy that most of the edible black ant species (i.e. *Carebara vidua* and *Polyrhachis vicina* Roger) are rich in iron. Remarkably, *Polyrhachis vicina* have a very high level of iron (118.00 mg/100 g of dry weight) even exceed the recommended daily intake amount (recommended dietary allowance: 8-27 mg/day for adults aged between 19 to 70 years) (USA NIH,

TABLE 7 Comparative mean value of mineral content (mg/100 g) of different edible black ant

Mineral content	Edible black ant species						
	Carebara vidua[a,1] (Black ant Smith)	*Carebara vidua*[b,1] (Black ant Smith)	*Carebara vidua*[c,1] (Black ant Smith)	*Carebara vidua*[d,2] (Black ant Smith)	*Polyrhachis vicina* Roger[3] (from Zhejiang) (Black ant)	*Polyrhachis vicina* Roger[3] (from Guizhou) (Black ant)	*Camponotus pennsylvanicu4* (Black carpenter ant)
Calcium	22.23	13.17	26.10	–	49.10	108.00	52.10
Potassium	51.73	70.40	46.37	–	–	–	3.49
Sodium	26.23	30.28	21.19	–	–	–	61.00
Iron	10.69	9.74	2.71	3.77	118.00	53.70	0.32
Zinc	5.69	5.51	6.65	–	17.60	11.90	0.49
Magnesium	10.41	10.50	10.93	–	65.30	67.60	4.03
Phosphorus	106.04	106.91	105.85	224.61	387.70	417.00	–

[a]Results recorded from female whole body of *carebara vidua* Smith; [b]Results recorded from female thorax of *carebara vidua* Smith; [c]Results recorded from female abdomen of *carebara vidua* Smith; [d]Results recorded from *carebara vidua* Smith after removing wings during processing.; "–" = not reported ([1]Ayieko *et al.*, 2012; [2]Jose *et al.*, 2022; [3]Rumpold and Schlűter, 2013a; [4]Abulude and Fagbayide, 2017).

2023). The required concentration of iron is dependent on the age and sex of the consumers. Edible black ants, except that *Camponotus pennsylvanicus* and female abdomen of *Carebara vidua* sufficiently supply iron for adult males (recommended dietary allowance: 8 mg/day). Meanwhile, pregnant women require 27 mg of iron per day (USA NIH, 2023) to prevent anemia during their pregnancy. Therefore, an edible black ant, especially *Polyrhachis vicina* sufficiently provides the iron element for pregnant women by consuming approximately 23 g of dried edible black ants per day. However, there is no considerable evidence on the safety aspect of insect consumption for pregnant women. In addition, the amount of iron (per 100 g) in an edible black ants contain much more iron than beef (1.9 mg/100 g), chicken (0.59 mg/10 g), pork (0.7 mg/100 g), and turkey (0.79 mg/100 g) (Marangoni *et al.*, 2015; Goran *et al.*, 2016).

Besides iron, an edible black ant can be considered as a valuable source of zinc. Zinc is important for maintaining the function of the body in reproduction, pregnancy, formation of bone, and hematopoiesis (Adámková *et al.*, 2020; Garner *et al.*, 2021). The average zinc concentration in edible black ants ranged from 0.49 to 17.60 mg/100 g of dry weight. Furthermore, Polyrhachis vicina Roger contains highest zinc (17.60 mg/100 g of dry weight) and able to provide a sufficient amount of daily intake of zinc according to the recommended dietary allowance for adults (8-12 mg/day for adults age ranging 19-70 years) (USA NIH, 2023). Surprisingly, it is higher than that of beef (3.9 mg/100 g), pork (1.8 mg/100 g), and oyster (0.2 mg/100 g) (Marangoni *et al.*, 2015; Zhu *et al.*, 2018; Zhang *et al.*, 2023). Thus, the consumption of edible black ants is possible to decrease iron and zinc deficiency in the population of developing countries. In addition, an edible black ant has appreciable amounts of magnesium (4.03-67.60 mg/100 g of dry weight) and phosphorus (106.04-417.00 mg/100 g of dry weight) (Table 7). In conclusion, edible black ants generally present insufficient amounts of macro-nutrients (i.e. calcium, potassium, sodium, magnesium, and phosphorus) but have the potential to supply adequate amount of micro-nutrients (i.e. iron and zinc) for fulfilling recommended minimum daily consumption of both macro- and micro-nutrients. The edible black ants could be a potential alternative source of minerals that are essential in regulating health and well-being of the body and can be processed as ingredients for food supplements.

Regarding vitamins, black ants Smith supply several vitamins (i.e. vitamin A, B, C, and E) (Table 8), in most cases provide an adequate amount for fulfilling vitamin requirements in human nutrition. For example, the edible black ants Smith provide a reasonable amount of vitamin A (0.77 mg/100 g of dry weight). This report is concomitant with the review by Kouřimská and Adámková (2016) that the escamoles (eggs of the ant) of the Formicidae family are rich in vitamins A with value 5.05 mg/100 g. Vitamin A plays several roles such as protecting eyesight and the overall immune system of the body. Moreover, according to Thirunavukarasu *et al.* (2022), severe deficiency of vitamins is often related to

TABLE 8 Mean value of vitamin content of whole body of female black ant Smith (*Carebara vidua*)

Vitamin	Concentration (mg/100 g)
Retinol (vitamin A)	0.77
α-Tocopherol (vitamin E)	0.59
Thiamin (vitamin B_1)	0.46
Riboflavin (vitamin B_2)	20.26
Niacin (vitamin B_3)	0.28
Ascorbic acid (vitamin c)	0.03
Folic acid	0.45

Source: Ayieko *et al.* (2012).

suffering from ocular disease (xerophthalmia) and dysfunctional immunity. Thus, consumption of black ants Smith along with other vitamin A rich foods can be practised to meet the daily uptake levels of vitamin A (recommended dietary allowance for adults age ranging 19-70 years is 0.7-0.9 mg/day) (USA NIH, 2023).

In general, a 100 g of edible black ant Smith is not an efficient source of vitamin B, especially vitamin B_1 (0.46 mg/100 g of dry weight) and vitamin B_3 (0.28 mg/100 g of dry weight), vitamin C (0.03 mg/100 g dry weight), and vitamin E, whereby, the National Institutes of Health (NIH), United States recommends a daily intake of 1.1-1.4 mg/day for vitamin B_1, 14-18 mg/day for vitamin B_3, 75-90 mg/day for vitamin C and 15-19 mg/day for vitamin E for adults aged between 19 to 70 years (USA NIH, 2023). Even vitamin B is present in an appreciable amount in black ant Smith, but it is often absent in many animal proteins (Ayieko *et al.*, 2022). In contrast, vitamin B2 is present in a high concentration in black ant Smith (20.26 mg/100 g). This indicates that a daily intake of approximately 7 mg of this black ant Smith could fulfill the recommended daily intake amount of vitamin B2 in the nutrition of adults aged between 19 to 70 years (recommended dietary allowance: 1.1-1.4 mg/day) (USA NIH, 2023). Sufficient uptake of vitamin B2 is important to prevent risk of several diseases such as migraines, cataracts, thyroid dysfunction, and anemia (Mahabadi *et al.*, 2022).

On the other hand, edible black ant Smith is rich in folic acid (0.45 mg/100 g dry weight) which recorded to be higher than insects of the order Coleoptera (i.e. beetles and grubs) (0.30-0.41 mg/100 g of dry weight) and Lepidoptera (i.e. months) (0.02-0.41 mg/100 g of dry weight). Nevertheless, the black ant Smith is insufficient for providing vitamin as compared to other edible insects belonging to the Orthoptera order (i.e. crickets, locusts and grasshoppers) (0.49-0.90 mg/100 g of dry weight) (Rumpold and Schlűter, 2013a). This suggests that the edible black ant Smith (~100 g) could be developed into a vitamin supplement which could supply sufficient daily intake of folic acid for males and female adults, aged between 19-70 years (recommended dietary allowances: 0.4 mg/day) (USA NIH, 2023).

7 Nutraceutical properties and medicinal uses of black ant Smith (*Carebara vidua*)

Ethnopharmacology or also known as enthnopharmacy is an interdisciplinary exploration of bioactive compounds and incorporation of concepts and methods of botany, anthropology, chemistry, toxicology, and pharmacology for traditional medicinal uses (Yeung *et al.*, 2020). Scientific research has proven that the body of insects has the potential in antibacterial, anaesthetics, diuretic, analgesic, antirheumatic, and immunological. These properties of insects suggest that it could be used as a source of therapeutic agent (Yamuna and Raja, 2019). Generally, Order of Hymenoptera, (family: Formicidae), for example, ants play a vital role in both the food and pharmacological sectors because of its therapeutic qualities (Agarwal *et al.*, 2022). The therapeutic properties are due to the presence of antioxidant capacity, bioactive peptides, defensive secretion (venom), bioactive compounds (alkaloids), and antimicrobial peptides (Supplementary Table S2). Particularly, ants have been utilised as traditional and modern medicine for the treatment of several diseases such as arthritis, asthma, cancer, and other microbial infections (Agarwal *et al.*, 2022).

Antioxidants are substances that are responsible to delay the cell damage caused by attacking from free radicals that released from body as an anti-pressure to environment (Aziz *et al.*, 2022). Intake of foods rich in antioxidant, for example fruits and vegetables have shown effectively prevent several oxidative stress-associated diseases (i.e. diabetes, cancer, and cardiovascular diseases) (Ho *et al.*, 2022a). Mattia *et al.* (2019) prepared the water-soluble extract (distilled water) and lipo-soluble extract (defatted using hexane washing) of edible black ant (*Lasius niger*) for analysing the antioxidant capacity of edible black ant (*Lasius niger*). The values are then compared to fresh orange juice and olive oil (as reference) for water-soluble extract and lipo-soluble extract, respectively. They reported that distilled water extract of black ant (*Lasius niger*) demonstrates higher values of trolox equivalent antioxidant capacity (TEAC) (0.57 mmol TE/100 g) than fresh orange juice (a reference) (0.40 mmol TE/100 g) (Mattia *et al.*, 2019). Moreover, *Lasius niger* has shown to contain comparable value of Ferric Reducing Antioxidant Power (FRAP) (mmol Fe^{2+}/100 g) and total polyphenols index (TPI) (452.00 mg GAE/100 g) to orange juice (0.94 mmol Fe^{2+}/100 g and 496.00 mg GAE/100 g, respectively). According to Hall *et al.* (2018), the antioxidant capacity of the insects might be associated with the presence of high proteins content. On the other hand, the liposoluble extracts of *Lasius niger* showed to contain 0.018 mmol TE/100 g of TEAC, which is lower than olive oil as reference (0.063 mmol TE/100 g) (Mattia *et al.*, 2019). Furthermore, the dietary behavior of insects directly influences their antioxidant capacity, whereby, insects (i.e. crickets, grasshoppers, silkworm, and caterpillars) with vegetarian diet are known to contain high antioxidant capacity. In another study by Ramakrishnan and Selvaraju (2020), they investigate the antioxidant activity of black ant (*Camponotus compresses*). The authors observed that the black ant extracted using ethanol as solvent at concentration of 4 mg/mL presented a maximum level (89.24%) in inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. This indicates that the extract of *Camponotus compresses* has ability in donating protons, thus could scavenge the free radical released by DPPH (Ramakrishnan and Selvaraju, 2020). The antioxidant properties of *Camponotus compresses* may be due to the presence of bioactive peptides (Pradeep *et al.*, 2012). It can be concluded that *Camponotus compresses* have an excellent potential to be used as therapeutic agents due to their antioxidant properties and capability in scavenging free radicals. Raza *et al.* (2022) reported that the adult weaver ant (*Oecophylla smaragdina*) has higher flavonoids content, ABTS radical scavenging activity, and ferric reducing power but lower in phenolics contetn and DPPH radical scavenging activity than its brood.

Recently, chemical bioactive compounds extracted from insects (particularly black ants) have received great attention from the food and pharmaceutical industries. Most studies have focused on Chinese black ants (*Polyrhachis dives* Smith/*Polyrhachis vicina* Roger) because these ants are considered among the most auspicious for potential use in pharmaceutical industry (Zhang *et al.*, 2022) and commercial production. It has been recorded that the Chinese black ants to contain anti-inflammatory properties, and renoprotective and immunosuppressivea activities (Su *et al.*, 2018; Li *et al.*, 2020) due to the presence of bioactive compounds (60 bioactive components are identified in the extract of *Polyrhachis vicina* Roger) (Zhang *et al.*, 2022). According to Ayieko *et al.* (2012), black ant Smith contains a variety of essential nutrients that believes able to treat several body ailments, whereby, it has properties similar to the ant species that has been employed as a traditional Chinese remedy (*Polyrhachis vicina* Roger) for thousands of years in China for treating several diseases such as tuberculosis, nervous breakdown, hepatitis B, and relief of menopause syndrome. However, the bioactive components and biological activities of the black ant Smith (species *Carebara vidua*) that are responsible for health-promoting effects are largely unexplored and require to be studied further to investigate their biological properties, which are important to provide information for the development of healthcare promoting foods.

The extraction of bioactive peptides from the source of insects had gained interest among the scientific community, food manufacturers, pharmaceutical, and cosmetic industries due to them possessing a positive effect on human health (Ho *et al.*, 2022a). In general, bioactive peptides are fragments of a specific protein that consist of two to twenty amino acids (Sanchez and Vazquez, 2017). Peptides derived from the source of insects have been revealed to demonstrate a large spectrum of bioactivities for maintaining the functions of immune, gastrointestinal, cardiovascular, and endocrine systems (Quah *et al.*, 2023). Earlier, a study done by Padmanabhan *et al.* (2012) demonstrates that the bioactive peptides extracted from *Camponotus compressus* ant has shown potential in treating diabetes mellitus. Further, Krishnan *et al.* (2012) reported that the bioactive peptides from *Camponotus compressus* ant act as insulin-like in decreasing the blood sugar level of an alloxan-induced diabetic albino mice model.

The bioactive peptides can be obtained through enzyme hydrolysis, whereby a small peptide and free amino acid can be obtained through cleaving of proteins during hydrolysis. This fine and free amino acid's structure could improve the functional properties of protein (i.e. viscosity, emulsifying, solubility, and foaming characteristics) (Tang *et al.*, 2018). Several edible insects from the order of Blattodea, Coleoptera, Isoptera, Lepidoptera, and Orthoptera had been used for bioactive peptide production. For example, *Bombyx mori* (silkworm and silkworm pupae), *Spodoptera littoralis* (African cotton leafworm) and *Tenebrio molitor* (mealworm larvae) have been identified for their bioactive peptides that obtained via protein hydrolysate (Nongonierma and FitzGerald, 2017). However, the bioactive peptides from *Carebara vidua* have not been extracted till date. Therefore, a study on determination of amino acid profile (i.e. essential and no-essential amino acid) of *Carebara vidua* should be conducted prior to extract bioactive peptides and determination of activity of bioactive peptides.

Ants are known to contain toxic compounds that can cause severe pain, vomiting, nausea, cardiac arrhythmias, and diaphoresis in humans as a result of envenomation caused by the ants (Agarwal *et al.*, 2022). According to Tani *et al.* (2019), venom of ants is composed of >75 different chemical compounds, including enzymes (venom dipeptidyl peptidases, hyaluronidase, phospholipase, and, etc.), alkaloids (solenopsin), proteins, biogenic amines (tryramine and histamine), pilosulins, formic acid, and hydrocarbonds. Alkaloids have been reported in several lineages from pygidial, mandibular, and venom gland secretions (Fox and Adams, 2022). Earlier, Chen *et al.* (2016) identified seven defensive chemical compounds (i.e. neocembrene ((E,E,E)-1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene, N-methylenedecan-1-amine, 9-decenyl-1-amine, N-methylenedodecan-1-amine, N-methyl-2-(hex-5-enyl)-5-nonanyl-1-pyrrolidine, ß-springene ((E,E)-7,11,15-trimethyl-3-methylene-1,6,10,14-hexadecatetraene), and 2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline)) that present in the venom (defensive secretion) of little black ants (*Monomorium minimum*). Furthermore, several chemical compounds of venom extracted from ants have been identified to have therapeutic potential. For example, the venom of ants such as *Pachycondyla senaarensis* and *Polyrhachis Lamellidens* have pharmacological properties including anti-inflammatory property, anti-cancer, and ability to heal damaged tissues (Agarwal *et al.*, 2022). Giant ant (*Dinoponera quadriceps*) was found to have anti-platelet, anti-coagulant properties (Madeira *et al.*, 2015). In addition, ants are commonly used as active ingredients in several tonics such as for kidney tonifying (Tang *et al.*, 2015). Tang *et al.* (2015) isolated 13 non-peptide nitrogen compounds, whereby these substances are alkaloids and some of them possess pyridine moiety. The peptides of dinoponeratoxins (M-PONTX-Dq3a and -Dq4e) of the giant ant showed effectively suppressed the developmental forms of *Trypanosoma cruz*i, including the major form for the maintenance of infection on chronic stage of the disease (amastigotes form). Ebaid *et al.* (2014) has proven that the venom extracted from gland of Samsum ant (*Pachycondyla senaarensis*) showed anti-neoplastic activity by protecting against carbon tetrachloride (CCL_4)-induced nephrotoxicity in mice model. Furthermore, in the study by Al-Tamimi *et al.* (2018), venom extract of Samsun ant has been found to be effective against breast and liver cancer cell lines-induced in mice models. Whereby, a significant decrease in transcription factors and key cytokines has been observed (Al-Tamimi *et al.*, 2018).

Ants are insects that can produce biocompounds with antimicrobial properties. Furthermore, carboxylic acids, fatty acids, and phenolic compounds are identified as the chemical compounds secreted from metapleural glands of ants (Evana *et al.*, 2019). According to Wu *et al.* (2018), insects are rich in antimicrobial peptides (AMP), a compound that functions to build the immune system. The inducible antibacterial peptides, known as insect defensins isolated from insect order of Hymenoptera (i.e. ants) show to have antibacterial properties in anti-Gram-positive bacteria (i.e. *Staphylococcus aureus*) (Wu *et al.*, 2018). Furthermore, Solenopsis and Monomorium alkaloids have been tested to have antimicrobial activity (Fox and Adams, 2022). Several studies have shown the extracts of ants effectively inhibit the growth of pathogenic bacteria. For example, Yamuna and Raja (2019) reported that the solvent extracts of ant (*Camponotus compressus*) effectively suppress the growth of pathogenic bacterial (i.e. *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). Padmanabhan *et al.* (2012) reported that the hemolymph of ant (*Camponotus compressus*) showed strong activity against the susceptible strains *Kleibsiella pneumonia* and *Staphylococcus aureus*. In another study by Lima *et al.* (2018) showed the crude venom of giant ants has antitrypanosomal activity which is effective against parasites (*Trypanosoma cruz*i). Vidhu and Evans (2015) found the venom extracted from the abdominal gland of adult worker ants *Oecophylla smaragdina* showed antimicrobial activity to inhibit the bacterial and fungal strains.

8 Risks associated with consuming black ant Smith (*Carebara vidua*)

Edible insects including black ant Smith are viewed as a favorable substitute for food resources which are beneficial towards human nutrition, nation's economy and environment. However, the food safety concern of edible insect utilization is still an ongoing debate due to the limitation of research. In developed countries, there is a growing concern about food safety as the ingestion of edible insects is beginning to be accepted by the consumers as an alternative protein source (Soggari *et al.*, 2018; Wilkinson *et al.*, 2018). Endogenous risk factors associated with edible insect consumption comprise chemical hazards (including allergens), antinutri-

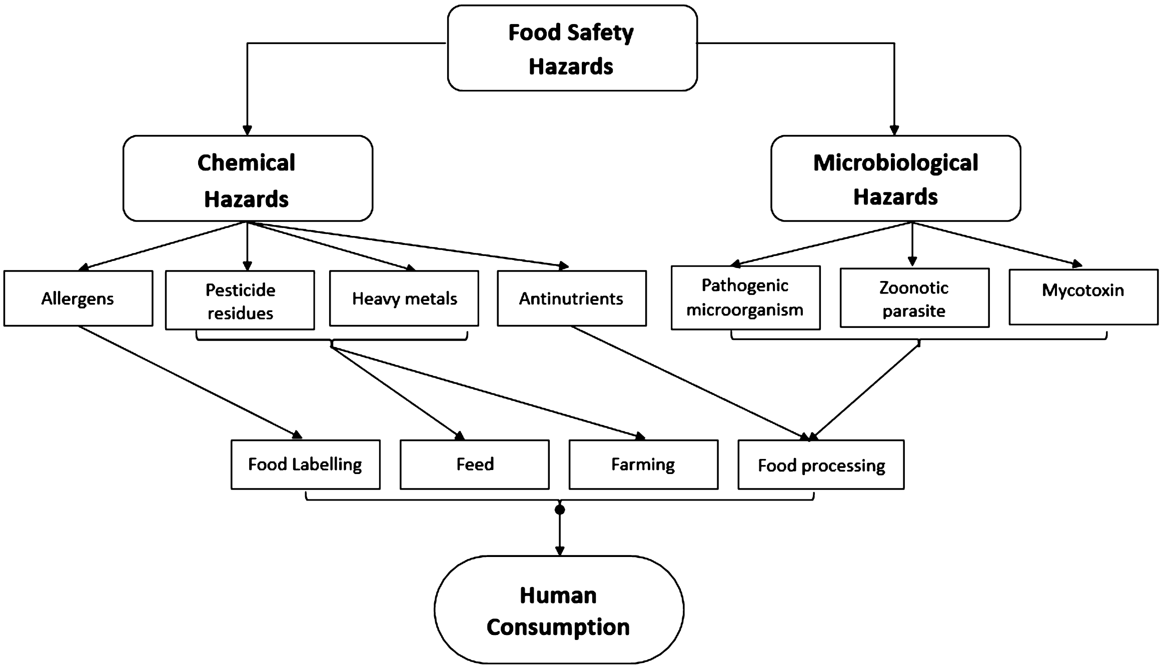

FIGURE 11 Strategies to reduce potential food safety hazards in consumption of *Carebara vidua*.

ents, and microbiological hazard (Aguilar-Toalá *et al.*, 2022) as summarised in Figure 11.

Microbiological hazards

Microbiology hazards that are associated with edible insects either raw or processed products, became a crucial focal point and food safety concern among the researchers. According to Rumpold and Schlüter (2013a), edible insects can naturally harbor spoilage and pathogenic microorganisms as symbiotic and gut-associated bacteria. Further microbial contamination may occur due to the harvesting of insects from the wild or farm, food processing handling, and hygiene practices. Osimani *et al.* (2017) reported that several spore-forming bacteria were detected in edible insect samples. For example, *Bacillus cereus* and *Clostridium thermopalmarium* were found in cricket powder. Whereas, *Bacillus weihenstephanensis* and *Bacillus subtilis are determined* in dried small crickets. On the other hand, in Uganda, bacteria such as *Bacillus* spp., *Campylobacter* spp., *Clostridium* spp., *Neisseria* spp., *Pseudomonas* spp., and *Staphylococcus* spp. were present in fresh grasshoppers which are responsible for foodborne illness. Moreover, the finding from a microbial analysis conducted on fresh, processed and stored edible insects revealed the existence of spore-forming bacteria that remained viable even after boiling. Besides, scientific literature has recorded cases of microbiological foodborne illnesses and intoxications originating from entomophagy, indicating the necessity of fostering effective good hygiene practises along the full value chain of edible insect foods to preserve the health of entomophagists (Garofalo *et al.*, 2017).

A zoonotic parasite called *Dicrocoelium dendriticum* is easily transferred from animals to humans by eating edible insects like ants (Boye, 2012). Metacercariae-containing ants are the source of the infection, whereas infected animal liver is the source of pseudo-infections which indicates the presence of *Dicrocoelium dendriticum* eggs in faeces without adult worms (Belluco *et al.*, 2018). In a well-run closed farm setting, there would not be any hosts left over to allow parasite life cycles to be fully completed. In any situation, but especially when it comes to species caught outdoors insects as opposed to those raised in farms, adequate handling before eating, relying on freezing and boiling, might reduce dangers (Belluco *et al.*, 2018; Dou *et al.*, 2019).

Papastavropoulou *et al.* (2022) revealed that mycotoxins are probably present in the substrates that edible insects feed on. To counter threats from ants and bees, certain edible bug species do, nevertheless, create poisons or other dangerous materials. Scientific research has shown that by adding sorbent materials, enzymes, or microorganisms in insects' feeds can detoxify mycotoxins effectively (Jard *et al.*, 2011). Insects would be regulated similarly to other foods of animal origin under the Codex Alimentarius standards of food hygiene since the need to ensure food security and safety is unbreakable (Hardy *et al.*, 2015). Beetles (31%), caterpillars (18%), bees, wasps followed by ants (14%) are the most frequently ingested insects by people as reported by van Huis (2013). Therefore, microbiological contamination and toxicological risks, which include chemical risks and antinutrients, should be taken into account to assure safety.

As a mitigation strategy, it is crucial to implement the food preservation techniques such as drying, thermal treatment and acidification are managed the proliferation and elimination of spoilage and pathogenic microorganisms in order to ensure the food safety and also prolong the shel-life of edible insect products. Acosta-Estrada *et al.* (2021) revealed that the application of hurdle technology in processing and production of insect-based products will retard the microbial growth. For instance, the combination of treatments like 30 minutes boiling, followed with 12 hours drying at 80 °C and 5 minutes boiling, then with 24 hours drying at 55 °C demonstrated a superior efficiency in terms of inactivating and reducing the spoilage microbial species including yeast, mold, staphylococci and enterobacteria that presence in *Tenebrio molitor* (Acosta-Estrada *et al.*, 2021). Moreover, the same author pointed out that the application of freeze-drying also one of the effective preservative techniques to stop the proliferation of microbial load as well as retains the nutrients of the edible insects (Acosta-Estrada *et al.*, 2021). In addition, lactic fermentation applied to composite flour and water mixtures, which incorporated with 10% of powdered roasted mealworm larvae, not only achieved successful acidification but also exhibited its effectiveness in extending the product's shelf life (van Huis, 2016). Hence, managing stringent hygiene practices throughout the cultivation, processing and storage of insects and its products may minimise risk of both cross-contamination and post-contamination by microbes.

Chemical hazards

The primary focuses of chemical hazards that are related to edible insect consumption are food allergens, heavy metals and pesticide residues. A food allergy is a negative immunological reaction to food that is brought on by allergens (a type of antigen), which can cause serious disease, sometimes even death. Since proteins compose the majority of edible insects, it is possible that some insects and foods produced from insects could be sources of allergies. Some types of protein that are found in edible insects namely α-amylase, arginine kinase, and tropomyosine are considered as allergens which are able to evoke adverse immune responses and severe allergic related health issues (Lange and Nakamura, 2021). Francis *et al.* (2019) reported that the genetic similarities between insects and crustaceans are higher possible to cause food allergies. In a clinical study performed by Sun *et al.* (2013) demonstrated that most adult individuals are able to consume about 15-20 g powder of *Polyarchies vicina* (Chinese black ants) without any adverse effects. However, few individuals with allergies faced certain allergic temporary symptoms such as diarrhea, skin sensitivity, and abdominal swelling which resolved within a couple of days after discontinued the powder. Furthermore, the consumption of edible insects has caused 18% of food-related deaths in China and 7.6% of allergic reactions in Lao (Barennes *et al.*, 2015). In Thailand, a survey conducted by Taylor and Wang (2018); approximately 2,500 respondents were participated to investigate the prevalence of allergic reactions associated with the consumption of edible insects. The survey findings revealed that 7.4% of respondents had an allergy to edible insects with adverse reactions. While, 14.7% of respondents experienced multiple reactions, indicating potential insect allergies (Taylor and Wang, 2018). Based on the survey responses, water bugs exhibited the highest allergic reaction followed by, scorpions, grasshopper, crickets, bamboo worms, red ants, silkworms, and red ant eggs which showed lowest allergic reaction (Taylor and Wang, 2018). Interestingly, notable differences were observed in post-consumption symptoms of edible insects between males and females. Furthermore, Taylor and Wang (2018) also reported that male respondents were more vulnerable to report gastrointestinal problems like diarrhea or vomiting, whereas female respondents were more prone to symptoms such as nausea and dizziness. Thus, physiological reactions to insect consumption differed significantly between males and females. In addition, larvae's body hair had negatively impacted the palatability of the larvae and the ingestion of its hair highly potential to release toxic secretion from the poison glands which lead to cryptotoxicity and haemorrhage (Gahukar, 2020). In addition, problems with allergenicity linked to various exposures, such as injection, ingestion, inhalation, and skin contact, should be taken into account (Raheem *et al.*, 2019b). As a mitigation strategy, it is crucial to implement effective product labelling practices for insect-based food products in order to inform potential consumers about the allergens and other ingredients that are contained in the products. Pali-Schöll *et al.* (2019) revealed that the Austrian Federal Ministry of Health and Women's Affairs strongly advised to implement labelling for edible insects and its product to alert the risk individuals from the adverse allergic reaction.

Furthermore, the exposure of pesticides residues is one of the greatest threats to the edible insect population when it is collected from the wild rather than from farms. This is because the wild-collected edible insects are free to migrate with uncontrolled diet sources. Wild-collected insects may ingest pesticide-sprayed crops that result in the accumulation of potential pesticides residues in their bodies (Gao *et al.*, 2013; Fraqueza and Patarata, 2017). Besides, accumulation of insecticides namely pirimiphos-methyl, chlorpyrifos and chlorpyrifos-methyl were determined in food that incorporated with black soldier fly larvae (Papastavropoulou *et al.*, 2022). Moreover, dioxins, especially polychlorinated biphenyls (PCBs) content have been detected in some edible insects in which their concentrations are in compliance with regulations set by the European Union (EU) (Papastavropoulou *et al.*, 2022). In Thailand, a food poisoning case was reported due to consumption of insects which were contaminated with pesticide residue (Lange and Nakamura, 2021). Consuming contaminated edible insects could lead to health issues like neurological disorders, diabetes, respiratory problems, cancer, oxidative stress and reproductive issues stress (Aguilar-Toalá *et al.*, 2022; Rani *et al.*, 2021). Although directly linking pesticide exposure to health problems is challenging, the seriousness depends on pesticide levels, detoxification ability, and consumer responses (Imathiu, 2020). Therefore, it is pivotal to implement and initiate insect farming to collect and produce insects free from pesticides residue.

In addition, heavy metals are trace elements namely lead, arsenic, cadmium, and mercury which are considered as chemical hazards related to edible insect consumption. According to Poma *et al.* (2017), edible insects have lower levels of heavy metals, dioxins and DDT (Dichlorodiphenyltrichloroethane), than chicken eggs, fish, and animal flesh. However, the minimal exposure of heavy metals may cause toxicity (Jan *et al.*, 2015; Cito *et al.*, 2017). The existence of heavy metals within edible insects mainly due to the species of the edible insects, the insects' life cycle, diet sources, and food processing (Papastavropoulou *et al.*, 2022; van Huis and Rumpold, 2023). Moreover, black soldier flies, *Gryllus assimilis* (cricket), and *Tenebrio molitor* (mealworm) are recognised as widely used food sources in western countries. Notably, cadmium detected in both black soldier flies and cricket while arsenic detected in mealworm (van der Fels-Klerx *et al.*, 2018; Papastavropoulou *et al.*, 2022). Köhler *et al.* (2019) demonstrated that Bombay locust, scarab beetle, house cricket, and mulberry silkworm are prevalent in Thailand in which lead, mercury, arsenic, and cadmium content were detected by using atomic absorption spectrometers. Fortunately, the heavy metal contents are below the maximum level which is safe for human consumption and animal feed (Köhler *et al.*, 2019). Idowu *et al.* (2014) reported that chromium and copper content were detected in *Macrotermes bellicosus* within the acceptable limits of 0.226 mg/L and 0.0076 mg/L, respectively. Therefore, it is pivotal to implement appropriate insect feeding practices in insect farming. This is because the utilization of contaminated soil, feed and soil may introduce the heavy metals into the edible insect's food chain (Fernandez-Cassi *et al.*, 2019). The insect-based processors and producers are important to assess the heavy metal analysis to ensure the safety and quality of its products and to safeguard against potential health risks for consumers. As a conclusion, it is crucial to employ and comply the Hazard Analysis and Critical Control Points (HACCP) system, pre-requite programs like Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) which embraced by commercial producers of edible insects and companies involved in the development of insect-based food items in order to inimize the chemical and biological contamination throughout the food production process.

Anti-nutrients factors

Though edible black ants have a high potential to be used as a therapeutic agent for the treatment of several diseases due to their nutritional values (particular high protein) but their anti-nutrients are relatively high (Abulude and Fagbayide, 2017). Anti-nutrients can affect the absorption of the nutrients such as protein and minerals (i.e. calcium, iron, and magnesium) from foods by reducing their level in foods (Chakravorty *et al.*, 2016; Giampieri *et al.*, 2022). This indicates that some of the nutrients might not be able to absorb in the body, hence not be available for the consumers. Abulude and Fagbayide (2017) reported the black carpenter ant to contain anti-nutrient compositions of oxalate (0.14 mg/100 g), phytate (6.28 mg/100 g), and tannins (0.07 mg/100 g). In comparison with an edible red ant, the black carpenter ant has a relatively lower anti-nutrients properties, whereby, an edible red ant is comprised of anti-nutrients of oxalate (3.79 mg/100 g), phytate (19.67 mg/100 g), and tannins (109.56 mg tannic acid equivalent/100 g) (Sailo *et al.*, 2020), which can cause reducing in nutrient bioavailability via formation of indigestible complexes with proteins (Chakravorty *et al.*, 2016). Several anti-nutritional factors such as tannin

(496.67 mg/100 g) and phytic acid (171.0 mg/100 g) are found in weaver ant (*Oecophylla smaragdina*).

According to Samtiya *et al.* (2020), the presence of anti-nutrients not only decreases the bioavailability of nutrients, but also can become toxic when present in large amount and harmful effects to human health (Zhou *et al.*, 2022). In most cases, the authors found that the level of anti-nutrient factors are far below the accepted levels established for human consumption. Anti-nutrient factors of an edible insect below 0.52% can be considered as a non-toxic concentration (Shantibala *et al.*, 2014). Although those studies have reported that these compounds are present in low concentrations or that some processing methods can decrease their content, consequently their presence should not be entirely overlooked (Aguilar-Toalá *et al.*, 2022). Moreover, to best of our knowledge, there are only few studies that have described the presence of anti-nutrient factors in edible insects. Particularly, the anti-nutrient factors that might be present in black ant Smith (*Carebara vidua*) are still limited and yet to be explored. Hence, the concentrations of anti-nutrient in edible black ants are necessary to be deeply investigated before consuming to prevent toxicity and other related health problems resulting from anti-nutrient factors.

Regulatory aspects of black ant Smith (Carebara vidua) *consumption*

Novel foods including edible insects are categorised as food or ingredients that had not been consumed by humans within the European Union before May 15th, 1997, as specified by European Union legislation (Pali-Schöll *et al.*, 2019; Imathiu, 2020). Insects are intriguing as ingredients in food and feed because they are resource-efficient, have a circular economy, and can combat climate change (Gasco *et al.*, 2020; Aidoo *et al.*, 2023). The antiquated food and feed rules that cover the usage of insects are a hindrance to this industry's expansion. Due to consumers' growing awareness of their rights to high-quality and safe food, many consumers' consumption habits have significantly changed recently. Accordingly, authorised parties in both developed and undeveloped countries should take responsibility to establish safety laws and regulation on edible insects including ants (Lähteenmäki-Uutela *et al.*, 2021). This is crucial to protect and minimise the risk supply of this food, from the production stage to consumption. In addition, it is certainly applying to edible ants and their food products, particularly for Western consumers who tend to exhibit greater reluctance towards practicing entomophagy (Dobermann *et al.*, 2017; Gasca-Alvarez and Costa-Neto, 2022). Van Huis (2016) reported that formal approval for the production and consumption of specific edible insect species has been obtained from various European countries such as the Netherlands, Belgium, and Switzerland.

Furthermore, in Belgium, the Scientific Committee of the Federal Agency for the Safety of the Food Chain reached an agreement that farmed edible insects are recognised as safe for human consumption. In addition, prior processing including a heating step is required for the edible insect before the market entry to ensure food safety and suitable for human consumption (Acosta-Estrada *et al.*, 2021). Besides, in the United States, edible insects are allowed to sell legally as a food source for human consumption. It is crucial to establish dedicated production lines, comply with the current good manufacturing practices, process the products under sanitary conditions and appropriately labelled in accordance with Food and Drug Administration (FDA) regulations (van Huis, 2016). Consumers in developed countries may be concerned about the safety of edible ants produced there due to lax or absent production, handling, and processing regulations. Concerns about food safety in well-regulated Western markets pose a significant challenge for edible ant producers in developing countries. Even if there are any potential markets in these regions, making a livelihood through exporting this commodity would be difficult. Żuk-Gołaszewska *et al.* (2022) revealed that limitation in commercialization and development of edible ants and their products in Europe due to the strict regulations in developed nations. Given these findings, it is urgently necessary to design and enforce harmonised edible insect food safety laws, especially in developing nations, to encourage the safe use of this product from farm to fork (Shelomi, 2015).

Based on Regulation (EU) No 2015/2283, an increasingly wide range of novel foods are considered to be included in the Union list of authorized Novel foods. However, until 2018 none of the edible insects have been included in the Union novel food list. But with substantiating data to back the safety and nutritional aspects of other edible insects like *Carebara vidua*, this is likely to change. These changes will inspire new policies and regulations that can be set in place to establish an effective value-chain from foraging and rearing to processing and sales. Additionally, some EU countries have their own food safety authorities to legislate regulations that can promote the trade of edible insects for human consumption, e.g. Austria, Belgium, Denmark, Netherlands and the UK. This can in turn provide models to other countries to develop and enforce policies and regulations, leading to partnership programs creating networks that can increase the economic viability at growing scales.

Relevant standards need to be created to support international trade aimed at establishing consistent value chains. The inclusion of *Carebara vidua* in the Codex Alimentarius would be a positive step towards international industrial standards, allowing member nations to build their own supplementary standards. In order to accomplish this, emphasis should be made on developing data collection approaches and procedures. Statistical data on safety and nutritional aspects of *Carebara vidua* is limited; worldwide, regional, and national overviews of monetary contribution to domestic and international trade are mostly unknown. The dearth of academicals, industrial and commercial information on this edible ant needs to be addressed to develop standards, which can be particularly challenging on a global level.

GREEiNSECT is a consortium of public and private institutions investigating how insects can be utilized as novel and supplementary sources of protein by means of mass production in small- to large-scale industries in Kenya. GREEiNSECT is funded for four years by the Danish International Development Agency (DANIDA). The consortium is organized through work packages addressing technological adoption, investigation of operational and implementable business models; development of institutional frameworks necessary for managing the risks related to mass rearing systems, and international trade and food security standards; modeling and assessing contribution of insect production systems to green economic growth and nutrition security. Exploring economic and political incentives for the development of climate-friendly food and feed sector, and capacity building of Kenyan research institutions. Dissemination of knowledge gained and development of a Kenya-based knowledge platform involving public and private sectors.

Furthermore, The Kenya Bureau of Standards has approved three national standards which are (1) Edible insects' products specification, (2) Products containing edible insects' specification, and (3) Production and handling of insects for food and feed (code of practice) to guide the primary production of edible insects. Specifically, this standard focused the food safety, quality, insect farming practices, environmental concerns, product labelling and packaging (Acosta-Estrada *et al.*, 2021). Moreover, in Thailand, the economic insect farmers are encouraged to register under the Department of Agricultural Extension (DOAE) which enables the government to maintain a database for strategic planning to promote, develop production and marketing the edible insects (Krongdang *et al.*, 2023). Hence, the policy of Thailand government consistently supports the economic farmers to industrialize the edible insects throughout the global market. Additionally, in both Australia and New Zealand, edible insect products are regulated under the Food Standard Code (1.5.1) categorized as novel food. Before introducing an edible insect food product in Australia and New Zealand, a comprehensive risk-based assessment must be conducted. In this evaluation, a few factors need to be considered including the product's chemistry, consumption pattern, and toxicological and nutritional aspects (Lähteenmäki-Uutela *et al.*, 2021). Furthermore, a strategic partnership between the Centre of Insect Physiology and Ecology (ICIPE) and the African Organization for Standardisation (ARSO) is established to develop and harmonize standards for edible insect-based food products in Africa. This collaboration also contributes to the continuous growth of insect farming, consumption, and high-value-added product development (Tanga and Ekesi, 2024). Therefore, these diverse policy regulations in various countries are collectively contributed to fostering economic growth and industrializing the food products derived from edible insects such as black ant Smith for human consumption.

9 Environmental benefits of consuming black ant Smith (*Carebara vidua*)

The cultivation of edible insects is a unique and viable approach to livestock production and has myriad advantages; the main one is it being a source of nutrition and essential protein for people plagued with food insecurity (Aiking and de Boer, 2019; Tanga *et al.*, 2021; van Huis *et al.*, 2021). As an environment-friendly option; the level of greenhouse gases (GHG) that insect farming emits is lower when compared with conventional livestock farming modalities. Meat shipping, slaughter, and storage contribute 17-25% of GHG (Oonincx and de Boer, 2012). Moreover, insect farming has the capacity to generate food that matches the nutritional value of traditional livestock while demanding fewer resources such as water and land. These characteristics make insect farming a climate-smart, forward-thinking, and sustainable agricultural practice (Lipper *et al.*, 2014; Alexander *et al.*, 2017).

In a more material sense, specifically in rural Africa, insect farming can create employment opportunities and sources of income for families engaged in this practice (Huis *et al.*, 2013). In spite of these benefits, harvesting of insects occurs mainly from the wilderness and consumption is sporadic and limited to specific seasons (Parodi *et al.*, 2018). However, wild harvesting creates biodiversity concerns in the form of overconsumption of specific insect species. To counter these issues, farming insects has emerged as a feasible solution, encouraging insect consumption through sustainable farming methods. Additionally, the global push for nutritious and eco-friendly food production also underscores insect farming's potential as an innovative avenue for animal-derived food production.

Raising any species of animals in a manner that is sustainable and with minimal environmental impact necessitates the careful consideration of the species' natural behaviors, habitat requirements, life cycle, mating behaviors, and optimal conditions for reproduction. Literature surrounding *Carebara vidua* is still limited, but there are instances of other edible insects, specifically other ants, being reared; therefore, using some methods used for the rearing of these ants can be helpful.

The first step is to ensure that the collection of *Carebara vidua* is done in an ethical manner, following local regulations and guidelines if any are prescribed, and in a way that doesn't cause harm to the natural population and ecosystem balance. Research should be done on their natural habitat – the types of vegetation, substrate, humidity levels, and microclimates they thrive in. Natural vertical elements like branches, twigs, or leaves simulate the verticality of their natural habitat and encourage movement. Appropriate temperature and humidity levels can be maintained using misting systems, controlled ventilation, and heat sources to create a stable microclimate. Moisture beyond a point can prove detrimental to these ants, so water needs to be let into the engineered environment slowly and in a way that doesn't lead to water wastage. It is important to note that rearing these ants requires less water than is required for rearing traditional livestock. *Carebara vidua* are omnivores, and they can be fed dead insects, vegetable matter from kitchen waste, composted organic waste, etc., thereby ensuring a circular economy. Environmental management methods should include using narrow-spectrum pesticides as an alternate component for agrochemicals (Ayieko *et al.*, 2012).

Potential of using edible black ant Smith (Carebara vidua) *to address global food insecurity*

Around 2 billion people are affected by the rapid rise in food insecurity worldwide, with the COVID-19 pandemic expected to add an additional 83-132 million people (Alhujaili *et al.*, 2023). Food insecurity will be increased further by global population growth, which is expected to increase from 7.7 billion in 2019 to 9.7 billion in 2050 (Alhujaili *et al.*, 2023). Fortunately, the market for edible insects is predicted to reach more than US $522 million by 2023, and entomophagy, particularly for humans, has significantly advanced thanks to productive research projects (Han *et al.*, 2017; Kim *et al.*, 2019). Substituting livestock production with insect production holds potential for addressing several critical challenges within the global food system, particularly in terms of land utilization and resource efficiency (Alhujaili *et al.*, 2023). This strategic reallocation of resources can contribute to alleviating food insecurity and enhancing overall food availability, especially in regions where resource constraints are a pressing concern. However, the viability of edible insects as food in the long-term is dependent on a variety of factors, including the species that are being utilised, the type of feed required, and the amount of energy expended while generating insect-based goods (Dagevos, 2021) Furthermore, insect production, like other novel foods, requires creating new value chains while also taking into consideration concerns such as customer acceptance, food safety, and production costs (van Huis, 2013; Henchion *et al.*, 2017; Cadinu *et al.*, 2020).

Carebara vidua's unique food value makes it the most sought-after edible insect in the Luo community. However, the opportunity cost of selling *Carebara vidua* or Onyoso (local name) is very high and therefore, collectors of this insect have been known to keep the catch for personal consumption that to sell it cheaply in their markets. The fact that the catch is often too small to sell it profitably compared to other edible insects like lake flies and elate termites and also the purported medicinal of *Carebara vidua* makes the locals prefer self-consumption than to take to open markets (Ayieko *et al.*, 2012).

The most pressing environmental issues surrounding edible insects are the right and ability to farm insects for commercial reasons, as well as where to cultivate them (Niassy *et al.*, 2022). As insects are considered wildlife, they are included as natural resources in the legislation of many countries, mandating the obtaining of permission and/or license from competent authorities before exploitation (Grabowski *et al.*, 2020). Grabowski *et al.* (2020) have found that except for Botswana, edible insects are not listed in national rules, which creates a complicated, nation-specific scenario about which insects may be utilised lawfully and for what purpose. In terms of collecting wild insects, several nations, notably those in East Africa, already have strong laws in place that might be extended to insects (Grabowski *et al.*, 2020).

Aside from overexploitation and insects being characterised as pests, the right taxonomic identification of insects to be farmed is an essential safety matter that requires legislative attention (Grabowski *et al.*, 2020). The number of edible insect species is a small percentage of the overall number of insect species. Many communities consider eating insects as part of their cultural history, and such cultures have established a profound understanding of desirable insects over many generations of insect-eating. Because new edible species are often reported, current lists of edible insects are not thorough. Although collectors, foragers, and gatherers identify insects based on scent, sound, season, and host plant (Bomolo *et al.*, 2019), national catalogues and criteria for identifying insects for food and/or cultivation are vital. To avoid misidentification (since certain insects can be dangerous) and overexploitation, developing profiles of insects that could be ingested and mass-reared is essential.

Environmental impact of food production influencing **carebara vidua** *consumption*

Recent studies have highlighted the need to change our diets as the primary source of environmental deterioration is food systems (Clark *et al.*, 2018; Vega Mejía *et al.*, 2018; Willett *et al.*, 2019; Kim *et al.*, 2020). In 2019, food waste was 931 million tons, including food service (26%), households (61%), and retail (13%), accounting for 17% of the total global food production (Food Waste Index Report 2021, 2021).

Carebara vidua is presently facing extinction, threatening the potential of being an alternative solution to the food and hunger crisis (Ayieko *et al.*, 2012). Black ant population abundance has decreased significantly, and they are now scarce in all agroecological zones of Western Kenya. Understanding the variables controlling the abundance and distribution of *Carebara vidua* remains a difficulty since, in many parts of Kenya; the species is considered an endangered species.

In a study of different agroecological zones of Homabay, Kisumu, and Siaya counties (Ondede *et al.*, 2022), several causes were identified contributing to the shift in the distribution and population decline of black ants appearing over the lower midland regions, including climate change, land degradation, using advanced agricultural equipment, and habitat loss. Because of these conditions, predicting the emergence of the insect proved difficult. The distribution of *Carebara vidua* has changed over time over the lower midland zones, and the number of emerging insects has decreased, resulting in the insect's extinction.

In recent years, it has been reported that the population of black ant Smith (*Carebara vidua*) has declined significantly, especially in the agro-ecological zones of Western Kenya. Stress from biotic and abiotic stress are the factors that influence the population of the black ants Smith (Ondede *et al.*, 2022; Ondede, 2023). Predation, parasitism, competition among the insects for food, and human activities (i.e. urbanization, agriculture, industrialization, fragmentation, and deforestation) are the biotic factors that district the activities (building the mounds under the ground, rocks, and buildings) of subterranean ants (anthropogenic disturbance) and thus affecting their habitat (Buczkowski and Richmond, 2012; Johansson and Gibb, 2012; Soare *et al.*, 2014; Sample *et al.*, 2015; Cecilia and Fredrick, 2018; Leweri and Ojija, 2018; Paolini *et al.*, 2020; Elliott, 2022).

On the other hand, abiotic factors such as climate change/global warming may affect the period of metamorphism, multiplication, and survival rate of the ants (Berman and Zhigulskaya, 2012; Kiritani, 2013; Kadochová and Frouz, 2014; Khaliq *et al.*, 2014). Wildfires cause microclimatic changes in burnt regions by reducing moisture and increasing temperature. Additionally, fire alters soil characteristics, causing a significant loss of biomass above the ground, which contributes to GHG emissions (Silveira *et al.*, 2010). Anthropogenic activities such as these endanger the availability and safety of ants as food sources (Payne and Van Itterbeeck, 2017).

Moreover, since existing land cannot feed the rising population, competing uses of lands for agricultural commitments can lead to the destruction of ant hills. Salts and kerosene can be utilised to destroy the ant mounds, which can also lead to ant deaths through clogging of the soil's air gaps by kerosene (Ondede *et al.*, 2022). Study participants kept reiterating the importance of conserving ant hills as they understood that to be the most important habitat of these insects.

10 Cultural attitudes toward edible black ant Smith (*Carebara vidua*)

Upon investigation of differences between participant groups, significant differences were revealed that males and young people, participants living in cities and nations including Mexico, Poland, and Spain. Participants with greater incomes and educational levels were individuals with higher knowledge levels. Participants who ate insects out of curiosity, a desire to protect the environment, a desire to enjoy eating insects, and because of their nutritional value were those with relevant knowledge. These findings evidently demonstrate that a number of variables have a significant influence on people's knowledge of the sustainability of edible insects, which may be helpful in formulating strategies to spread awareness and eventually boost people's willingness to consider edible insects as a serious alternative to other protein foods, even in nations that are not accustomed to them (Guiné *et al*., 2022).

Attitudes in different regions of the world

The insect is found in Asian countries like India, Pakistan, Southern China, and Thailand, as well as Afrotropical countries such as Botswana, Eritrea, Kenya, Malawi, South Sudan, Sudan, and Zambia. Ants are the third most often consumed insect worldwide, particularly in the region of Latin America, where the larval or pupa stages are preferable for consumption (Raheem *et al*., 2019a). Thailand is one example of a country where insect eating is constantly rising. Thailand now consumes over 200 insect species. Several species are harvested from the wilderness and sold in local markets on a seasonal basis. Since the mid-1990s, numerous Thai regions have successfully grown house crickets and palm weevils. Nowadays, insect consumption has grown in popularity and has spread across the country. Insect consumption patterns are evolving, and the focus has lately turned to convenience and eating insects as snacks (Olsson *et al*., 2019). In Thailand marketplaces, the cost of insects is sometimes three to four times that of meat per kilogram. Surprisingly, despite the trend of high consumption growth, a considerable proportion of Thai people remain skeptical about insects as food due to revulsion and feared side effects or hazards.

As part of the novel foods' regulation, insects considered to be food must be authorised through a specific EU process. The legislation's goal is to safeguard customers from unforeseen dangers such as allergies, toxins, and diseases. However, some EU countries, including the Netherlands, Belgium, France, the United Kingdom, and Denmark, have interpreted the law more liberally, allowing for the sale and rearing of insects, with the most common form of sale is insects that are pulverised or sold as dried or freeze-dried products. Selling and breeding insects for human consumption is still illegal in Sweden (Olsson *et al*., 2019). According to the author, unfavorable impressions stem mostly from social/cultural construction and a lack of exposure to insects. Another cross-cultural qualitative research looked at the reasons for Thai and Dutch customers' acceptance and rejection of certain insects and insect-containing meals. The conflict here was between a culture in which insects feature often in the cuisine and one in which insects are typically not considered food (Olsson *et al*., 2019).

Many studies in India have reported on the entomotherapy practice of various indigenous communities in states like Arunachal Pradesh, Assam, Chhattisgarh, Kerala, Madhya Pradesh, Manipur, Nagaland, and Tamil Nadu. Furthermore, an estimated 15-20% of Ayurvedic material medica was derived from animal products, including insects. Many researchers have demonstrated that insects are employed in a variety of capacities among Indian ethnic tribes. Some insects are eaten and may have medicinal properties (Devi *et al*., 2023).

Role of cultural factors in black ant Smith* (Carebara vidua) *consumption

Nyberg *et al*. (2020) found that men displayed a lower fear of trying new things, such as eating insects, and a higher degree of curiosity and interest than women; hence, they were more inclined to engage in the study than women.

The study's middle-aged participants outnumbered the young by 58% to 42%. Herbert and Beacom (2021) Megan and Emma (2021) found that middle-aged and young people are adventurous, prefer experiencing new sensations, and have a positive attitude, therefore they are willing to try new things.

According to Payne *et al*. (2016) insects possess a high protein concentration when compared to other protein sources such as eggs, meat, and milk. Most families consume insects, including ng'wen (31%), onyoso (32%), onjiri (19%), oyala (1%), dede (10%), and sam (4%) (Ochieng *et al*., 2023). Furthermore, children are mostly involved in insect harvesting, reducing the parents' time spent on economic activities through insect gathering for the home, making insects even cheaper (Dürr and Ratompoarison, 2021).

Others, on the other hand, do not enjoy the taste of insects or do not like them in general. Potential consumers state that they dislike insects and insect-based meals and are consequently unmotivated to consume them. The dislike of insects contributes to the unacceptability of insect consumption. Neophobia, disgust towards eating insects, unfamiliarity, and concern for cleanliness, contamination, and safety of insects and insect-based meals are all typical contributors to a dislike for insects.

Another disadvantage of insect eating is its seasonality and limited distribution. Dürr and Ratompoarison (2021) found that many edible insects are seasonal. Nevertheless, the seasonality of insects does not imply that there is a period of the year when insects are unavailable; rather, different species can be found at various times throughout the year. Furthermore, despite the fact that insects are inexpensive and easily available, they are not in sufficient abundance to meet demand and are only found in certain places; therefore, their poor dispersion impacts their consumption.

Many religions, including Christianity, Islam, and Judaism, practice insect consumption. Christians are permitted to consume insects according to the Bible. The Bible stipulates in Leviticus that flying insects that walk on all fours cannot be ingested. It does, however, allow the ingestion of flying insects that walk on all fours, with jointed legs, and ground hops. Grasshoppers, locusts, crickets, and katydids are among the insects mentioned in Leviticus 9: 20-25. The New Testament identifies locusts as Saint John's protein source (Mark 1:6) (Ochieng *et al.*, 2023).

Consumption of insects is regarded normal for certain people and prohibited for others. Some societies, such as Western cultures, forbid insect consumption, especially small-sized termites, which might cause deafness. The fear of insects stems from the perception that insects are unpleasant and destructive, causing problems such as disruptions, infections, and injury. Other attitudes surrounding insect consumption include the notion that it is a poor man's diet, making it inappropriate to consume them (McDade and Collins, 2019).

Individuals with a high level of income showed lower levels of neophobia than those with a low level of income. Individuals with high incomes are exposed to a wide range of cultures; therefore, their growth and understanding of a variety of stimuli, including foods, leads to a low degree of insect phobia and consumption.

Some survey participants claimed that they were aware of numerous cultural ideas and values that forbade the intake of insects, while others claimed they were unaware of any cultural beliefs that forbade the consumption of insects. Religious practices of respondents were not identified as a role in the restricted intake of insects, in addition to cultural views (Durst and Hanboonsong, 2015; Cicatiello *et al.*, 2016). As a result, the behavior was acceptable in the various families based on religious beliefs and a lack of understanding of any cultural customs that prevented the consumption of insects. Furthermore, taboos played a considerable impact on the pattern or rate of insect consumption.

Finally, the findings revealed that societal variables influenced insect consumption in households. Insect consumption was impacted by age, education, and family size (Ochieng *et al.*, 2023). On the contrary, Oyaro *et al.* (2022) discovered that age played an essential role in impacting insect consumption and insect-based meals.

In comparison to the current study, Oyaro *et al.* (2022) found that social factors such as education and family size had no effect on the acceptability of insect consumption. The degree of education achieved and the number of family members in a home had no effect on the consumption of insects or quantity consumed. However, this conclusion contradicts previous research by Mwiinga *et al.* (2022) which found that the level of education was highly related to household insect consumption.

Because of the growing problem of food insecurity in many homes, people must accept and embrace insect consumption. To do this, it is critical to focus on the cultural, economic, and societal reasons linked with low acceptance of insect consumption. First, it was suggested that farmers, regardless of land size, be encouraged to breed and cultivate insects (Ochieng *et al.*, 2023). Insect breeding and farming do not require a large space and are not expensive. As a result, insect rearing is inexpensive and sustainable, and when combined with consumption, it is extremely advantageous (Kelemu *et al.*, 2015).

This study demonstrated disgust to be a significant barrier in the context of the United Kingdom. This is also the most crucial element in deciding whether or not individuals were willing to consume insects, use an insect-based alternative, or buy an insect-based product (Russell and Knott, 2021). Furthermore, people's readiness to ingest locusts and crickets, notably in the form of insect-based flour-baked cookies, rather than mealworms or cockroaches, may indicate a lack of resistance to adopting flour-based meals composed by *Carebara vidua*.

Ochieng *et al.* (2023) revealed that moral concern and repulsion predicted a lower tendency to consume insects, indicating that both these variables may operate as barriers. "We also observed that when people were convinced that insect consumption was more common and ordinary, they were more inclined to partake in the practice" (Ochieng *et al.*, 2023).

The consequences of distaste, moral concern, and cultural pressure on people's willingness to consume insects are the most critical and fundamental concerns that demand further investigation. Ochieng *et al.* (2023) found that social impact, or being around individuals who eat insect-based foods, has minimal influence on one's own inclination to do so.

Cultural variations, according to Ochieng *et al.* (2023), frequently influence how familiarity with food items is perceived. Varied cultures have varied preferences for and against specific meals, even down to the perspectives of the young. Familiarity often increases acceptability since it makes describing sensory aspects simpler. Understanding the benefits of unusual foods, on the other hand, can boost adoption. When doing cross-cultural research, it is necessary to include factors other than culture, such as social and environmental circumstances.

Understanding the influence that familiarity, culture, environment, language, and information have on customers' sensory perception and acceptance is critical for increasing the value of food products in a globalised society. Future research should look at these cross-cultural characteristics, maybe employing focus groups to better understand food perceptions, product value, and motivations for consumption across cultures (Ochieng *et al.*, 2023).

11 Harvesting and rearing of edible black ant Smith (*Carebara vidua*)

The growing industry of cultivating edible insects suffers from a clear lack of information that is verifiable about key elements of the chains of food production in comparison to conventional domestic animals-based food production systems (Cortes Ortiz *et al.*, 2016; Dobermann, 2017; van Huis, 2017). Therefore, it has become urgently necessary to acquire knowledge of insect husbandry and facility design; wild collection will serve as the basis for large-scale insect raising for markets. (Miech *et al.*, 2016; van Huis, 2013).

Traditional ways of collecting and harvesting in the wild

Many black ant species have been collected by communities in Laos, Myanmar, Vietnam, and Thailand (Rastogi, 2011) for personal consumption and rarely sell in markets due to small amounts of catching. Black ants are not easy to detect because their bodies are perfectly camouflaged like the ground and their emergence by tiny, small workers and soldiers which are not easily observable on the ground. Moreover, they build mounds underground and sometimes under the rocks which make it more difficult for them to be caught (Ayieko *et al.*, 2012).

Under the conventional method, the edible black ants are harvested by picking/catching from their habitat located in termite mounds; grassland or emergence hole during the season (Ayieko *et al.*, 2012; Hlongwane *et al.*, 2021). In Southeast Africa (particularly Zimbabwe and Zambia), the nourishing alates (queens) of the *Carebara vidua*, commonly known as the thieving ant, are often collected at winged sexual stage after the nuptial flight as the ants emerge in large numbers from their nests after heavy rains (Rastogi, 2011; van Huis, 2021). During harvesting, the heads of the ants are often smashed to prevent them from flying as they emerge from holes near termite hills (Ayieko *et al.*, 2012; van Huis, 2021). Thus, before eating, the head is often removed due to destruction during harvesting. Small ants (workers) are a hindrance during collection since they emerge from the holes at the same time. They establish their nests in close proximity to termite nests, on which they forage and prey (van Huis, 2021).

In African countries, traditional harvesting of edible insects is generally done by women, and occasionally, young ones also help (van Huis, 2003; Netshifhefhe *et al.*, 2018). Due to consumer demands and the nutritional value of insects, there has been a transition from domestic small-scale production for consumption at the local market level to a more elaborate close cycle set up of farms to produce large quantities for a wider market in traditional consumption regions (Halloran *et al.*, 2016). Since labor costs are high in the regions of Northern America and Europe, it is vital to intensify and automate manufacturing to lower production costs and make edible insects more affordable (Fraqueza and Patarata, 2017).

In the Northeastern and Northern regions of Thailand, people harvest ants' larvae and pupae using a long bamboo pole equipped with a basket or bag attached to the top using strings. The basket is gently shaken over the ant nest, causing the larvae and pupae to fall into the bag. After harvested, the ants are placed into a container filled with tapioca flour or rice to prevent the ants from escaping from the container and potentially biting the collector. Adult ants are encouraged to return to their nest by providing a branch on the container,

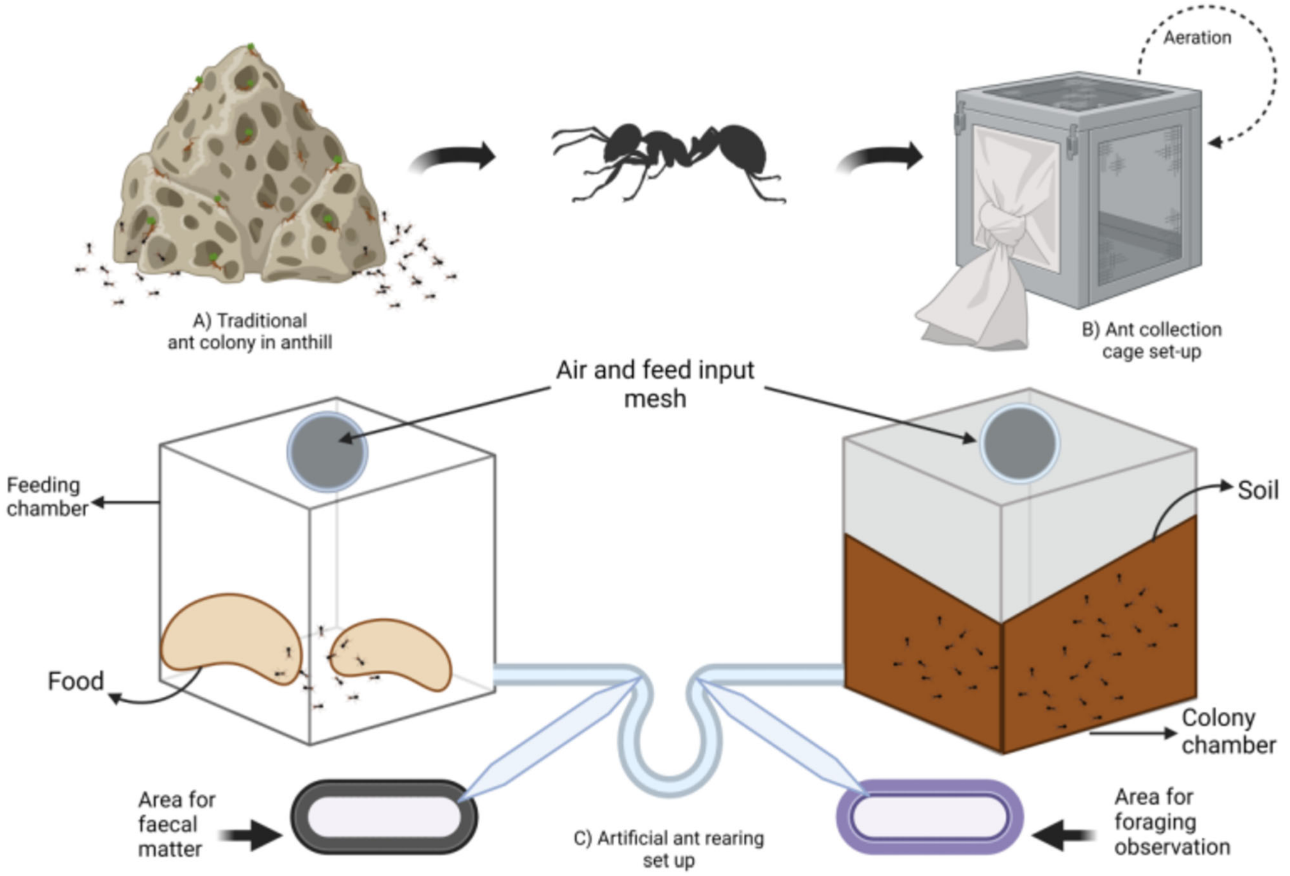

FIGURE 12 (A) Traditional ant colony habitat, (B) Ant cage collection set-up, (C) Semi-natural/artificial ant colony set-up.

while the larvae and pupae are collected for consumption (Raheem *et al.*, 2019a).

Semi-natural rearing/artificial rearing system

Insect housing and rearing techniques so far have been improved for the pet industry, as well as for the production of sterile insects for pest management and medical applications (Dossey *et al.*, 2016). The established small-scale firms whose present focus is growing edible insects for general consumption (on a local scale) are another important reservoir of knowledge regarding insect farming (Berggren *et al.*, 2018). Sustainable insect farming plays a pivotal role in economic significance of black ant Smith (*Carebara vidua*) which associated with the mass production of black ant-based food products.

Identifying a wild colony with proper guidance or obtaining one from a reliable supplier that is specialised in providing starter ant colonies would be a desirable start to harvesting the *Carebara vidua* ants. A suitable housing set-up then needs to be prepared for the ants, which can replicate the environmental establishment to serve as an ant farm or be a mimicking artificial nest. This preparation must ensure that the housing set-up provides adequate temperature and moisture/humidity control as well as sufficient ventilation to leverage in semi-natural rearing facilities (Musundire *et al.*, 2016a; Berggren *et al.*, 2018). Along with this, allocation of access to the nesting areas, foraging areas, and a source of food and water for the ants must be provided. As the colony will reproduce and grow, more space and resources must be made available to sustain the development of the colony (Raheem *et al.*, 2019a) (Figure 12 a-c). The female nests in a suitable site to lay her brood of eggs, and the warmth that these eggs receive through the shallow soil layers enable them to hatch within 5 to 7 days (Ayieko *et al.*, 2012). The harvesting for the semi-natural reared/artificial reared colonies provides the access to harvest them whenever the ants of the colonies are noted to be in safe numbers for harvesting and emerge from the grounds in excess or post their nuptial period (Ayieko *et al.*, 2012). With sufficient observable progress of the colony's growth, the harvesting of the black ants can be done by gently nudging or directing the ants to shift towards a clean container or surface used for collection. They are collected from pasture fields and pooled to provide one composite catch. Any debris or substrate collected along with them is separated or cleaned off from the ants. The ants are cleaned with a gentle rinse under running water to remove any unnecessary material (Musundire *et al.*, 2016a).

The majority of research on edible insects focuses on insects that are caught in the wilderness than those that are raised on farms; nevertheless, indoor farming and semi-domestication have enhanced the edible insect production's sustainability and availability (Aguilar-Toalá *et al.*, 2022). In fact, current food standards in the EU and the United States require that insects designated for human consumption be bred in approved insect breeding facilities rather than collected in the wild. (Liceaga, 2021). A key justification for advising against eating wild insects is that the controlled environment of a farm facility allows for the regulation and prevention of contamination sources, contrary to conditions for insects that were harvested in the wild (Melgar-Lalanne *et al.*, 2019).

Factors affect the success of mass-rearing

Artificial feeds and/or diets generally comprise of a mix of nutrients, which the insects consume roughly 70-75% of food for life stage maintenance, and once these nutrition requirements are supplied anything additional are excreted as excess or stores as unwanted fat. Therefore, avoiding over-supply of nutrients to prevent this wastefulness and essentially cutting costs is imperative as the diet expenses are relatively higher than costs in insect production. In fact, oversupply of nutrients is counterproductive as it contributes to build-up of metabolites that may result in antagonistic imbalances leading to increased metabolic stress (Ngomane *et al.*, 2022). Similarly, an undersupply may lead to reduced productivity, fecundity and fertility, and immune suppression. Diversified diet mixtures are recommended to observe expediated development, higher adult weight and healthy female fecundity (Malinga *et al.*, 2018). Mass-rearing automated facilities that produce stable, reliable, and safe products must be created due to the massive amounts of insect biomass needed to replace the current protein-rich sources, such as fish and soybeans (Huis *et al.*, 2013).

Carebara vidua is believed to feed on humus and decaying matter of plants. They used to occur in more numbers in the years of the 1970s due to the existing types of tree species. Indigenous trees like *Markhamia lutea*, *Abizia coriaria*, *Prunus africana*, and soap berry trees were known to enhance humus formation that was available to *Carebara vidua*. The growth of exotic trees in the stead of these indigenous trees played a role in the depletion of *Carebara vidua*'s current distribution, as the decay matter from these tree species made the humus formation more through toxication of the overall matter, which also produced a non-ecofriendly smell. *Carebara vidua* environmental management should include the constant use of pure organic substances or the restricted use of agrochemicals, to also conserve the soil ecology. Practices like these will have a good impact on the insect's diminishing population, resulting in the zones of emergence to sustain their availability. *Carebara vidua* could also emerge twice in a year, in the month of April, then emerge again in the month of August for the second time, where there is sufficient rainfall. In certain circumstances, *Carebara vidua* is possible to emerge more than thrice a year; April, May, August, and other months with intermittent rainfall (Illgner and Nel, 2000; Onyeike *et al.*, 2005; Ayieko *et al.*, 2012; Obopile and Seeletso, 2013; Kelemu *et al.*, 2015; Musundire *et al.*, 2016a; Hlongwane *et al.*, 2020; Ondede, 2023). In another report by Jose *et al.* (2022), *Carebara vidua* is available in the month of November to December, while dormant during summer (Musundire *et al.*, 2016a). On the other hand, according to Hlongwane (2021), *Carebara vidua* is accessible all year round in KwaZulu-Natal and Limpopo provinces of South Africa (Table 9).

Additionally, Ma *et al.* (2018) studied the influence of the pH of a feed affecting rate of development and longevity in insect production. They noticed that the larva's weight grew as the feed's pH rose, indicating that the ideal pH for observing the larvae' optimal growth time is 8.0. Controlling the temperature, relative humidity and pH of feed, is a key factor affecting growth rate and metabolism, growth efficiency and macronutrient composition as well (Bjørge *et al.*, 2018). Understanding how temperature may be adjusted is essential since temperature has such a significant influence on production quality, efficiency, and time. Temperature affects insect survival, growth and developmental rate, and fecundity (Lehtovaara *et al.*, 2018). The relative humidity and photoperiod are important factors that will impact the growth phases of *Carebara vidua*, however, there is no research providing this information yet. Investigating for optimal conditions for both these factors can make key advancements in artificial and semi-natural mass rearing set-ups (Lehtovaara *et al.*, 2018; Cadinu *et al.*, 2020).

The mimicry of their new habitat or their artificial environment must be so the conditions being provided to the ants encourage the sustenance of their normal behavior with reduced stress from any variations (Berggren *et al.*, 2018). Optimal levels of temperature and humidity within the confines of the enclosure

TABLE 9 Availability of the edible black ant smith (*Carebara vidua*)

Species	Order/family	Locations	Months and time of occurrence	Reference
Carebara vidua Black ant smith	Hymenoptera/ Formicidae	Botswana, Burundi, Kenya, Malawi, Mozambique, Namibia, South Africa, Sudan	April, May-June, August-December	Illgner and Nel, 2000; Onyeike *et al.*, 2005; Ayieko *et al.*, 2012; Obopile and Seeletso, 2013; Kelemu *et al.*, 2015; Musundire *et al.*, 2016a; Hlongwane *et al.*, 2020; Ondede, 2023

are imperative to support the different developmental stages of the entire colony. The mass-rearing of edible black ants can be successfully developed by providing a conducive set-up that addresses key factors contributing to their health, development, and the reproduction of the colony. The highly competitive nature of this developing industry and a lack of willingness to spread innovative rearing methods can be attributed as key reasons for the limitations of open research on western-based insect rearing strategies (Wilkie, 2018).

12 Processing and packaging of edible black ant Smith (*Carebara vidua*) as human food

Processing of edible black ant Smith

Consuming insects also means consuming the microorganisms found in various ecologies at every stage of industrial insect rearing, from the live insect to its processing, freezing, and consumption (Wade and Hoelle, 2020). A series of processing steps for a large-scale production of edible insects has been established to produce easy consumption or easily prepared food products (Rumpold and Schlüter, 2013a). Ojha *et al.* (2021b) proposed the traditional processing pathway to be applied for processing edible insects in future. The four main pre-processing technologies such as harvesting insects/separating from substrate residuals, inactivation of insects/killing, wing/leg removal, and washing-make up the first stage of each edible insect processing pathway (Ojha *et al.*, 2021b; Rumpold and Schlüter, 2013b). Generally, edible insects are either manually or mechanically picked from their rearing cage once they have reached the applicable size or maturity. Edible insects tend to be contaminated with microbial, therefore, isolation the insects from their diet prior to harvest, a period known as starvation is applied ahead of harvesting to reduce the contamination (Garofalo *et al.*, 2019). The starvation requires the insects to go through a 24-48 hour fasting period by not giving them their food hence has a positive impact on product quality such as producing a better taste and flavor as well as a hygiene end product (Fernandez-Cassi *et al.*, 2019). Before being consumed, the insects go through a few post-harvest procedures. The insects need to be killed. Some of the most popular procedures used for this purpose are freezing, whereby freezing can be opted for the advantage of facilitating the removal of legs and wings (Fraqueza and Patarata, 2017). In addition, submerging in hot or boiling water, and steaming also can be applied to kill the insect. Killing is an important stage in insect processing because it helps to reduce the microbial load and get rid of any adhering impurity as well as influence the proximate composition, color, and flavor of the finished product (Jensen *et al.*, 2017; Larouche *et al.*, 2019; Melgar-Lalanne *et al.*, 2019) (Figure 13). Boiling insects before roasting proves effective to prevent bacterial spore formation and dehydrating insects can also lessen microbial contamination by lowering the water content, in turn preventing bacterial growth (Meyer-Rochow *et al.*, 2021). However, a specific commercial processing step for *Carebara vidua* is yet to be reported, but edible insects should be processed and stored by undergoing the same hygiene protocol as for processing of other traditional foods to ensure food safety and suitability for human consumption (Imathiu, 2020). After a series of processing, the insects can be prepared and consumed in three different ways: as whole insect, in paste or granular form and in fortified food in the extract of protein or fat (van Huis *et al.*, 2013). Although *Carebara vidua* is high in nutrient content, its incorporation in food or processing to food products are not widely researched and documented yet.

The traditionally household methods for processing edible insects are common cooking practices like frying, roasting, steaming, smoking, stewing and brais-

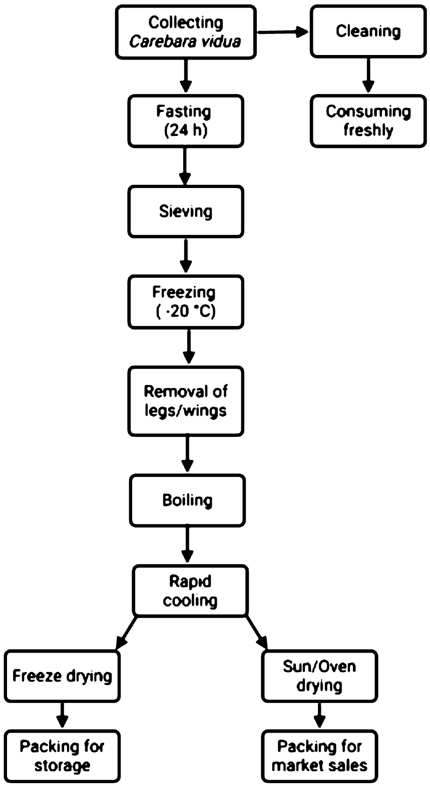

FIGURE 13 Typical process flow for processing *Carebara vidua*.

ing to enhance palatability (Belluco *et al.*, 2013; Nyangena *et al.*, 2020). Additionally, sun-drying, frying, or roasting the bulk are traditionally typical processing steps done without proper hygienic handling to prepare "ready-to-eat" that are kept for sale at local markets (Melgar-Lalanne *et al.*, 2019). Efforts in ramping up insect production must ensure the safety of the food, prolong its shelf-life, and explore various product variations or novel presentations to attract diverse potential consumers and overcome any resistance (Fraqueza and Patarata, 2017). The traditional processes of edible insects prior consuming like boiling, drying at 60 °C overnight in an oven, and toasting were investigated, which improved safety but had an impact on nutrition (Musundire *et al.*, 2016a). When compared to boiling, toasting increased protein while decreasing fat (Nyangena *et al.*, 2020).

Simple processes such as decontamination, heat treatment steps or dehydration to reduce microbial count, then followed with drying, and grinding prior processing edible insects into extudates, powders or whole dried insects are appropriate processing steps for a new small-scale business (Grabowski and Klein, 2017). These powdered forms are utilised in the production of foods like biscuits, pasta, energy bars, cereals and meat preparations (Reverberi, 2021). This simple powdering technique, however, has drawbacks as the high lipid content causes storage and logistical issues. Additionally, the overall composition restricts use in food products and may not be well received by consumers (Huis, 2022).

Furthermore, the general processing related aspects for producing downstream food product from edible insects involves protocols isolating protein, fat and chitin (van Huis, 2022) (Figure 14). After proteins, lipids are the largest nutrient composition of edible insects (Lee *et al.*, 2021). Making insect oil extraction essential for obtaining high quantities of high-quality edible oil and facilitating later protein isolation (Purschke *et al.*, 2017). Extraction methods can include gentle mechanical pressing (Urbizo-Reyes *et al.*, 2019; Urbizo-Reyes *et al.*, 2021), using food grade solvents that are environmentally friendly like ethanol and methanol (Zhao *et al.*, 2016), or a three-phase partitioning utilizing water, tert-butanol and ammonium sulphate; as applied for oil extraction from rice bran and soybean (Dutta *et al.*, 2015) which also can be applied for extraction of fat from edible insects. Additionally, various methods like Folch extraction, Soxhlet method, water extraction, and supercritical CO_2 extraction, have also been relied upon to obtain lipid components (Lee *et al.*, 2021). Although, the supercritical CO_2 is more expensive, the low temperatures used allow for prevention of oxidation of solutes and helps obtain thermally sensitive components. Contrary to proteins, where the extraction procedure has little to no impact on the fatty acid composition, it is crucial to note that the extraction process affects the types of lipids and the extraction yields that are achieved (Jantzen da Silva Lucas *et al.*, 2020).

Other processing methods to be explored can include enzyme-based technology, sonication, extrusion, pasteurization, fermentation, microwave assisted extractions (Fraqueza and Patarata, 2017; Ojha *et al.*, 2021b). Besides, heavy metal contamination from lead, arsenic, mercury, and cadmium can accumulate, although polycyclic aromatic hydrocarbons (PAHs) and mycotoxins do not appear to accumulate, and insects destroy mycotoxins and veterinary medications such lincomycin (Luo *et al.*, 2022). Processing can sufficiently reduce target pathogens, but caution needs to be exercised about a possible trade-off between food safety and replacing energy-consuming techniques (e.g. freeze drying) with innovative thermal technologies (e.g. cold plasma, high hydrostatic pressure, low energy electron beams pulsed electric fields, intense light pulses, low energy electron beam, and so on) (van Huis, 2022).

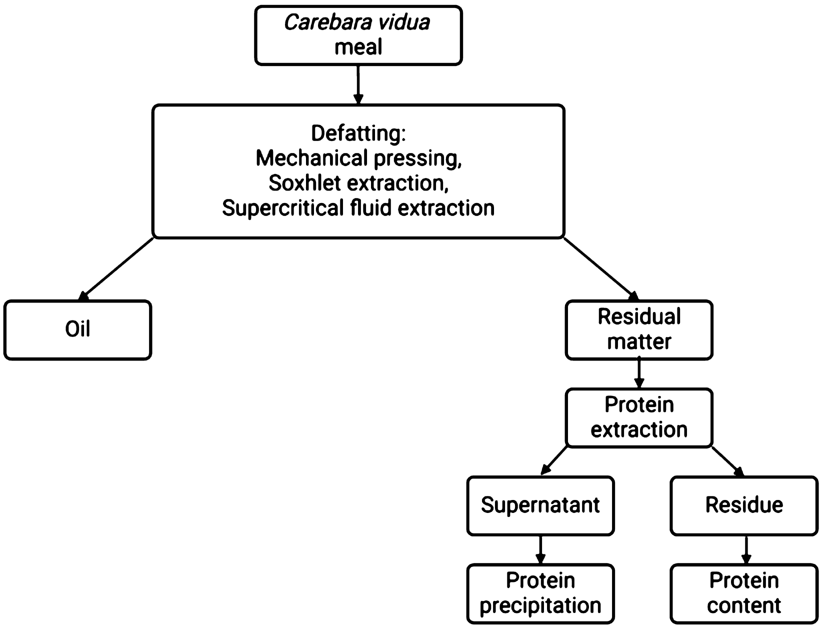

FIGURE 14 Typical fat extraction process of *Carebara vidua*.

Physico-chemical changes

Generally, heat treatments increase the likelihood of lipid oxidation, proteolysis, and the solubilization of vitamins and minerals (Caparros Megido *et al.*, 2018). To consider an example of common extraction methods, those using hexane exhibit a favorable impact on physio-chemical characteristics like for color, yield, crude protein, ash content and available carbohydrates. Utilizing hexane for extraction is also effective in defatting processes (Ndiritu *et al.*, 2017). On the other hand, proteins in food that cause allergy reactions may undergo physicochemical changes as a result of food processing. For example, heat treatment during food processing can alter antigens' immunological characteristics by altering the protein's three-dimensional structure. The secondary and tertiary structures of the proteins can alter as a result of heat and non-thermal processing, interactions at interfaces including oil-water (emulsion) and air-water (foams) systems, and more (Aguilar-Toalá *et al.*, 2022). Furthermore, according to Purschke *et al.* (2018), pretreatment steps also considerably influence the final dried powders' color, size, density, and other properties of dried and fractionated powders of edible insects. The protein recovery yield can be impacted, and the macronutrient contents of the sieving fractions may vary (Table 10). Fractionation is a potential method to help standardise the production of insect-based intermediates for consumer acceptance and industrial applications.

Additionally, the impact of processing methods or supplementary strategies should be further explored and could assist in eliminating anti-nutrient factors. A proper food processing method can be applied for reducing the level of anti-nutrient factors such as cooking, puffing, soaking, and fermentation (Handa *et al.*, 2017) to increase the digestibility of protein and thus improving the biological value of an edible ant. According to Megido *et al.* (2018), the protein digestibility level of mealworms increased after boiling and followed by oven cooking. On the other hand, Ojha *et al.* (2021a) reported that the digestibility or bioavailability of the protein derived from edible insects is influenced by the species of insects. For example, protein digestibility showed a significant reduced in edible grasshoppers prepared by toasting and drying method, but the concentration of protein digestibility remained unchanged for edible termites after toasting and drying as compared with raw sample. Kröncke *et al.* (2019) examined an *in vitro* digestibility on the bioavailability of mineral (total Zinc) of larvae from yellow mealworm (*Tenebrio molitor* L.). The author reported a decrease up to 60% and 80% of bioavailability of total zinc in samples prepared by freeze or vacuum (60 °C for 24 hours) drying and oven (120 °C for 1 hour) drying solely, respectively (Kröncke *et al.*, 2019).

Storage

Edible insects present a significant obstacle to year-round inclusion in diets due to their seasonality and short shelf life, which causes quick spoilage. Freshly harvested edible insects have a very short postharvest shelf life; edible grasshoppers, for instance, may only

TABLE 10 Effect of processing methods on the nutritional value and shelf life of edible black ant Smith (*Carebara vidua*)

Processing technique	Treatment	Main findings	References
Sun drying	Placed under direct sun to remove water and prevent microbial activity	Traditionally renowned method to eliminate microbial risks and improve nutritional quality compounds, such	Nyangena *et al.*, 2020
Oven drying	Used primarily for insect flours and powders at 60 °C	· Industrial grade usage to increase shelf-life for storage and distribution · Increased protein solubility and decreased lipid oxidation · Color changes · Monosaturated fatty acid content reduces	Khan *et al.*, 2020
Blanching	A pretreatment to reduce microbes and inactivate enzymes affecting food spoilage through quick heating then cooling	· Is paired with other treatment steps to reduce bacterial spore formation	Caparros Megido *et al.*, 2017; Purschke *et al.*, 2018
Smoking	· A thermal process curing process with fuming vapours	· Paired action of heat and enzymes promotes improvement of protein and lipids · Most traditional method known to be used with animal meats	Tiencheu *et al.*, 2013
Freeze-drying	· Oxidative and microbial degradation is reduced to a large extent	· Nutritional qualities retrained along with good shelf-life period for further research · Final product is high quality	Lenaerts *et al.*, 2018
Microwave-assisted drying	· Reducing water activity (aw < 0.30) to significantly reduce the microbiological count	· More studies need to be carried out on how to maintain the sensory quality · Further studies required to be conducted to maintain sensorial quality	Sun *et al.*, 2018

keep for a day or two (Ssepuuya *et al.*, 2016). Without thermally induced pre-treatment, edible insects may contain a microbiological load that can cause cross-contamination and even recontamination. Therefore, it is imperative that they are processed, packed and stored properly (Grabowski and Klein, 2017; Melgar-Lalanne *et al.*, 2019). For insects that are to be consumed freshly, the recommended storage method is freezing (–20 °C) instead of refrigeration (5 to 7 °C) to maintain their microbial quality (Belluco *et al.*, 2013). However, refrigeration is the ideal method for avoiding microbial and oxidative degradation for dried and powdered edible insects (Melgar-Lalanne *et al.*, 2019). When refrigeration is utilised simultaneously with vacuum or modified atmospheres, there is a significant extension in the shelf-life of the items. The duration for which whole edible insects can be stored at room temperature is enhanced through storage under vacuum and darkness (Ssepuuya *et al.*, 2016). The application of vacuum packing enhances the microbial quality of the product, while the absence of light prevents the oxidation of lipids. A recommended approach to maintain the nutritional quality of dried edible insects is to store them in a mix of salt and pepper (Meyer-Rochow *et al.*, 2021).

Modified atmosphere packaging (MAP) is an alternative to packaging methods generally relying on vacuum. It was shown that MAP (60% CO2 40% N2) prevented microbial growth in insect paste kept at 4 °C, maintaining microbiological stability for at least 21 days. However, they found no decrease in the number of bacterial spores by (Stoops *et al.*, 2017). Reduced mold contamination in insect powder or whole dried insects may also benefit from the use of MAP. MAP can be an important step in increasing the shelf life of food insects because molds create mycotoxin, which can be a substantial safety risk in edible insects when the water activity is higher than 0.6 (Kamau *et al.*, 2018a). Modified packaging solutions are crucial in preventing further contamination and extending the time that the edible insects can be preserved (Musundire *et al.*, 2016b). The type of packaging used is also important for safety. For instance, polypropylene (PP) packing resulted in a shelf life of only 45 days for boiled, sun dried and milled crickets stored for two months at room temperature, whereas other materials kept it for 2 months. Adding an interior layer of polypropylene can lower permeability of air and water-vapor, hence improving shelf life, even though plastic packaging with caps were preferable to bags (Kamau *et al.*, 2018b).

The effect of temperature of powdered insects and acidity during storage, as unfavorable temperatures exhibit and increase in off-flavors. The relation between temperature and acidity can be attributed to the oxidation of fat (Kim *et al.*, 2016). Stoops *et al.* (2017), examined the effect of different storage conditions on microbial growth in mealworms and concluded that replacing air in the storage environment with carbon dioxide and nitrogen reduced microbial growth during storage.

13 Future prospects for edible black ant Smith (*Carebara vidua*)

Potential as a sustainable protein source

Carebara vidua's potential as a source can be realised depending on increasing reception from the markets. Results from a study reported by Guiné *et al.* (2022) shed light on economic, social, geographic, and demographic factors and how they influence the perception on the sustainability of edible insects. This could serve as the first step in devising strategies that can promote a better flow of knowledge to different segments of the population. Hopefully, the impact of this information and reduction of ignorance can lead to an increase in willingness to replace other animal protein foods with insects as a more suitable substitute – even partially, and even in countries where this consumption shift does not belong to their traditional practice.

Insects can potentially be considered in partly substituting other more conventional protein sources, particularly those with the biggest effect, specifically bovines to offer protein-based alternatives to address demands on a global scale (Guiné *et al.*, 2021; Molfetta *et al.*, 2022). Therefore, insects and insect-based meals emerge as meat substitutes with significantly reduced environmental impact (Mitchaothai *et al.*, 2022; Vinci *et al.*, 2022). Insect farming produces less greenhouse gas and ammonia emissions than traditional cattle farming and requires a lot less feed, water, and land. Furthermore, eating insects could significantly improve global food security and reduce hunger in lower socioeconomic (Bao and Song, 2022; Jantzen da Silva Lucas *et al.*, 2020; Ordoñez-Araque and Egas-Montenegro, 2021).

Research on black ant **(Carebara vidua)** *Smith farming and its processing*

Human activity like raising crops, domestic animals, and constructing housing for an increasing number of households in the region of Lake Victoria, this is one of the nutrient-rich food insects that is in risk of going extinct (Ayieko *et al.*, 2012). Many households in the lake region rely on fish for more than 50% of their animal protein. However, the increased demand for fish for human and animal consumption has resulted in a shortage and skyrocketing fish market prices for low- and middle-class households. Demand changes have led to a shortfall of protein, especially in metropolitan areas where food production and supply are not keeping up with population growth.

Additionally, the research material available on the topic of the edible black smith is sparse and scanty. From the perspective of the producers, there is more to be desired regarding research on different rearing techniques and conditions that can be leveraged to improve development of more colonies. There is much more headway to be made in terms of the physicochemical properties of proteins from this source, with more depth required regarding the nutritional composition of this ant. That will hopefully motivate more culinary journals to expect gastronomical investigations.

However, insect food items pose challenges like being prone to rapid unsaturated fatty acid oxidation, which causes these products to go bad quickly. To guarantee the high quality of the finished product, this needs to be monitored, particularly during processing (Kinyuru *et al.*, 2015). The dearth of information on host plants and habitat relationships threatens the sustainable use of insects as food in sub-Saharan Africa (Musundire *et al.*, 2016a).

Due to fragmented and, in some cases, poor knowledge, consumers of insects currently face limits on the intended execution of operations for capturing insects from their native habitats. Habitat conservation and delicious insect conservation efforts are also affected (Musundire *et al.*, 2016a). The majority of edible insects researched have well-established relationships with host plants and habitats. As a result, conservation activities for these insects can be carried out successfully. Current efforts to capture insects in the field and combine conservation efforts for these insect species may be limited by a lack of knowledge about the habitat and host plant linkages of *Carebara vidua*. The species will become prone to extinction if nothing is done to protect them.

Market potential and challenges

Future forecasts suggest that this sector might be worth US $8 billion by 2030. Although over 50% of the market is in Asia-Pacific and Latin America, the fastest growth is anticipated to occur in Europe and North America (Goldstein, 2018; Bombe, 2019). Furthermore, insect farming is becoming important in Cambodia's efforts to combat rural poverty. Many people in the edible insect supply chain, including collectors, farmers, retailers, and wholesalers, earn a living from selling insects in many countries in Africa, including Burundi, Cameron, Kenya, and Uganda (Odongo *et al.*, 2018; Baiano, 2020). The edible insect market in the Lake Victoria basin, which includes parts of Uganda and Burundi, was examined (Odongo *et al.*, 2018). Their findings showed that edible insects were primarily traded in urban areas and were regarded as treats. Additionally, they contend that the rising demand can spur this industry's growth, as the demand currently far outpaces the supply, driving up insect prices compared to beef, pig, or poultry (Odongo *et al.*, 2018).

A report released by RABO Bank projected the insect-based protein demand to grow from 1.2 thousand tons to half a million metric ton by the year 2030 (De Jong and Nikolik, 2021). The report also projected the price of a metric ton of insect protein to increase from EUR 3,500-5,500 in the scale up phase (2020) to 1,500-2,500 in the maturity phase (2030). Though another report (IPIFF, 2021) identified a similar trend, it is more optimistic than the report from RABO Bank. Additionally, interest in academia also grew significantly. Over 80% of all articles that were relevant throughout the last five years were published with the keyword "edible insects" in the title. The patents cover a wide range of subjects; many of them relate to rearing techniques and include attractants, conversion, environmental control, feed composition, hatching, and oviposition. Additionally, they also cover chitin, cosmetics, frass, and processing (van Huis, 2022).

For insects as food in particular, marketing is arguably more about a market push than a market pull. One should take into account that insects are more readily accepted by pigs and poultry because they are a natural source of food. Insects in pet food were never subject to legal regulations because pets are typically not consumed. Additionally, it is becoming more widely acknowledged that meat-based pet food can have a significant negative impact on the environment in relation to CO_2 equivalents and land usage (Leenstra *et al.*, 2018).

14 Future perspectives and conclusions

Carebara vidua is a sought-after source of nutrition in some cultures, and in others, a valuable commodity with medical properties that gets kept or consumed by the collectors even before it is sold into the markets. Beyond the context of these cultures, it has been reported as an edible insect that can be a serious consideration for human consumption, with potential to become another important solution to address potential food insecurity. Suffering in the past from near-extinction due to intensive agricultural practices and other disruptive human intervention, demand for pertinent regulatory interventions to promote natural and semi-artificial rearing of the black ant smith is imminent. As an excellent source of macro-nutrients (protein and fat) and micro-nutrients (vitamin B-complex) and therapeutic claims, black ant smith is receiving attention from researchers. Given its history of consumption across many Asian and African countries, and with expressed interests from western countries, the economic potential of this edible ant species remains underexplored. Additionally, its commercialization should be supported through substantiating studies that can educate various members in the value chain to participate actively. Studies have revealed its physicochemical attributes to support its value for human consumption. It is however imperative that this ant species grows further in popularity as research subject to unlock its potential as a viable source, through further studies that can investigate more dimensions like health- and safety-related issues. These efforts will improve the prospects of the production and processing practices that utilise the black ant smith to convert it into an efficient and sustainable source of insect protein. The west has begun to follow the footsteps of Asian and African regions that have already been infamous for adopting entomophagy much earlier; therefore, efforts directed towards *Carebara vidua* can likely be met with positive reception. As market challenges with entomophagy are related to neophobia and the association of disgust with insects in general, relevant culinary interventions can be crucial in broadening the range of the edible black ant smith in transforming its acceptability to the consumers. Ultimately, the future of the edible black ant smith rests on the whim of social perceptions and the dynamic demands of the consumers. But relevant steps must be taken by the producers, industry and academia alike to ensure *Carebara vidua*'s potential as a food source for humans is not dismissed. It is another important component in our collective efforts to create a more sustainable and inclusive food system. Projects like GREEiNSECT need to be encouraged to address creation of institutional frameworks to create mass rearing and production systems that can make *Carebara vidua* more accessible as a research subject. Policy interventions like the Regulation (EU) No 2015/2283 and unique models by food safety authorities of countries that are in a position to promote trade of edible insects of human consumption will play a crucial role in establishing market spaces. However, before countries can begin formulating laws surrounding the commercialization and consumption of black ant Smith, they need to address formulating of laws to safeguard the environment that they grow in and support the natural, semi-artificial and artificial rearing of them. Relevant standards need to be created to support international trade aimed at establishing consistent value chains. The inclusion of C.vidua in the Codex Alimentarius would be a positive step towards international industrial standards, allowing member nations to build their own supplementary standards. In order to accomplish this, emphasis should be made on developing data collection approaches and procedures. Statistical data on safety and nutritional aspects of *Carebara vidua* is limited; worldwide, regional, and national overviews of monetary contribution to domestic and international trade are mostly unknown. The dearth of academic, industrial and commercial information on this edible ant needs to be addressed to develop standards, which can be particularly challenging on a global level. These are crucial for the development of relevant processing techniques, industrial interventions, and strategies to build producer/consumer awareness and subsequently pique their interests.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.25211291

Author contributions

S.A.S. – conceptualization, methodology, writing – original draft, writing – review and editing, validation, formal analysis, resources, visualization, data curation, project administration, investigation, supervision. L.-H.H. – writing – original draft. S.C.A. – writing – original draft. A.N. – validation. B.Y. – review and editing. R.C-M. – formal analysis. S.A.I. – funding and validation.

Conflicts of interest

The authors declare no conflict of interest.

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