UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

ANTÔNIO BISCONSIN JÚNIOR

INSETOS COMESTÍVEIS: ESTUDO DO CONSUMIDOR E DESENVOLVIMENTO DE INGREDIENTE ALIMENTÍCIO

EDIBLE INSECTS: CONSUMER STUDY AND FOOD INGREDIENT DEVELOPMENT

CAMPINAS

2023

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EDIBLE INSECTS: CONSUMER STUDY AND FOOD INGREDIENT DEVELOPMENT

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Ciência de Alimentos.

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Science.

Orientadora: Lilian Regina Barros Mariutti

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO ANTONIO BISCONSIN JUNIOR, ORIENTADA PELA PROFESSORA DOUTORA LILIAN REGINA BARROS MARIUTTI

CAMPINAS

2023

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

B541i

Bisconsin-Junior, Antonio, 1985-
Insetos comestíveis : estudo de consumidor e desenvolvimento de ingrediente alimentício / Antônio Bisconsin Júnior. – Campinas, SP : [s.n.], 2023.

Orientador: Lilian Regina Barros Mariutti.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Insetos comestíveis. 2. Estudo de consumidor. 3. Representações sociais. 4. Concentrados proteicos. 5. Tecnologias não térmicas. 6. Segmentação de mercado. I. Mariutti, Lilian Regina Barros. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações Complementares

Título em outro idioma: Edible insects : consumer study and food ingredient development
Palavras-chave em inglês:
Edible insects
Consumer study
Social representations
Protein concentrates
Non-thermal technologies
Market segmentation
Área de concentração: Ciência de Alimentos
Titulação: Doutor em Ciência de Alimentos
Banca examinadora:
Lilian Regina Barros Mariutti [Orientador]
Cinthia Baú Betim Cazarin
Juliana Aparecida Fracarolli
Adriano Gomes da Cruz
Bruno Ricardo de Castro Leite Júnior
Data de defesa: 26-06-2023
Programa de Pós-Graduação: Ciência de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0001-9474-1691
- Currículo Lattes do autor: http://lattes.cnpq.br/6295730338779399

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A Ata de Defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertações/Teses e na Secretaria do Programa de Pós-Graduação

Aos meus pais Odinéa e Antonio, minha filha Sofia e minha parceira de vida Carol.
A todos que acreditam na educação e ciência como caminhos para um mundo melhor.

AGRADECIMENTOS

Agradeço à minha orientadora Profa. Dra. Lilian Regina Barros Mariutti, que me apoiou desde a primeira reunião sobre a ideia de trabalhar com insetos, por toda atenção, conhecimento e tempo disponibilizados, além da paciência e do suporte emocional.

Agradeço ao Dr. Oliver Schlüter, por ter me recebido na sua equipe de pesquisadores na Alemanha, pela confiança em meu trabalho e pelas contribuições significativas nesta tese.

Agradeço ao Prof. Dr. Jorge Herman Behrens e ao Dr. Heber Rodrigues, pelas contribuições fundamentais para a realização do estudo de consumidor.

Agradeço à Profa. Dra. Flavia Maria Netto e à Dra. Jéssica Viana, pela importante contribuição científica ao trabalho e tempo disponibilizado.

Agradeço aos membros da banca examinadora pelas contribuições valiosas que ajudaram a aprimorar este trabalho.

Agradeço a todos os 780 participantes do estudo de consumidor, por terem doado parte do seu tempo para a realização das entrevistas.

Agradeço aos colegas desta jornada acadêmica na Unicamp, Suênia Felex, Juliany Bordignon, Mayara Ponte, Lorena Januário, Larissa Duarte, Fernandus Silva, Ligia Martins, Adriele Hacke, Celso Neto e Marcella Marques, e aos colegas de pesquisa da ATB-Potsdam, Marios Psarianos, Giacomo Rossi, Shikha Ojha e Robert Sevenich, por terem compartilhado conhecimentos, risadas e desabafos.

Agradeço aos técnicos de laboratório do Departamento de Ciência de Alimentos e Nutrição, Elizabeth Silva (Bete), Francisco Carraro (Chico) e Rosemar dos Santos (Rose), por terem dado um importante suporte na realização deste trabalho.

Agradeço a todos os professores, pesquisadores e colaboradores da Faculdade de Engenharia de Alimentos da Unicamp, que de alguma forma auxiliaram no desenvolvimento desta tese.

Agradeço a minha filha, Sofia Bisconsin, e a minha parceira de vida, Caroline Rocha, que foram compreensivas e deram o apoio necessário para a realização do meu doutorado.

Aos meus pais, irmãos e amigos que sempre acreditaram e apoiaram a minha escolha pela vida acadêmica.

Agradeço às seguintes entidades que deram suporte para viabilizar este doutorado:

Ao Instituto Federal de Educação, Ciência e Tecnologia de Rondônia (IFRO), pela concessão do afastamento integral para desenvolver o meu doutorado.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa de doutorado (142153/2018-4).

À editora acadêmica Elsevier pela bolsa concedida para participar do 13º Pangborn Sensory Science Symposium na Escócia.

Ao Deutscher Akademischer Austauschdienst (DAAD) pela bolsa de intercâmbio na Alemanha (91793370).

À Associação Brasileira dos Criadores de Insetos Alimentícios (ASBRACIA) pelo suporte com informações sobre o cenário brasileiro de insetos comestíveis.

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de financiamento 001.

RESUMO

Insetos comestíveis surgem como uma sólida alternativa para atender à crescente demanda por proteína animal decorrente do aumento da população mundial. Eles se destacam por apresentarem altos teores de proteínas e lipídeos, além de serem criados de forma ambientalmente sustentável. No entanto, apesar dessas vantagens, os insetos comestíveis ainda não conquistaram uma ampla aceitação nos países ocidentais. Nesse contexto, este estudo teve como objetivo avaliar inicialmente a representação social dos brasileiros em relação aos alimentos com insetos comestíveis, a fim de identificar estratégias para a inserção desses produtos no mercado nacional. Constatamos que consumidores de diferentes regiões do Brasil possuem percepções distintas sobre insetos comestíveis, sendo que as regiões Centro-Oeste e Norte mostram maior predisposição ao consumo, em comparação com as regiões Sul, Sudeste e Nordeste. Essa disparidade pode ser atribuída às diferentes experiências e características culturais das populações dessas regiões. Além disso, identificamos e caracterizamos grupos de consumidores, nos quais jovens com maior escolaridade e do sexo masculino apresentaram atitudes mais favoráveis e menor percepção de risco em relação ao consumo de insetos. Por fim, este estudo indicou uma preferência dos brasileiros pelo uso de grilos e por tratamentos que evitem a percepção do inseto inteiro no alimento. Assim, usamos um grilo nativo brasileiro (*Gryllus assimilis*) para produzir um concentrado proteico em pó com o auxílio das tecnologias não térmicas de ultrassom, campos elétricos pulsados e alta pressão. O concentrado obtido por ultrassom destacou-se pela maior extração proteica, melhor solubilidade, capacidade de retenção de água e excelentes propriedades de formação e estabilidade de espuma. Além disso, o uso das tecnologias não térmicas resultou em redução significativa no tempo e na quantidade de reagentes necessários para a obtenção dos concentrados proteicos. Esta tese apresenta resultados inéditos e importantes para a comunidade científica e para a indústria de alimentos, fornecendo informações que podem orientar a introdução de produtos alimentícios à base de insetos no mercado brasileiro, bem como a produção de um ingrediente rico em proteínas derivado de insetos, que pode ser utilizado na formulação de novos alimentos.

Palavras-chave: insetos comestíveis; estudo de consumidor; representação social; segmentação de mercado; concentrado proteico; tecnologias não térmicas.

ABSTRACT

Edible insects emerge as a solid alternative to meet the growing demand for animal protein driven by the increase in the world population. They stand out for their high protein and lipid content, and their environmentally sustainable farming practices. However, despite these advantages, edible insects have yet to gain widespread acceptance in Western countries. In this context, this study aimed to initially evaluate the social representation of Brazilians regarding foods with edible insects to identify strategies for introducing these products in the domestic market. We found that consumers from different regions of Brazil have distinct perceptions of edible insects, with the Central-West and North regions showing a greater tendency for consumption compared to the South, Southeast, and Northeast regions. This disparity can be attributed to the different experiences and cultural characteristics of the populations in these regions. Furthermore, we identified and characterized consumer groups, where young males with higher education exhibited more favorable attitudes and lower perceived risk towards insect consumption. Lastly, this study revealed a preference among Brazilians for using crickets and treatments that avoid the visible presence of insects in food. Thus, we used a native Brazilian cricket species (*Gryllus assimilis*) to produce a powdered protein concentrate, employing the non-thermal technologies of ultrasound, pulsed electric fields, and high pressure. The ultrasound-assisted concentrate stood out for its higher protein extraction, improved solubility, water-holding capacity, and excellent foam formation and stability. Additionally, using non-thermal technologies significantly reduced the time and reagents required for obtaining the protein concentrates. This thesis presents novel and important findings for the scientific community and the food industry, providing insights that can guide the introduction of insect-based food products in the Brazilian market, as well as the production of a protein-rich ingredient derived from insects that can be used in the formulation of new food products.

Keywords: edible insects; consumer study; social representation; market segmentation; protein concentrate; non-thermal technologies.

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INTRODUÇÃO GERAL E OBJETIVOS

1. Introdução geral

O consumo de insetos, conhecido como entomofagia, tem sido amplamente estudado como uma alternativa viável para atender à crescente demanda global de alimentos. Esta prática remonta à história e pré-história humana (Ramos-Elorduy, 2009; Bodenheimer, 1951) e os insetos comestíveis continuam sendo incluídos na dieta tradicional de mais de 2 bilhões de pessoas em todo o mundo, principalmente na Ásia, África e América do Sul (van Huis et al., 2013).

Diversos aspectos positivos dão suporte para promover o uso de insetos como uma fonte alimentar sustentável. Em comparação com animais de corte tradicionais, os insetos necessitam de menos espaço e água, apresentam maior fecundidade, melhor taxa de conversão alimentar e podem ser criados em resíduos orgânicos, contribuindo para a valorização da biomassa (Vinci et al., 2022). Além disso, dependendo da espécie, estágio metamórfico e alimentação, alguns insetos contêm proteína de alta qualidade e aminoácidos essenciais, além de alto teor de vitaminas e minerais (Riekkinen et al., 2022; Köhler et al., 2019). Ainda, apresentam baixa concentração de colesterol comparado a outros produtos de origem animal e exibem uma proporção favorável de ácidos graxos ômega 6/ômega 3 e ácidos graxos poliinsaturados/saturados (Hlongwane et al., 2020; Rumpold e Schlüter, 2013). A combinação de alto valor nutricional, menor impacto ambiental e baixos custos de produção tornam os insetos particularmente interessantes como “mini rebanhos” voltados para a alimentação humana (van Huis et al., 2013).

Apesar das vantagens nutricionais e ambientais evidentes, a maioria dos ocidentais não parece convencida a adotar os insetos como uma fonte de alimento. Dagevos (2021) revisou diversos estudos que avaliaram a percepção de consumidores sobre insetos comestíveis e constatou que existe uma grande resistência em adotar a entomofagia como um comportamento alimentar comum nas sociedades ocidentais, já que os insetos não são reconhecidos como parte da dieta tradicional do Ocidente moderno. No entanto, é importante destacar que a adoção da entomofagia varia entre culturas e países e que os consumidores podem demonstrar diferentes atitudes, motivadores e barreiras para consumir insetos (Ardoin e Prinyawiwatkul, 2021). Portanto, tratar a entomofagia como uma cultura alimentar homogênea seria uma generalização equivocada.

Para desenvolver estratégias de inserção de alimentos à base de insetos no mercado brasileiro, é fundamental compreender a percepção dos consumidores sobre o tema. Embora existam estudos publicados sobre a percepção de consumidores na ingestão de insetos comestíveis, a maioria foi realizada na Europa e América do Norte (Ardoin e Prinyawiwatkul, 2021) e nenhum deles utilizou a abordagem da representação social para avaliar o consumidor brasileiro.

Com base nos resultados obtidos no estudo de consumidor (Bisconsin-Junior et al., 2020; Bisconsin-Junior et al., 2022), verificamos que os grilos são mais aceitos para o consumo do que outras espécies de insetos e que a adição de insetos aos alimentos como ingrediente em pó reduz a rejeição a este tipo de produto.

O inseto *Gryllus assimilis* é um grilo nativo do Brasil, criado comercialmente para alimentação animal e considerado adequado para o consumo humano (Magara et al., 2021). Durante a fase adulta do seu ciclo de vida, *G. assimilis* apresenta um alto teor de proteína, superior aos demais nutrientes, contendo aproximadamente 65% de proteína em peso seco (Soares Araújo et al., 2019).

Os insetos geralmente passam por processamentos térmicos para garantir a segurança no consumo como alimento (Melgar-Lallane et al., 2019). No entanto, altas temperaturas podem levar a efeitos indesejados no produto, como oxidação lipídica e escurecimento (Mishyna et al., 2020), e nas propriedades das proteínas, como a redução da solubilidade (Kröncke et al., 2018). Tecnologias não térmicas, como ultrassom, campos elétricos pulsados e alta pressão, são alternativas viáveis ao processamento térmico convencional, capazes de evitar os efeitos adversos das altas temperaturas. Além disso, as tecnologias não térmicas fornecem tempos de tratamento mais curtos e têm menor impacto ambiental (Chakka et al., 2021).

2. Objetivos

Estudar a percepção dos brasileiros sobre alimentos produzidos com insetos comestíveis e desenvolver um ingrediente alimentício em pó, com alto teor de proteína, a partir do *Gryllus assimilis*.

De maneira específica, os objetivos foram:

a. Avaliar as diferenças entre as regiões do país na representação social de insetos comestíveis, considerando a distância física e a identidade cultural regional dos brasileiros entrevistados;
b. Explorar a representação social dos insetos comestíveis para os brasileiros, identificando os grupos de consumidores com relação às suas características sociodemográficas e às suas associações com o tema;
c. Definir o perfil do potencial consumidor de insetos comestíveis no Brasil segundo o local de origem, características sociodemográficas e principais associações que favorecem o consumo deste alimento;
d. Comparar os efeitos das tecnologias não térmicas de ultrassom, campos elétricos pulsados e alta pressão no rendimento, tempo, e reagentes usados na extração das proteínas de *G. assimilis*;
e. Produzir concentrados proteicos de *G. assimilis* utilizando as tecnologias não térmicas e avaliar seus efeitos na estrutura e propriedades funcionais das proteínas.

3. Estrutura da tese

A estrutura desta tese consiste na apresentação de seis capítulos. Os detalhes de conteúdo de cada capítulo são descritos a seguir:

O Capítulo 1 apresenta uma introdução geral, os objetivos e o detalhamento da estrutura da tese.

O Capítulo 2 apresenta uma revisão bibliográfica sobre insetos comestíveis, a fim de descrever as vantagens de consumo, o contexto histórico e econômico, fatores de segurança alimentar e os principais desafios para a ampliação da aceitação desta matéria-prima. Parte desta revisão bibliográfica foi publicada na *Food Research International* (Mariutti et al., 2021).

O Capítulo 3 e 4 discorrem sobre os resultados obtidos no estudo de consumidor sobre insetos comestíveis. Estes resultados foram publicados em dois artigos, sendo um na *Food Quality and Preference* (Bisconsin-Junior et al., 2020) e outro na *Appetite* (Bisconsin-Junior et al., 2022). O Capítulo 3 refere-se ao estudo dos efeitos da cultura e da distância geográfica na representação de insetos comestíveis no Brasil. Enquanto, o Capítulo 4 refere-se ao estudo da representação social de alimentos com insetos comestíveis e a segmentação do mercado de consumidores no país.

O Capítulo 5 apresenta os resultados do uso das tecnologias não-térmicas de ultrassom, campos elétricos pulsados e alta pressão no processo de extração proteica de *G. assimilis*, além dos efeitos destas tecnologias na estrutura e propriedades funcionais das proteínas.

O Capítulo 6 refere-se à discussão geral e conclusão geral da tese.

CAPÍTULO II
REVISÃO BIBLIOGRÁFICA

Edible insects as an alternative food source

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Esta revisão foi publicada na *Food Research International* como parte do artigo:
Mariutti, L. R. B., Rebelo, K. S., Bisconsin-Junior, A., de Morais, J. S., Magnani, M., Maldonade, I. R., Madeira, I. R., Tiengo, A., Maróstica, M. R., & Cazarin, C. B. B. (2021). The use of alternative food sources to improve health and guarantee access and food intake. *Food Research International*, *149*, 110709. https://doi.org/10.1016/j.foodres.2021.110709

Abstract

As the world population continues to grow, the challenge of producing enough food to sustain everyone becomes more pressing. One potential solution to this problem is the use of insects as an alternative food source. In this review, we provide an overview of the use of insects as human food, including historical and current trends, and the prospects for this food sector. We also examine the nutritional content of edible insects, with a particular focus on their protein and lipid. Furthermore, we discuss the current challenges related to food safety, including biological, chemical, and allergenic hazards. Finally, we explore the major obstacles that need to be addressed before insects can be widely adopted as a food source, including legislation, consumer acceptance, pricing, and sustainability.

Keywords: alternative protein; nutrients; food safety; food legislation; consumer acceptance.

1. Introduction

The United Nations (UN) projects that the world population will reach 9.7 billion people in 2050 (United Nations, 2019), consequently, there will be a proportional increase in the demand for proteins of good nutritional quality, being the edible insects one of the alternatives to conventional products of animal origin. Furthermore, consumption of edible insects has advantages related to sustainability, such as the conversion rate of feed to protein, which is about 1.7 kg of feed per kg of insect, compared with 10 kg of feed per kg of cattle. Another advantage is the significantly low water consumption, being 1 L/kg of protein for insects in comparison with 1500 L/kg of protein for cattle; generation of fewer greenhouse gases and ammonia; and need of considerably smaller spaces (such as vertical farms) than other systems or proteins production, including cattle, poultry, and pigs (Baiano, 2020; van Huis et al., 2013).

2. History, current scenario, and perspectives

Entomophagy has become a trend in food research, creating the impression that edible insects are a new food fashion; yet they are not. Insect consumption by humans goes back thousands of years. Analysis of fossilized feces (coprolites) revealed that humans already consumed termites and diving beetles in the region of Great Basin in the U.S.A. in 9,500 B.C.; while in Mexico, ants, caterpillars, and dung beetle larvae were found in coprolites dating at least 5,400 B.C. (Sutton, 1995). Also, as stated by Yi et al. (2010), China has a long history of insect consumption, with records of up to 3,200 years. In Europe, the first reference of entomophagy is attributed to Aristotle (384–322 B.C.), in *Historia Animalium*, in which he reported that cicadas were considered delicacies and females had a superior flavor after copulation due to the presence of mature eggs (Bodenheimer, 1951). In South Korea, Jun Heo, considered the greatest Eastern medicinal physician (1546–1615 A.D.), wrote the book “Donguibogam” which described 95 different types of edible insects (Shin, Baker & Kim, 2018). Whilst the seventeenth and eighteenth centuries, some outbreaks of insects motivated its consumption in Europe: caterpillars of *Plusia gamma* were eaten in France, while locusts were consumed at a feast after a plague in Germany (Bodenheimer, 1951).

This food culture has persisted until the present day in many regions of the world. Entomophagy is still part of the traditional diets of at least 2 billion people, who belong to ca 3,000 ethnic groups in more than 100 countries, mainly in Africa, Asia, and Latin America (van Huis et al., 2013). Insects are consumed among different cultures because of their taste, cultural importance, nutritive value, and as a supplementary food when staple food is limited.

In Ghana, nine edible insects were found to be consumed by at least thirty percent of the population, being the palm weevil larva, the most consumed insect followed by termites and ground crickets (Anankware, Osekre, Obeng-Ofori & Khamala, 2016). While in the capital of the Democratic Republic of the Congo (Kinshasa), seventy percent of the 8 million inhabitants eat caterpillars and/or beetle larvae (Vantomme, Göhler & N'deckere-Ziangba, 2004). In North-East India (Nagaland region), 82 insect species are consumed in most of the tribes as a major component of their nutritional intake, representing an essential regular food type (Mozhui, Kakati, Kiewhuo & Changkija, 2020). Whereas Thailand developed a viable and thriving insect farming sector, which the most marketed and consumed insects are grasshoppers, crickets, beetles, weaver ants, silkworm pupae, and bamboo caterpillars. They are consumed as snacks in rural villages and on the crowded streets of Bangkok (Hanboonsong, Jamjanya & Durst., 2013). Besides, traditional Mexican cuisine is rich in edible insects, particularly in the state of Oaxaca, where chapulines (toasted grasshoppers) are notorious as a symbol of Mexican identity. Other popular edible insects in Mexico are maguey worms (Lepidoptera), chicatanas and escamoles (Hymenoptera) (Shockley, Lesnik, Allen & Muñoz, 2018).

Nowadays, most countries from the western world avoid the consumption of insects. As stated by DeFoliart (1999), the main hypothesis is linked to the European colonization. Large animals and plants were broadly domesticated in Europe and gave its people a considerable advantage, which favored them to colonize other regions. Thus, Europeans had a major influence on western food production, especially about habits, knowledge, techniques, and species which were regarded as edible. So, insect harvesting has been considered a primitive form of food acquisition and insects have come to be perceived as threats to the productivity of crops, as pests.

However, after FAO publication of the report entitled "Edible insects: future prospects for food and feed security" (van Huis et al., 2013), in 2013, a new movement promoting the consumption of insects started around the world. This report contains the state-of-the-art about edible insects for humans and animals, describing a wide range of research and information on topics such as culture, history, consumption, sustainability, nutritional value, rearing, processing, and economy. Following, the number of published research documents and patents about edible insects had a constant increase, year by year (**Fig. 1**). From 2010 to 2020, 558 documents using the term "edible insect" in title or keywords were retrieved in the Scopus database, with an expressive increase after 2014. For patents, 153 were published with "edible insect" in title or abstract or claims on Google Patents database, during the same period (2010-2020). In addition, the international conference "Insects to feed the world" (IFW) was created, together with several regional events, such as Insecta in Germany, Insetec in Brazil, and Insectinov in France, with all aiming to support the use of insects for human consumption and animal feed. Besides, the first scientific periodical about the subject, Journal of insects as food and feed (ISSN: 2352-4588), was released in 2015 by Wageningen academic publishers.

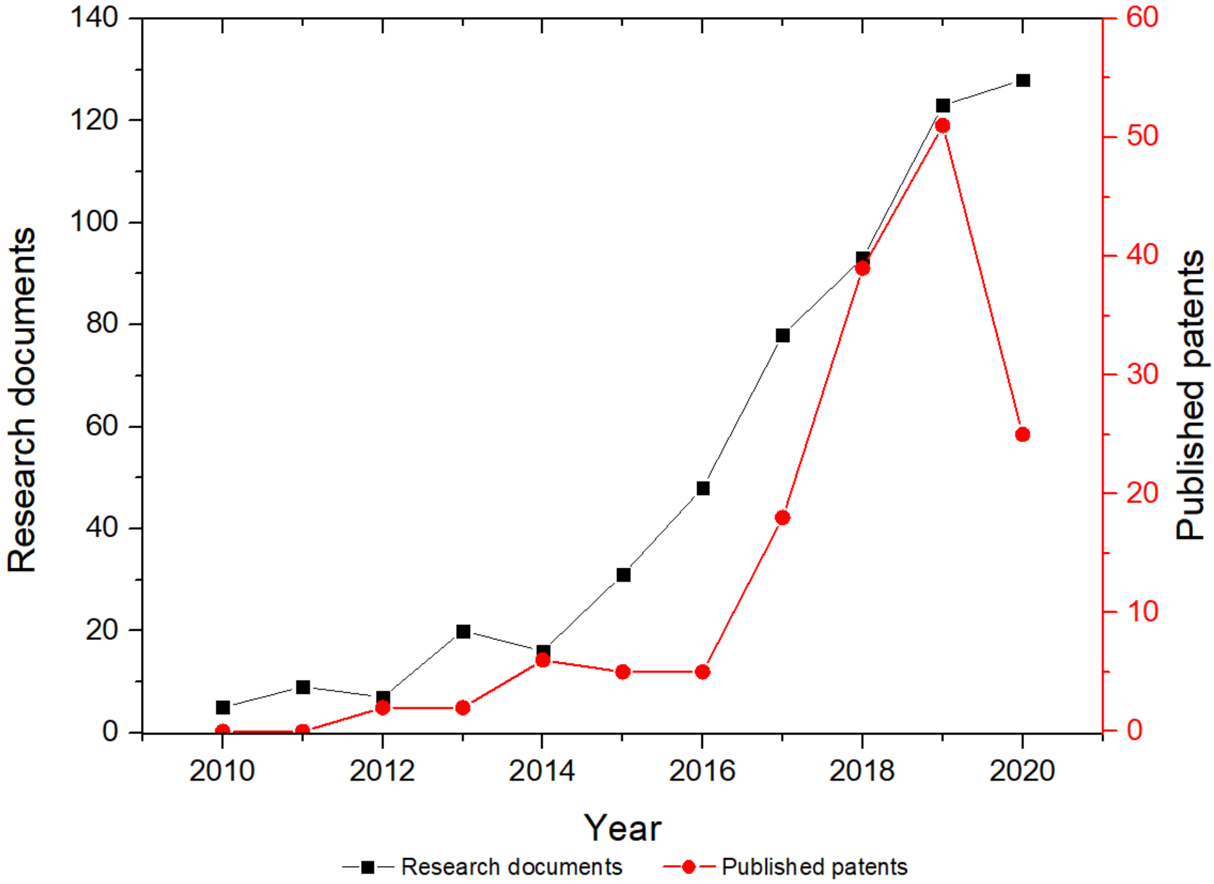

Figure 1. Research documents (Scopus database) and published patents (Google patents database) with the term "edible insect" in title or keywords or claims from 2010 to 2020 (Search on 1st October 2020).

Together with the increase of interest by academia and the third sector, the small-scale production and trade of edible insects scaled up, leading several companies and startups, mainly in the U.S.A., Europe, and Asia, to create and market insect food products for human consumption. Thus, requests for proper regulations of these food products emerged around the world (Wilderspin & Halloran, 2018).

Worldwide, the Codex Alimentarius provides a collection of standards, guidelines, codes, and recommendations to contribute to food production and food safety. However, at present, the Codex Alimentarius does not consider insects as food. Whereas the local regulations display substantial differences between each other, and most of them do not specifically address insects. In fact, some western countries have legislation that refers to insects only as impurities in food, establishing permissible levels, such as the U.S.A. (Food and Drug Administration of the United States of America, 2018) and Brazil (Agência Nacional de Vigilância Sanitária, 2014). In order to allow a successful and sustainable expansion of the edible insect industry, regulators, legislators, and policymakers should give considerable attention to create a standardized legal framework across the world (Baiano, 2020).

As pointed out by Mariod (2020), there are three major legal trends regarding edible insects: (a) the Anglo-Saxon countries, such as the U.S.A., U.K., Canada, New Zealand, and Australia, which edible insects are not perceived as novel food, and therefore the food agencies allow the production, import and sales; (b) the non-English-speaking Western countries, especially the European Union, which aims to establish rules to evaluate and approve insect products before allowing marketing; and (c) non-Western countries, for whom insects are usually regular food, which is rarely prepackaged, exported, or imported, and there is little or no regulation about edible insects.

Even without a clear set of standards, edible insects and its products are becoming popular in the U.S.A. Nowadays, it is possible to buy protein bars, snacks, insect powders and other processed foods. After several inquiries submitted to F.D.A. concerning the regulation of insects as food, the agency has not taken an official position, but made public its opinion during the IFT (2015) panel discussion on edible insects. According to Ramaswamy (2015), “insects are considered food if they are to be used for food or as component of food”, also insects shall be “clean and wholesome (i.e. free from filth, pathogens, toxin), must have been produced, packaged, stored and transported under sanitary conditions, and must be properly labeled (Sec. 403). The label should include the scientific name of the insect". Moreover, to be allowed in the market, insects must be raised specifically for human consumption, following current good manufacturing practices (cGMPs), so the collection of insects in nature for this purpose is not allowed. Besides, Mariod (2020) noted that the import from other countries is allowed, and that FDA already updated its Import Prior Notice with a list of edible insect products.

In the European Union, the new Regulation 2015/2283 on Novel Foods, and its implementing Regulations 2017/2468 and 2017/2469 came into force to clarify and integrate the rules concerning edible insects. Before that, the European Member States had different approaches to the old regulation on novel foods, resulting in a very heterogeneous marketing scenario between those countries. Now, edible insects are clearly considered as "novel foods". From January 2018, insects and insect products must be authorized by the European Commission before being placed on the market.

According to Pisanello and Caruso (2018), the novel food regulation provides two procedures to authorize the placing on the market of a novel food: a general procedure; and a notification procedure for traditional foods from third countries. The general procedure applies to any type of novel food and it takes at least 17 months. Therefore, a scientific dossier must be submitted to the European Commission, following the Regulation 2017/24696 and the EFSA guidelines (EFSA Panel on Dietetic Products et al., 2016; European Food Safety Authority, 2018). While the notification procedure applies only to the traditional food from a third country which satisfy two conditions: first, it is part of the regular diet of a significant number of people for at least 25 years, and second, it is derived from primary production as defined in Regulation 2002/178. The notification procedure requires only 5 months since there is no need to prove the safety of the product, but only the history of safe consumption in a third country.

Following the general procedure, on January 2021, EFSA published a scientific dossier about the safety on the use of dried *Tenebrio molitor* larva (EFSA Panel on Nutrition, Novel Foods and Food Allergens et al., 2021) and submitted it to the European Commission, which authorized the placing on the market of dried *Tenebrio molitor* larva as a novel food in June of 2021 (Regulation 2021/882).

The creation of regulatory frameworks will support the development of the global edible insect market, by increasing the consumers' and investors' confidence in insect food and feed products. Therefore, it is not easy to predict the future of the edible insect market. According to the market research report of Statista (2018), the edible insect market will go from an estimated value of USD 406 million in 2018 to USD 1.181 billion in 2023, almost a threefold increase in just five years. The most prominent region will be Asia-Pacific, representing 40% of the market value, followed by Europe (22%) and Latin America (21%). Besides, insect powder is the product type expected to grow at the highest rate in the next years, due to factors such as consumer unwillingness to eat the whole insect, high shelf life, and wide range of applications in several food and feed products. In another market research report (Meticulous Market Research Pvt. Ltd, 2019), the main drivers responsible for the growth of this market are: the increasing population and the decrease of food resources; the increasing demand for protein food combined with the high cost of animal protein; environment sustainability of production and consumption of insects; high nutritional value; and low risk of transmitting zoonotic diseases.

3. Insects consumed by humans and their nutrients

Nowadays, around 2000 insect species are consumed worldwide in different maturity stages, such as eggs, larvae, pupae and adults, being the most consumed beetles and caterpillars, followed by bees, wasps, ants, grasshoppers, locusts, crickets, cicadas, scales, true bugs dragonflies, termites, and flies (**Fig. 2**) (Ordoñez-Araque & Egas-Montenegro, 2021; Patel, Suleria & Rauf, 2019; van Huis et al., 2013).

Insects can be obtained both collecting in nature and by farming. However, most edible insects are harvested from the wild (92%), while only small amounts are raised. That is an alarming scenario, because the unsustainable collection of insects in nature can lead to serious ecological damage, such as habitat destruction or insect resource collapse (Yen, 2008). Thus, insect agriculture or “mini-livestock” cultivation, can sustain the increasing demand for edible insects and ensure food safety (Stull & Patz, 2020). Since, mini-livestock cultivation allows controlling the environment in which the insects are reared; it is possible to diminish the sources of contamination, as opposed to wild harvesting edible insects (Murefu, Macheka, Musundire & Manditsera, 2019).

Figure 2. Dried edible insects: a) house cricket, *Acheta domesticus*; b) Jamaican field cricket, *Gryllus assimilis*; c) banded cricket, *Gryllodes sigillatus*; d) grasshopper, (species not specified) e) buffalo worm, *Alphitobius diaperinus*; f) yellow mealworm, *Tenebrio molitor*.

The number of insect species currently being reared for human consumption is limited. Most of those insects belong to the Orthoptera, Coleoptera and Diptera orders and are mostly consumed in the larval (Coleoptera and Diptera) or adult (Orthoptera) stages. Also, those species have a history of already being reared as pet food or as bait for fishing (van Huis, 2020). From the Orthoptera order, the most common reared insects are crickets, such as *Acheta domesticus* (house cricket), *Gryllodus sigillatus* (banded cricket), *Gryllus assimilis*, *Gryllus testaceus* and *Gryllus bimaculatus* (field crickets), and grasshoppers, like *Locusta migratora* (migratory locust). While, from the Coleoptera, *Tenebrio molitor* (mealworm) and *Alphitobius diaperinus* (lesser mealworm) are the most often reared species. And finally, *Hermetia illucens* (black soldier fly) and *Musca domestica* (housefly) are more frequently reared in the Diptera order (Raheem et al., 2019).

Safety, flavor, appearance, size, availability, and nutritional profile are some of the factors that influence an insect to be considered edible (Ghosh, Jung & Meyer-Rochow, 2018). In order to define the best species for human consumption, Gere, Radványi and Héberger (2019) conducted a study comparing the nutritional profiles of edible insects. Yet, the authors concluded there are no “best” or “healthiest” insect species because it depends on the desired nutrients. For example, the adult *Tenebrio molitor* should be chosen based on the proximate composition, while *Galleria mellonella* larvae had the highest mineral content. Also, the nutritional profiles are highly diverse and are mainly influenced by the species and the developmental stage. However, to choose the best edible insect species for human consumption, it is not sufficient to consider just the nutritional profile, it also necessary to examine the rearing characteristics, flavor, and the potential for processing.

Edible insects are known to have a high concentration of high-quality nutrients, especially proteins containing all the essential amino acids (**Tables 1** and **2**). In addition, they are also rich in polyunsaturated fatty acids (**Table 3**) and fibers, mostly chitin. The consumption of 25 g/day of whole crickets in the form of flour for breakfast had a positive impact on humans’ microbiota and on the reduction of TNF-α in blood plasma, indicating a reduction in systemic inflammation (Stull et al., 2018).

Table 1. Proximate composition of some edible insects.

Insect	Country	Protein (g/100g) - dry basis	Lipids (g/100g) - dry basis	Carbohydrates (g/100g) - dry basis	Ashes (g/100g) - dry basis	Reference
Mysore thorn borer - *Anoplophora chinensis*	China	31.23	55.34	9.60	2.89	Wu et al. (2020)
Yellow Mealworm - *Tenebrio molitor*	China, Portugal, Poland	45.6-52.35	24.7-34.5	4.17-11.22	3.62-4.40	Costa et al. (2020); Wu et al. (2020); Zielínska et al. (2015)
Adult stage beetles - *Tenebrio molitor*	Mexico	54.86	12.50	nd	3.24	Flores et al. (2020)
Adult stage beetles - *Ulomoides dermestoides*	Mexico	40.36	8.33	nd	2.12	Flores et al. (2020)
Adult cricket - *Gryllodes sigillatus*	Poland	70.0	18.2	3.75	4.74	Zielínska et al. (2015)
Adult locust - *Schistocerca gregaria*	Poland	76.0	12.97	4.23	3.33	Zielínska et al. (2015)

nd – not determined

Table 2. Amino acid composition of some edible insects.

Amino acid	Stick insect (*Cladomorphus phyllinum*)[1]	Mysore thorn borer (*Anoplophora chinensis*)[2]	Yellow Mealworm *(Tenebrio molitor)*[2]	Yellow Mealworm *(Tenebrio molitor)*[3,4]	Adult cricket (*Gryllodes sigillatus*)[4]	Adult locust (*Schistocerca gregaria*)[4]
Histidine	9.7	6.65	8.37	16.1-28.9	17.2	20.6
Isoleucine	18.3	12.34	13.10	21.4-32.1	26.6	28.2
Leucine	36.7	19.48	22.06	45.8-75.9	57.8	77.7
Lysine	14.2	16.15	15.81	26.2-26.7	38.4	35.1
Methionine	5.4	5.63	6.01	9.6	15.9	8.2
Phenylalanine	4.4	11.94	13.09	16.1-34.3	22.0	18.7
Threonine	14.9	11.49	12.66	26.1-54.8	36.8	35.5
Tryptophan	Nd	1.8	2.98	nd	nd	nd
Valine	26.5	16.24	18.91	39.7-49.2	47.0	56.6
Arginine	22.2	13.26	18.85	25.6-45.6	46.6	39.8
Cysteine	4.3	12.69	11.86	5.5	11.1	3.6
Tyrosine	34.6	19.45	21.46	28.8-67.2	31.8	33.1
Alanine	50.6	14.38	24.83	44.3-100.9	58.0	88.8
Aspartic acid	35.3	19.10	15.44	50.5-97.4	72.8	66.1
Glutamic acid	52.1	34.46	39.19	79.7-125.3	106.6	107.5
Glycine	32.7	14.12	17.06	31.8-66.0	40.7	49.4
Proline	32.6	22.48	20.01	43.4-95.9	54.2	67.1
Serine	32.2	14.10	13.61	28.8-58.1	40.4	33.7

nd – not determined. [1] Botton et al. (2021) expressed in mg/g protein. [2] Wu et al. (2020) expressed in mg/g. [3] Costa et al. (2020) expressed in mg/g protein.[4] Zielínska et al. (2015) expressed in mg/g protein.

Table 3. Major fatty acids of some edible insects.

Fatty acid (%)	Stick insect (*Cladomorphus phyllinum)*[1]	Mysore thorn borer (*Anoplophora chinensis*)[2]	Yellow Mealworm *(Tenebrio molitor)*[2,3,4]	Adult cricket (*Gryllodes sigillatus*)[4]	Adult locust (*Schistocerca gregaria*)[4]
Myristic acid (14:0)		0.59	2.12-4.0	1.65	1.68
Palmitic acid (16:0)	15.94	25.08	15.3-18.0	23.5	23.26
Palmitoleic acid (16:1)		7.48	1.94-2.8	3.78	1.80
Stearic acid (18:0)	10.67	0.30	0.69-3.84	7.35	9.27
Oleic acid (18:1)	57.03	60.07	37.8-43.77	29.14	36.22
Linoleic acid (18:2)	13.76	4.28	29.39-33.2	29.78	14.04
Linolenic acid (18:3)		0.94	1.5-2.27	2.13	11.35

[1] Botton et al. (2021). [2] Wu et al. (2020). [3] Costa et al. (2020).[4] Zielínska et al. (2015).

Table 4. Minerals from some edible insects

Insect	Mineral (mg/100g dry weight)						
	Calcium	Magnesium	Zinc	Copper	Iron	Manganese	Reference
Stick insect (*Cladomorphus phyllinum)*	2548.28	275.11	43.99	0.66	4.96	8.81	Botton et al. (2021)
Alate termites (*Macrotermes subhyalinus*)		104.8	10.3	8.5	13.4	422	Verspoor et al. (2020)
Alate termites (*Macrotermes* spp.)			12.0-13.8	5.1-8.2	9.8-10.3	292-418	Verspoor et al. (2020)
Alate Termites (*Odontotermes* spp.)		95	9.2-12.9	6.6-7.6	8.8-13.9	271-515	Verspoor et al. (2020)
Tobacco Cricket (*Brachytrupes membranaceus*)			16.6	1.0	65.7	2.8	Verspoor et al. (2020)
Locust (*Locusta migratoria*)		85	25	6.0	9.2	1.0	Verspoor et al. (2020)
House cricket (*Acheta domesticus*)		68.1	26.6	5.3	9.2	3.8	Verspoor et al. (2020)
Water scorpion (*Lethoserus indicus*)		111.3	11.5	2.3	33.4	1.2	Verspoor et al. (2020)
Queen leafcutter Ant (*Atta* spp.)		64.6	19.0	2.8	11.0	2.1	Verspoor et al. (2020)
Mopane worm *(Gonimbrasi belina)*			16.6	6.4	54.5	3.9	Verspoor et al. (2020)
Silkworm pupae (*Bombyx mori*)		305.5	17.7	2.2	3.8	1.9	Verspoor et al. (2020)
Yellow Mealworm (*Tenebrio molitor*)	32-41	233-304	9.65-14.4	0.78-2.5	3.29-18.42	0.5-1.89	Costa et al. (2020); Verspoor et al. (2020); Wu et al. (2020); Zielínska et al. (2015)
Mysore thorn borer (*Anoplophora chinensis*)	26.93	188.10	22.36	0.90	13.13	3.50	Wu et al. (2020)
Adult cricket (*Gryllodes sigillatus*)	130	101	13.9	4.79	4.23		Zielínska et al. (2015)
Adult locust (*Schistocerca gregaria*)	70	82	18.6	6.32	8.38		Zielínska et al. (2015)

Protein represents the main component of the nutrient composition of insects; however, most of the reported values are overestimated. The protein content is often determined based on the total nitrogen content and most authors use a nitrogen-to-protein conversion factor of 6.25. Insects contain a varying amount of non-protein nitrogen, then the most appropriate factors should be determined for each species, for instance, 4.76-5.41 for mealworms, 5.25 for crickets and 5.33 for locusts (Boulos, Tännler & Nyström, 2020; Costa et al., 2020); therefore, care must be taken comparing protein values, and to not overestimate them.

The micronutrients of edible insects, such as minerals (**Table 4**) and vitamins vary enormously and are extremely influenced by the insect diet (Baiano, 2020). Insects can be sources of riboflavin, pantothenic acid, and biotin, but are usually deficient in vitamins A, C, and E (Rumpold & Schlüter, 2013).

It is important to highlight that the edible insect's composition is affected by the species, maturity stage, feed, and pre-processing and processing conditions, and their flavor in is influenced by their habitat in the case of wild insects. A recent systematic review presents a compilation of the nutritional data from 54 edible insects consumed in Africa, providing insightful information on proximate composition, vitamins A, C and B2, Fe, Ca, Zn, P, and Mg contents (Hlongwane, Slotow & Munyai, 2020).

The consumption of insects is more frequent in the tropics than in temperate regions of the world. As verified by Lesnik (2017), there is an inverse correlation between the latitude and the consumption of insects; so, as latitude increases, use of edible insects reduces. This pattern is related to the higher abundance of insect species in tropical environments, allowing a greater chance of finding appealing edible insect options.

Numerous methods have been applied for the traditional preparation and preservation of edible insects. Post-harvest procedures, such as removal of head, legs, wings, and other appendices are usually employed during the preparation; while steaming, boiling, baking, deep-frying, sun-drying, smoking, and transforming into a paste are the most common traditional methods for preparation and preservation (Rumpold et al., 2014).

Although, in the western market, insect-based food products are a new phenomenon. Over the last decade, numerous companies and startups, mainly in Europe, South Asia, and North America, have been founded with the aim of marketing edible insect products. The main edible insect products are flavored snacks, energy bars, and powders, while crickets, grasshoppers, and mealworms are the most commercialized insects (Melgar-Lalanne, Hernández-Álvarez & Salinas-Castro, 2019). Currently, the huge variety of edible insect products commercialized worldwide can be seen in “The big list of edible insect products!” (https://www.bugburger.se/guide/the-big-list-of-edible-insect-products/), organized by Anders Engström and last updated in July 2020.

4. Food safety of edible insects

In addition to the nutritional, environmental, and economical benefits associated with edible insects, they may host endogenous and exogenous risk factors to human health, just like any other type of food. The main route of contamination of food safety hazards in edible insects is the rearing environment, especially the feed substrate (van der Fels-Klerx, Camenzuli, Belluco, Meijer & Ricci, 2018). Therefore, to improve the food safety aspects of edible insects, the production setting and the feed need to be assessed and controlled, which is only possible if these insects are under cultivation.

There are three main categories of potential food safety hazards related with edible insect consumption: (i) biological, such as harmful microorganisms, viruses, and parasites; (ii) chemical, such as mycotoxins, heavy metals, and pesticides; and (iii) allergens.

Insects might act as vectors of microorganisms by mechanical contact with contaminated body surface or due to microbial growth inside the insect, even without any sign of disease (Wasala, Talley, Desilva, Fletcher & Wayadande, 2013). Generally, insect-specific pathogenic microorganisms are particular for invertebrates, thus being harmless to humans. Since there is a large taxonomical distance between insects and humans, these microorganisms can colonize most probably only in invertebrates’ tissues (Eilenberg, Vlak, Nielsen-Leroux, Cappellozza & Jensen, 2015).

Regarding human pathogenic bacteria, *Salmonella* sp. and *Listeria monocytogenes* seems to be major concerns of contamination during the rearing of edible insects, due to their pathogenicity and ability to colonize the gastrointestinal tract of insects (Wynants et al., 2019). Thus, these bacteria present an immediate health risk to the consumers, particularly in communities with the habit of consuming raw insects.

There is scarce literature evaluating the dynamics of viruses in edible insects. Thereby, two major concerns emerge from that lack of studies: (a) if the arboviruses, such as Dengue, West Nile Disease, Rift Valley Fever, Hemorrhagic Fever, and Chikungunya, are able to replicate in edible insects and be transmitted to humans through ingestion; and (b) if the foodborne viruses, such as rotavirus, norovirus, hepatitis A, and hepatitis E, can be introduced during insect rearing and be transferred to humans. Concerning the potential of edible insects to transmit the SARS-CoV-2 virus, responsible for the COVID-19 pandemic, Dicke et al. (2020) evaluated the mechanisms of virus fixation in insect cells. Thus, they recognized that the receptors of the angiotensin-converting enzymes ACE2 of insects are different from the receptors of vertebrates, making it impossible for the SARS-CoV-2 virus to replicate inside the insect cells. Also, Balaraman et al. (2021) tested SARS-CoV-2 replication in several insects' species and confirmed that those insects and their cells cannot support the virus replication. Still, the hygiene practices should be observed, as insects such as flies and cockroaches can carry and transfer viral particles through contact with contaminated surfaces.

As pointed out by Gałęcki and Sokół (2019), insects are an underestimated reservoir of human parasites. They evaluated 300 live edible insects (mealworms, house crickets, Madagascar cockroach, and migrant locust) and found 30% with parasites potentially pathogenic for humans. Also, several cases of human infestation by the nematode *Gongylonema pulchrum* have been reported in many places around the world and were occasionally related with raw insect consumption (Molavi, Massoud & Gutierrez, 2006). In Southeast Asia, where insect-eating is common, human autopsies and insect analyses revealed that trematodes from the family of Lecithodendridae and Plagiorchidae can be transmitted orally (Chai, Shin, Lee & Rim, 2009). Some cockroach species can harbor pathogenic protozoa like *Entamoeba histolytica*, *Giardia lamblia*, *Toxoplasma* spp. and *Sarcocystis* spp. (Belluco, Mantovani & Ricci, 2018).

It is mandatory to use subsequent treatments, such as boiling or frying to eliminate the pathogenic microorganisms, viruses and/or parasites to reduce the biological hazards after killing the insects. Also, those risks could be mitigated through strict hygienic rearing, handling, and processing.

Edible insect food products may contain chemical residues, such as, mycotoxins, heavy metals, and pesticides. However, there is limited literature of these substances in reared insects, also on excretion or accumulation of chemical contaminants from the feed substrates.

Mycotoxins in edible insects may originate from contamination of substrate by *Aspergillus*, *Penicillium* or *Fusarium*, or when handled or stored at sub-optimal conditions (Camenzulli et al., 2018; Musundire, Osuga, Cheseto, Irungu & Torto, 2016).

Heavy metals from the environment or feeding substrate have been found in the tissues and organs of insects, where they can be bioaccumulated. Larvae of *T. molitor* and *H. illucens* can accumulate cadmium, lead, and arsenic when they are reared on contaminated substrates (Vijver, Jager, Posthuma & Peijnenburg, 2003), but do not accumulate chromium, arsenic, nickel, and mercury (Purschke, Scheibelberger, Axmann, Adler & Jäger, 2017). Truzzi et al. (2019) investigated the presence of cadmium, lead, nickel, arsenic, selenium, and mercury in *T. molitor* larvae fed with substrates made from residues of olive processing and found that only mercury bioaccumulation was evident, and that selenium protected from mercury toxicity. A report shows an outbreak of lead poisoning in California was caused by excessively high lead levels in dried chapuline grasshopper (*Sphenarium*), which acquired lead from silver mines in Mexico (Handley et al., 2007).

Pesticides applied in agriculture are potentially dangerous for insect consumers, especially if the insects came from wild harvesting instead of controlled farming. Locusts collected in Kuwait contained high levels of organophosphorus pesticides, probably due to the unrestrained pesticides use in that area (Saeed et al., 1993). In addition, pesticide residues may be present in the feed substrate of insects. Chlorpyrifos was found in *Musca domestica* larvae fed on milk powder and sugar, while piperonyl butoxide was detected in *Calliphora vomitoria* (Charlton et al., 2015).

A wide range of foods causes allergic reactions, especially those containing protein. Since, insects are rich in proteins, edible insects and their products may be potential allergenics, and may cause allergic reaction by contact, inhalation, and oral ingestion (Imathiu, 2020). Food allergy to insects has been reported for silkworm, mealworm, caterpillars, *Bruchus lentis*, sago worm, locust, grasshopper, cicada, bee, *Clanis bilineata*, and the food additive carmine, which is derived from female *Dactylopius coccus* (Gier and Verhoeckx (2018). Edible insect allergic reactions have also been registered by mopane cartepillars (*Imbrasia belina*) in Africa (Kung, Fenemore & Potter, 2011) and silkworm in China (Ji, Zhan, Chen & Liu, 2008). According to a report of allergies provoked by food consumption in China (Ji et al., 2009), between 1980 and 2007, insects were the fourth most frequent cause of anaphylactic shock, for which locusts, grasshoppers and silkworm pupae were the mainly responsible.

Some types of proteins present in edible insects are considered allergen, like arginine kinase, α-amylase and tropomyosine, and can induce IgE-mediated allergic reactions in sensitive humans (Mitsuhashi, 2008). House dust mite allergic patients have a high risk for cross-reactions to desert locust and house cricket, while the same happens with crustacean allergics' associated with desert locust, house cricket and stable flies, and flies allergics' with house cricket, desert locust and migratory locust (Pali-Schöll et al., 2019). Thus, a warning must be given for dust mite, crustacean or flies allergic patients before they consume edible insects.

5. Major challenges and concerns

The edible insect sector is emerging. The numbers of companies, startups, investors, and insect-based products in the market are growing fast, and to maintain this growth sustainable it is necessary to deal with the current major challenges: western acceptance; the price of edible insects; and food safety and legislation. Moreover, two important concerns need further investigation to establish edible insects as an ethical food sector: the sustainability of processed insect foods; and the insects' welfare.

A crucial aspect that could threaten the edible insect sector is the aversion that the Western population shows for insects as food. Regarding that eating insects is a new phenomenon for Western consumers, it is not unexpected that the main factors which explain this aversion are neophobia and disgust (Looy, Dunkel & Wood, 2014). Food neophobia leads to the rejection of food when it is recognized as unfamiliar, while, the disgust associations tend to be passed between generations, reinforcing cultural differences in what evokes disgust. To overcome the food neophobia barrier, La Barbera et al. (2018) suggest that with the introduction of more insect food products on the market, public and private discussions will take place, and more people will start eating them. Eventually, the insect food products will be regarded as familiar. This process is slow and could take a generation to happen. While, to deal with the disgust factor, Jensen and Lieberoth (2019) proposed that Westerners' insect disgust may be reduced by availability of insect food products in the market combined with positive social models, through broadcasting insect-eating behavior by persuasive role models and the numbers of people eating insects.

Others key drivers to enhance the acceptance of insects as food are positive first eating experiences, which increase future willingness to eat them; target consumers who seek for novelty and sensation in their diet; information about the environmental benefits of replacing traditional animal protein for insect protein; and increasing the availability and reducing the price of insect products; and using processed insects which are unrecognizable inside the food. (Hartmann & Bearth, 2019).

The use of processed insects as an ingredient in foods is a well-known strategy to reduce disgust associations with edible insects. However, to obtain insect powders or their specific components, there are several processing steps that consume substantial amounts of energy, such as blanching, freeze-drying, oven-drying, and grinding. For that reason, eating the whole insect is a better option from a sustainable point of view, but they have higher rejection compared with processed insects. Thus, the sustainability aspect of processed insect foods should be evaluated in-depth to determine the real environmental gain of such products.

For being competitive with traditional livestock, the cost of edible insect farming needs to be reduced. This is linked with the efficiency of production, not only in terms of techniques and methods to rear insects, but also with the species used and the feeding substrate (van Huis, 2020). The technology development for insect production is just starting to be studied and refined. There are scarce data describing rearing mechanisms to increase feed conversion ratio, energy efficiency, and water use. Therefore, more research and experiments are mandatory to evolve the cultivation systems for rearing a variety of insect species on a wide range of feed substrates (Stull et al., 2020). One of the most promising solutions to lower the price is the use of organic side streams with a negative value as feed substrate for insects, such as agricultural byproducts, manure, crop residues, urban and catering waste. At the present, there are legal impediments to use these organic materials, but it should be investigated to which degree they may pose a real problem for food safety (van Huis, 2020).

A relevant concern related to the rearing and slaughter of insects is their welfare. There is no scientific consensus on whether insects are able of feeling pain or even are sentient beings (van Huis, 2019), which makes it impossible to evaluate the effects of the rearing and slaughter practices on the insect's welfare. Therefore, it is recommended to adopt the cautionary principle, giving invertebrates the benefit of the doubt. Thus, all practices related to the health, breeding, and slaughter of insects must be established to minimize their possible suffering.

Overall, there is a lack of literature to understand the real potential of food safety hazards for edible. So, more studies evaluating the dynamics of pathogens and toxins on edible insects are essential. These studies should be carried on a case-by-case basis using insect species that are promising for use as food and reared in an appropriate environment (van der Fels-Klerx et al., 2018). Even been recognized as food in most parts of the world, only European Union has a food safety regulation on edible insects. Thus, it is paramount to establish a worldwide legal framework to ensure the safety of insect food products (Murefu et al., 2019). Consequently, the consumption of insects will increase by boosting the confidence of consumers and investor in edible insects.

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CAPÍTULO III

Examining the role of regional culture and geographical distances on the representation of unfamiliar foods in a continental-size country

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Este artigo foi publicado na *Food Quality and Preference*:
Bisconsin-Júnior, A., Rodrigues, H., Behrens, J. H., Lima, V. S., da Silva, M. A. A. P., de Oliveira, M. S. R., Januário, L. A., Deliza, R., Netto, F. M., & Mariutti, L. R. B. (2020). Examining the role of regional culture and geographical distances on the representation of unfamiliar foods in a continental-size country. *Food Quality and Preference*, *79*, Article 103779. https://doi.org/10.1016/j.foodqual.2019.103779

Abstract

This study provides an experimental-exploratory investigation about the role of regional culture and Euclidean distances on the consumers' representation of edible insects in Brazil, a country with an extensive geographical surface. Seven hundred and eighty participants were recruited on the streets of eight cities from different Brazilian states: Manaus in Amazonas; Porto Velho in Rondônia; Macapá in Amapá; Cuiabá in Mato Grosso; Aracaju in Sergipe; Rio de Janeiro in Rio de Janeiro; Campinas in São Paulo; and Santa Maria in Rio Grande do Sul. These participating cities were considered from their cultural identity differences and geographical distances. Through a continual restricted word association task, participants were instructed to promptly verbalize the first five terms that came to their minds when stimulated with the expression "food made with edible insects". Following, they had to score the valence of each term they produced. The dictionaries produced in each city were compared and classified into groups using the Ellegård's index. Each group presented distinct ways of expression and attitude with respect to the inductive expression. Basically, Brazil was divided into two main groups according to their representation of edible insects: one consisted by the cities situated near the shore of the Atlantic Ocean, which present a cultural formation influenced by the European immigrants; and the other comprised the cities from the continental region that have strong cultural influence from the Amerindians. Thus, the cultural formation was more decisive to explain the similar representations among the cities than their geographical proximity. Given that, to effectively introduce a novel food in a country with varied regional culture, the marketing strategy should be focused on the values and beliefs of their culture subgroups instead of a single strategy for the whole country.

Keywords: regional culture; food representation; restricted continued word association; Ellegård's index; entomophagy; unfamiliar food.

1. Introduction

In traditional consumer research, a range of variables is taken into account for marketing, persuasion and publicity strategies before introducing novel foods in different societies. These variables are commonly socio-demographic characteristics, personality, habits of consumption and culture of consumers. Culture has already been considered (de Mooij & Hofstede, 2011) and plays a fundamental role in the way individuals interact with consumer goods, impacting their perceptions (Prescott & Bell, 1995; Prescott et al., 1997; Parr, Ballester, Peyron, Grose, & Valentin, 2015), attitudes (Charters et al., 2011), acceptance (de Magistris, Groot, Gracia, & Albisu, 2011) and representations (Son et al., 2014).

The concept of "culture", in cultural psychological research, was well-defined by Hofstede (2001) as "the collective programming of the mind that distinguishes the members of one group or category of people from another" (p. 9), which refers that people belonging to the same cultural group share similar characteristics that differentiate them from another cultural group. Hofstede and McCrae (2004) further explained this concept in three characteristics, as follows: culture is a collective, not individual, attribute; not directly visible but manifested in behaviors; and common to some but not to all people. More specifically, for Claval (1995), culture is deeply conceived according to some criteria, among which the following stand out: (a) it is the mediation between man and nature; (b) it is the inheritance, the result of a game of communication; (c) allows individuals and groups to project themselves into the future; (d) it is done by words, articulated by speeches and performed in representation; (e) it is an essential factor of social differentiation. Thus, we can conceive culture as a useful concept in food studies consisting of a set of beliefs and values that guide the actions of a particular social group. In turn, these beliefs and values give the singular characteristics which define the social group through contrast, giving rise to cultural identity (Neto & Bezzi, 2008).

In almost all food science studies, culture gains the notion of "nationality" (Rodrigues, Otterbring, Piqueras-Fiszman, & Gómez-Corona, 2019; Tuorila, 1998). Many scientific works developed in the 1990s and the 2000s exemplify this phenomenon (Rozin, Fischler, Imada, Sarubin, & Wrzesniewski, 1999; Ferdenzi, Mustonen, Tuorila, & Schaal, 2008). At this time, an inspiring study developed by Prescott et al. (1997) aimed at disclosing the perceptions of sweetness in food by participants from two different cultures: Japan and Australia. The perception of sweetness was manipulated in three foods, namely ice cream, cornflakes and orange juice. Through sensory analysis, participants were invited to rate sweetness intensity, sweetness liking, sweetness just right intensity, overall liking for all samples and a range of other sensory attributes. Results showed that hedonic responses to manipulated sucrose levels in the three foods showed clear differences between cultures and foods, despite the general agreement in the perception of the sweetness levels. For cornflakes, the Australian had greater sweetness liking ratings than the Japanese; while for ice cream, the Japanese liked the majority of the sweetness levels more than the Australian; and for orange juice, a high degree of agreement between the two cultures was observed, but the Japanese were more willing to tolerate sweeter juices. Finally, they concluded that an unbiased assessment of preference for sweetness may only be possible in foods that are novel or unfamiliar to both cultures, which was not the case of the referred study. Thus, the authors pointed out the difficulty to escape from the influence of food familiarity in crosscultural studies, showing the importance to take this parameter into account at the moment of planning cross-cultural experiments.

In a different line, a qualitative study developed by Guerrero et al. (2010) compared consumers-driven associations from six European regions (Flanders – Belgium, Mazovia – Poland, Burgundy – France, Catalonia – Spain, Akershus and Østfold – Norway and Lazio – Italy) about their driven-definition of traditional food products. Through word association, they were asked to provide single-word responses and avoid brands when they were stimulated with the word "traditional". Concerning the comparison among European regions/countries, the results showed some similarities and contrasts. In general, southern European regions tended to associate the concept of "traditional" more frequently with broad concepts such as heritage, culture or history. Central and Nordic European regions tended to focus mainly on practical issues such as convenience, health or appropriateness.

In those cross-cultural studies, as seen before, a static definition of culture was approached by the researchers: consumers from the same country speaking the same language belong to the same culture. From them, like many other studies, it is now widely accepted by the food and consumer research communities that the culture in which we were born and within which we were socialized plays a significant role in how we respond analytically and hedonically to the incoming information from our senses (Prescott, Young, O'neill, Yau, & Stevens, 2002; Rodrigues & Parr, 2019).

However, thanks to the diversity of cultural identities on a given society, the concept of culture, as demonstrated before, has been repeatedly revisited and is well accepted as much more than classifying people by nationality or by speaking the same language. In a recent review, Ares (2018) resumes Sobal (1998) arguing the importance of identifying cultural groups within a society because different sub-cultures retain their own identity, like in the United States due to its large migratory flux. For example, several works have explored health and nutritional aspects of consumption among different ethnic groups in USA (Hispanic-Mexican and Africans) and their tendency to develop, from their food consumption habits, coronary diseases, diabetes, among other diseases (Eicher-Miller, Fulgoni, & Keast, 2015; Blumberg, Frei, Fulgoni, Weaver, & Zeisel, 2017; Casagrande et al., 2018). In the same line, Chang, Thach, and Olsen (2016) aimed at exploring the influence of the ethnicity of American consumers (with other demographic characteristics as gender and age group) and their influence on wine and health representation. An online survey was used to collect data from more than 1000 U.S. wine consumers divided in African American, Hispanic, Asian and White/Caucasian. The results were interesting, and regarding the ethnicity of consumers, there was a difference in the level of health consciousness. African and Asian American people are more concerned about their health when compared with other ethnical groups. Although no differences were found concerning the beliefs that wine is healthier than other alcoholic beverages such as beer and spirits, African Americans tend to believe that sparkling wine is healthier than other wine categories (thanks to the beliefs of the effervescence effect on digestion), disclosing differences in their choices when compared to other studied cultures. That is important because the system of beliefs of an ethnic group seems to drive their behaviour towards different objects of consumption, which may be different on another ethnical group in the same country.

Historically, ethnic groups have been moving around the world for centuries, either through colonization, at the time of navigations; known as European colonialism (Sluyter, 2001); through the Diaspora (dispersion of a people as a result of political, religious or ethnic prejudice or persecution) (Huyssen, 2003); or even by force, which is the case of the slavery of the African natives sent to Europe or to the European colonies for forced labour (Burin, 2008). These migratory waves helped to fragment countries forging strong regional identities, that when comparing regions of the same country, may differ in terms of gastronomy, people behaviour and even manufacturing production, due to this ethnic fragmentation. This was the case of many countries in the Americas colonized by British, Spanish and Portuguese. For instance, Brazil was colonized by Portuguese and has received throughout its history a diversity of immigrants from different nationalities for the formation of this big country. They contributed to the formation of regions with different cultural identity. In accordance with Neto and Bezzi (2008), identity exists as a function of culture, as a resultant product, capable of expressing its most distinctive characteristics, attributing "cultural values". In this case, the identity of a region refers to the cultural (e.g., history, ethnic formation, art, music) and physical (e.g., geography) characteristics which distinguish regions (Kotler & Gertner, 2011). Allied to these regional identities in a country with a continental surface such as Brazil, could the geographic distance between regions also be a factor of accentuation of cultural dissimilarity on food representations?

Even nowadays, with television perpetuation and all the advent of the connected world that has contributed to the reduction of cultural distances, countries separated by long distances such as Brazil and Russia, for example, can have an "isolation" of the inhabitants of certain localities due to the difficulty of transport networks (e.g. exorbitant price of air tickets, poor roads and travel time). In business, marketing and technology sciences, this factor is always considered and has a long history (Armstrong & Cole, 2002; Holmstrom, Conchúir, Agerfalk, & Fitzgerald, 2006; Ambos & Ambos, 2009). In marketing, for example, Audretsch and Stephan (1996) suggested that geographic proximity facilitates informal social interactions. This purported link is also supported by research on interpersonal communication, which has found that physical proximity is positively related to greater amounts of communication (Conrath, 1973; Gullahorn, 1952), suggesting that the proximity and/or geographical distance may impact communication, understanding and the change of social and cultural norms and finally, the sharing among social actors. This means that geographical proximity and, above all, communication between people from different regions within the same country can be a vector of transformation of social norms. Consequently, there is a reduction of cultural barriers between different groups that gravitate in the same region, which hypothetically, would reduce the cultural dissimilarity within the country.

Keeping this in view, the general goal of this work was to investigate the effect of regional-culture differences and geographical distances among cities from different regions on food representation. As a practical outcome for marketers and consumer scientists, we would like to verify if, in order to decide whether strategies for introducing novel foods should be uniform across the same country or it is worth considering specific strategies to each situation/region. To achieve our goal, we used different cities in different regions of Brazil (politically known as "states"), a country with a continental size and remarkable cultural distances (different ethnicities in its formation). More specifically, we aimed at addressing the following questions:

(a) Will consumers from different cities of different states have similar or different representations of unfamiliar foods?

(b) If differences in food representations exist, what else could influence these differences: regional cultural identities or geographical distances?

2. Material and Methods

2.1. Brazil: The study area

Brazil, officially the "Federative Republic of Brazil", is the largest country in the Latin American region, with a territory of continental size (8,511,960 km2 area), being the fifth largest country in the world (equivalent to 47% of South American territory) and sixth in population, with more than 200 million of inhabitants (Instituto Brasileiro de Geografia e Estatística, 2018). It is the only country in America where the population speaks the Portuguese language, and the largest Lusophone country on the planet. Brazil is formed by the union of the Federal District and the 26 states. According to Pena et al. (2011), Brazil is a country with different regions and has diverse population histories. Its colonization starts at 1500, when Portuguese explorers discovered the lands of Brazil and were the first Europeans to arrive and claim its possession. Before that the region was inhabited exclusively by hundreds of Amerindians tribes. Between sixteenth and eighteenth centuries other Europeans countries, notably France and Netherlands, performed incursions in the coast to establish colonies. During the same period, African slaves were brought to Brazil for forced labor in agriculture, mining and cattle ranching (Bethell, 1987). In the nineteenth century, Brazil opened its borders to immigration: about five million people from more than 60 countries migrated to Brazil between 1808 and 1972, most of them from

Portugal, Italy, Spain, Germany, Japan and the Middle East (Levy, 1974). In the 1970s and 1980s, several Brazilian policies were implemented to occupy the lands of the continental region of Brazil, increasing the population of North and Central-west by more than three times. These policies aimed at supporting the migration of people from the Northeast, Southeast and South of Brazil to the North and Central-west regions (Brasil, 1997; Cunha, 2002). From this context, the North had a large influence of the Amerindian root, the Northeast had a history of strong African presence due to slavery and the South was mostly settled by European immigrants. In the present experiment, we planned to work with a considerable diversity of participants and regions in Brazil, who have in its cultural constitution different constructions of ethnic identity. **Fig. 1** presents the detailed constitution of each state that the city took part in the study, in terms of ethnic basis.

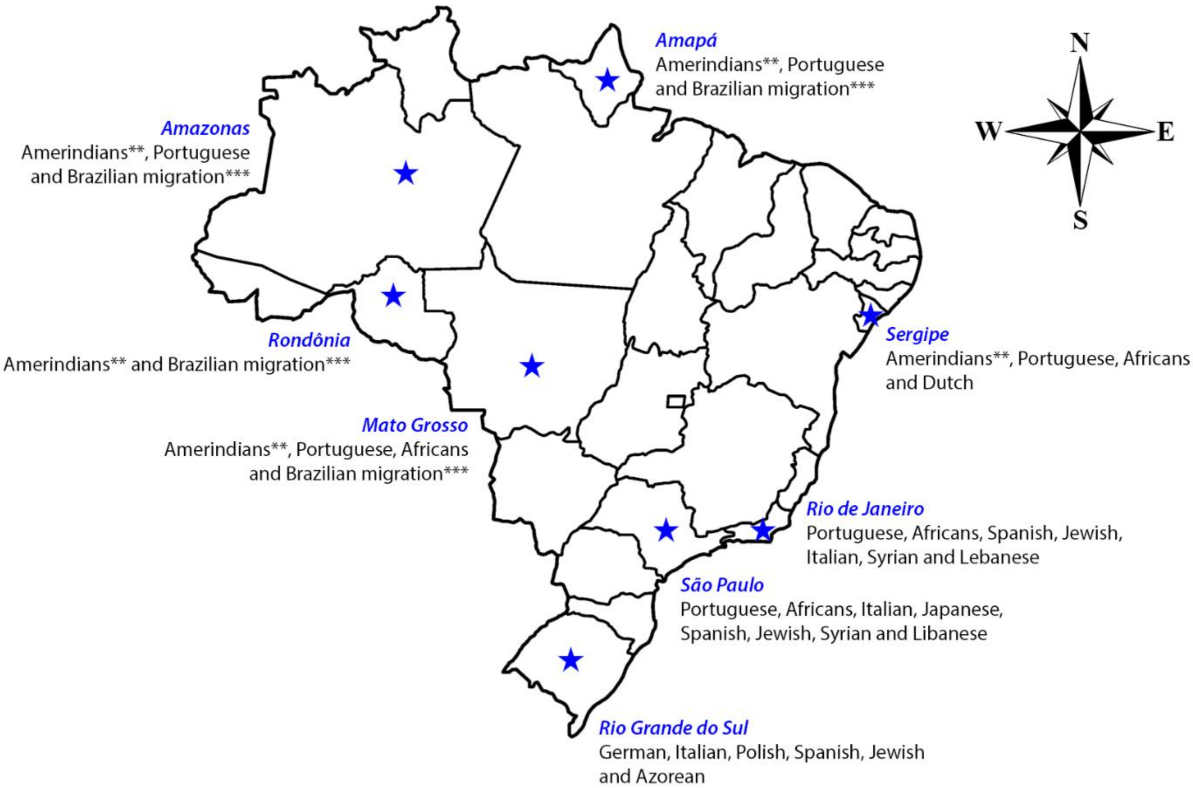

Figure. 1. Ethnic foundation in the eight Brazilian states that the city took part in the study. *Data were based on history research studies published by the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística, 2007); ** Amerindians are the native people that lived in Brazil before the European colonizers arrived; ***Brazilian migration represents a recent event (1970s and 1980s) in which people from South, Southeast and Northeast were encouraged to migrate to the Central-west and North regions.

2.2. Participants

A total of 780 consumers participated in the study. They were from eight cities (urban areas), strategically chosen from different states of Brazil: Manaus in Amazonas (AM); Porto Velho in Rondônia (RO); Macapá in Amapá (AP); Cuiabá in Mato Grosso (MT); Aracaju in Sergipe (SE); Rio de Janeiro in Rio de Janeiro (RJ); Campinas in São Paulo (SP); and Santa Maria in Rio Grande do Sul (RS). In each city, approximately 98 participants were recruited by an intercept sampling procedure at affluence points; the interviewers stopped any possible participant and invited them to take part in the study. Only participants who reported being born in the state where they were intercepted were considered in the study. All participants signed the Free and Informed Consent Term (TCLE) of this research, which was approved by the Research Ethics Committee of the University of Campinas under protocol (CAAE 80665117.5.0000.5404). The socio-demographic characteristics of the participants by Brazilian city are shown in **Table 1**.

2.3. Induction expression choice to word association task

Edible insects were chosen as the central object of the experiment because they are not part of the Brazilian national diet (Brasil, 2012), being an unfamiliar food to Brazilians. In cross-cultural research, the notion of "familiarity" must be considered when designing an experiment (Prescott, 1998). For example, when comparing the responses of two cultural groups, if group 1 was exposed to a given food and group 2 was not, the responses of these groups will be different, probably due to the familiarity or unfamiliarity with the particular food. Thus, if we seek to know the cultural drivers of these two groups, the cultural differences will be masked by the effect of familiarity. The concept of "familiarity" has a close relationship with research in food science (Pilgrim & Kamen, 1963) and a long history in psychology research (Maslow, 1937). As a definition, familiarity is a result of cumulative consumer product related experiences (Alba & Hutchinson, 1987) being part of the cognitive architecture of the individual on a particular product (Marks & Olson, 1981). Its importance on food and consumer research has been demonstrated by different ways: Familiarity has an influence on people's dietary choices (Steptoe, Pollard & Wardle, 1995) and it's a factor that drives sensory liking and preferences (Williamson, Robichaud & Francis, 2012) and individual's affective and cognitive image of food (Seo, Kim, Oh & Yun, 2013).

Table 1. Characteristics of participants in the eight Brazilian cities (%).

	Manaus (**AM**) (n=100)	*Porto Velho* (**RO**) (n=100)	*Macapá* (**AP**) (n=100)	*Cuiabá* (**MT**) (n=94)	*Aracaju* (**SE**) (n=94)	*Rio de Janeiro* (**RJ**) (n=94)	*Campinas* (**SP**) (n=100)	*Santa Maria* (**RS**) (n=98)	Total (n=780)
Gender									
Female	50.0	50.0	50.0	48.9	56.4	47.9	56.0	60.2	52.4
Male	50.0	50.0	50.0	51.1	43.6	52.1	44.0	39.8	47.6
Age (years)									
18-30	33.0	43.0	36.0	37.2	48.9	36.1	60.0	62.2	44.6
31-40	19.0	22.0	13.0	14.9	20.2	24.4	17.0	19.4	18.6
41-50	38.0	22.0	39.0	30.9	14.9	18.1	10.0	8.2	22.8
50 and older	10.0	13.0	12.0	17.0	16.0	21.3	13.0	10.2	14.0
Education									
Incomplete elementary school	-	-	-	2.1	1.1	4.9	5.0	2.0	1.8
Complete elementary school	-	8.0	3.0	6.4	2.1	4.9	7.0	1.0	4.0
Incomplete high school	6.0	14.0	2.0	10.6	17.0	-	3.0	2.0	6.9
Complete high school	14.0	47.0	13.0	45.8	29.9	32.2	24.0	5.1	26.5
Incomplete higher education	38.0	15.0	53.0	21.3	12.8	22.2	39.0	46.0	31.4
Complete higher education	32.0	13.0	19.0	13.8	16.0	21.0	14.0	16.3	18.1
Postgraduate	10.0	3.0	10.0	-	18.1	14.8	8.0	27.6	11.3

2.4. Procedure

2.4.1. Experimenters' training program

Before starting the experiment, the experimenters that have applied the word association task in the different cities were trained by an experienced researcher to apply the procedure in the same way in every city. The training session lasted around 2 h. The experimental training program comprised: (i) explanation of the word association technique; (ii) how to approach people on the street - stopping one in two randomly; (iii) how to explain to the participant the procedure before its starting and (iv) how to enter the data on the Excel program. Finally, after all the theoretical explanations, the experimenters practiced the technique with each other, under the supervision of the experienced researcher. A total of seven experimenters have applied the task in the same way in the different cities of Brazil. The data collection started in February and finished in July 2018.

2.4.2. Word association procedure

The interviews were conducted individually and lasted about 15 min. The participants were first asked to read and sign a consent form of their voluntary participation in the study. Afterwards, they were told that there were no right or wrong answers. In order to make participants feel comfortable and familiarized with the procedure, the experiment started with a warm-up phase (Son et al., 2014; Rodrigues et al., 2015). The experimenter told the participants: *"I'll tell you a word. Please write down (or tell me) everything that comes spontaneously to your mind when you hear that word*". The participants were then asked to mention the five first words or expressions that came spontaneously to their minds when the interviewer said the word "*sky*" and then the word "*car*". Following Moliner and Lo Monaco (2017), they were stimulated by a "restricted continued association", that means the experimenter limits the production of terms in the association process. The task started with the following instruction: "When I say *food made with edible insects*, what comes to your mind?" Then, participants were asked to evaluate their positive or negative connotation to each term related to the inductor expression using seven-point scales ranging from −3 (completely negative) to +3 (completely positive). After the end of the procedure, we ensured that all participants were not familiar with edible insects to eliminate the effect of food familiarity in the distortion of responses. For this, at the end of the task, the participants had to declare if they had ever eaten insects or not. All participants stated they had never eaten insects in any way.

2.5. Data analysis

The terms evoked by participants from each Brazilian city were firstly formatted and analyzed to verify the distances and proximities among the dictionaries (Moliner & Lo Monaco, 2017) of the different studied cities (similarities or dissimilarities). That was possible following a simple term counting in each case. Then, in order to facilitate the understanding of these produced dictionaries, the terms were grouped into categories. Finally, the frequencies of category mentions were calculated for each Brazilian city. Only categories mentioned by at least 5% of the participants were considered.

2.5.1. Ellegård's index: Examining similarities and/or dissimilarities between the evoked dictionaries

The Ellegård's index was calculated (Eq. 1) to allow the identification of the proximity/distance relationships maintained among the different studied dictionaries. According to Moliner and Lo Monaco (2017), this index is effective in assessing convergence or divergence of associations between the different types of mentioned dictionaries. The challenge is to compare these dictionaries in pairs. The Ellegård's index is based on the ratio of the number of common terms between the two dictionaries divided by the square root of the product of the total number of terms present in the two dictionaries:

(Eq. 1)

$$r^n = n_c / \sqrt{(n_1 \times n_2)^2}$$

Where, r^n = Ellegård's index, which varies from 0 (totally distinct dictionaries) to 1 (identical dictionaries); n_c = number of terms common to the two dictionaries; n_1 = number of different terms in the first dictionary; n_2 = number of different terms in the second dictionary.

A Hierarchical Cluster Analysis (HCA) was performed in the distance matrix made with the Euclidean distances between the Brazilian cities aiming at observing the geographical distances among them. Following, all the possible pairings of city dictionaries were done to produce a similarity matrix in order to demonstrate the similarities/dissimilarities among the evoked dictionaries. The Ellegård's index was recently approached in some social representation studies to disclose similarities/dissimilarities between dictionaries (Brunel et al., 2018; Robieux et al., 2018).

2.5.2. Polarity index

The polarity index (P) was calculated by participant to define a positive or negative valence of their representation according to Eq.2:

(Eq. 2)

$$(P) = \frac{\textit{Number of positive terms - Number of negative terms}}{\textit{Number of total evoked terms}}$$

The polarity index of each participant was calculated as the number of times that each participant had positive connotations minus the frequency of the negative connotations, divided by the total frequency of elicitation of the terms. This index ranges between –1 and +1; a value between –1 and –0.05 indicates that most of participants have accorded negative connotations in their evoked terms. A value between –0.04 and +0.04 indicates an equal number of positive and negative connotations. A value between +0.05 and +1 indicates that most terms had a positive connotation (de Rosa, 2002).

2.5.3. Lemmatization, categorization and translation

For each produced dictionary in each city, a lemmatization (Bécue-Bertaut, Alvarez-Esteban, & Pagès, 2008) was operated, which converted every term into its standardized form, known as a *lemma*: a) by deleting all connectors, auxiliary words and adverbs from each comment, and b) standardizing the evoked term in the infinitive form for the verbs, singular form for the nouns and masculine-singular for the adjectives. The third step was to group synonyms and words that belong to same semantic universe into categories through a triangulation process (Denzin, 1978; Apostolidis, 2003). The terms with the higher frequency of elicitation were used to group and rename all their synonyms. The fourth step was to deal with ambiguous terms difficult to regroup. So, these terms were carefully analyzed by three researchers who decided if they should be regrouped or left as independent terms (with a low frequency of elicitation). This step was done cautiously to avoid over-interpretation or over-grouping of terms (Symoneaux, Galmarini, & Mehinagic, 2012). Finally, the fifth step was the translation of the final terms into English. This task was done by two Brazilians who were fluent in English. The first one translated the terms from Portuguese to English and, afterward, the English words were given to the second person, who translated them to the Portuguese. If a perfect match was found, the translated term was kept; otherwise, the translators changed the term several times until an agreement was reached.

In order to better visualize the differences between cities, correspondence analysis (CA) was performed on the frequencies of terms in each category using the XLStat software (Addinsoft, New York, USA, 2014). Additionally, chi-square tests were conducted to examine differences between cities.

3. Results

3.1. Characteristics of the dictionaries

The total number of mentioned terms was 2,864, ranging from 484 terms in Manaus (**AM**) to 199 in *Campinas* (**SP**). An average of 3.7 associations per participant was obtained in the study; participants from the cities of *Aracaju* (**SE**) and Manaus (**AM**) tended to produce more terms, 5.0 and 4.8 respectively, whereas fewer terms appeared in *Campinas* (**SP**) (2.0). Some cities produced a higher variety of terms than others, which can be described by the diversity index. *Rio de Janeiro* (**RJ**), *Porto Velho* (**RO**) and *Santa Maria* (**RS**), for example, had diversity indexes higher than 0.55, while in *Campinas* (**SP**), participants were succinct in their association (diversity index of 0.4). The characteristics of the dictionaries from the eight Brazilian cities are presented in **Table 2**.

Table 2. Characteristics of the elicited dictionaries in the eight Brazilian cities.

	Manaus (AM)	*Porto Velho* (RO)	*Macapá* (AP)	*Cuiabá* (MT)	*Aracaju* (SE)	*Rio de Janeiro* (RJ)	*Campinas* (SP)	*Santa Maria* (RS)
Total elicited terms	484	341	391	309	470	224	199	446
Number of different terms	246	190	202	129	231	138	78	253
Diversity index	0.51	0.56	0.52	0.42	0.49	0.58	0.39	0.57
Average evocations by participant*	4.84^{ab}	3.41^{d}	3.91^{c}	3.29^{d}	5.00^{a}	2.70^{e}	2.01^{f}	4.55^{b}

Diversity index = *Total elicited terms/Number of different terms*.
* Means with different superscript letter in the same row are significantly different ($p < 0.05$).

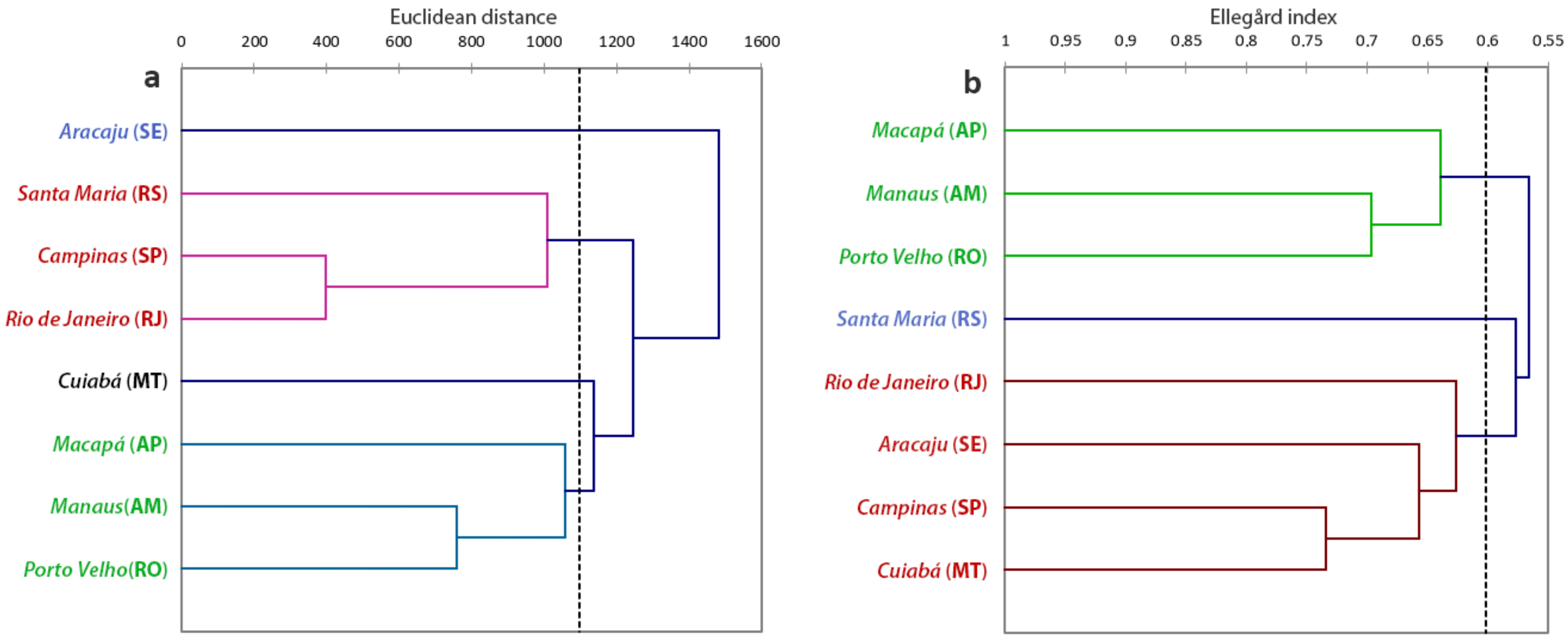

Fig. 2. Hierarchical Classification Analysis (HCA) on the Euclidean distances between Brazilian cities (**a**) and dictionaries using Ellegård indexes (**b**).

3.2. Proximities/distances between studied cities: Influence of geographical distances and regional culture

To get understanding on the relationship between the geographical distances and the regional culture, we analyzed the similarities between the dictionaries of the Brazilian cities. According to Lo Monaco, Delouvée & Rateau (2016) social representations appear to the experimenter in the form of a discourse, so by comparing the evoked dictionaries (discourses) between different regions, we can know how close or distant these regions are to each other in terms of people's representation. For that purpose, the Euclidean distances between the cities were compared with the distances between the different dictionaries calculated by the Ellegård's Index. Therefore, two distance matrices were generated using the Euclidean distances (km) and Ellegård's Indexes, comparing the cities in pairs – the table containing the distance matrices can be found in the Supplementary material of this paper. Then, from each matrix, HCA was used to group the cities in order to expose the dictionaries and geographical distances that were near and/or distant between them.

Considering the Euclidean distances (**Fig. 2a**), the results showed that *Macapá* (**AP**), *Manaus* (**AM**) and *Porto Velho* (**RO**) cities, politically pertaining to the Brazilian North region, were grouped because these cities were in average distant less than 1100 km from each other. Similarly, *Campinas* (**SP**) and *Rio de Janeiro* (**RJ**), from the Southeast region, were grouped together with *Santa Maria* (**RS**), from the South region, also due to their geographical proximity; while, *Cuiabá* (**MT**), from the Central-west region, and *Aracaju* (**SE**), from the Northeast, stood alone as separate clusters.

On **Fig. 2b**, assuming that two dictionaries with Ellegård's index close to zero indicate that they are divergent and, conversely, dictionaries with indexes close to one indicate a convergence/similarity between these dictionaries (Moliner & Lo Monaco, 2017). Therefore, *Macapá* (**AP**), *Manaus* (**AM**) and *Porto Velho* (**RO**) were grouped together, similarly to the HCA of Euclidean distances, showing Ellegård's indexes higher than 0.6 suggesting a more consensual form of representation between these cities. On the other hand, *Cuiabá* (**MT**) and *Campinas* (**SP**), were grouped together with *Rio de Janeiro* (**RJ**) and *Aracaju* (**SE**). In this case, the geographical distances between these cities were not determinant to affect the way those participants communicate their representation of a novel food. In cultural terms, this similarity of expression might be due to the mutual Portuguese influence during the colonization, which shaped a resembling culture among these cities. In opposition, *Santa Maria* (**RS**), which was grouped together with *Campinas* (**SP**) and *Rio de Janeiro* (**RJ**) by geographical proximity, stood as an isolated cluster on the Ellegård's Indexes HCA. This singular form of representation possibly occurred because the culture of *Santa Maria* (**RS**) has a strong and distinct identity, known as "gaucho". This identity was shaped by the state's isolation in the south of Brazil, a strong German, Italian and Polish colonization and the influence of the cultural habits from the neighborhood countries of Uruguay and Argentine.

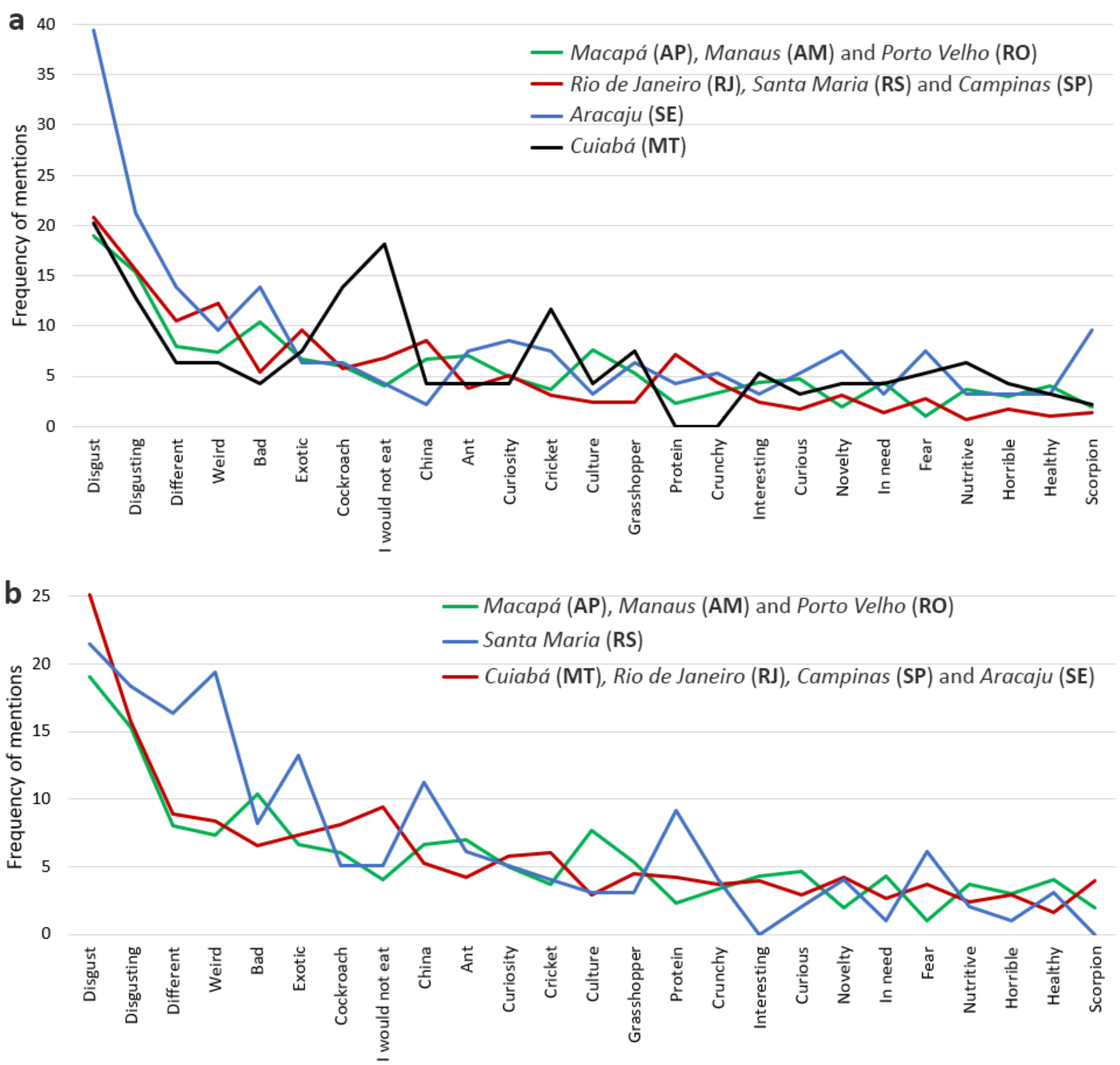

Fig. 3. Relative frequency (%) of the 25 most mentioned terms of the clusters from the dendrogram of Euclidean distances (**a**) and Ellegård indexes (**b**).

3.3. Mentioned terms by cluster

Comparing the 25 most mentioned terms in the cities clustered by the Euclidean distances (**Fig. 3a**) and Ellegård indexes **(Fig. 3b**), some similarities and differences were observed. As shown in **Fig. 3**, *disgust* was clearly the most salient term in all clusters regardless of the grouping method. In **Fig. 3a**, *Aracaju* (**SE**) stood out with the terms *disgust* and *scorpion*, while *Cuiabá* (**MT**) participants pointed out the terms *cockroach*, *I would not eat* and *cricket*. *Aracaju* (**SE**) and *Cuiabá* (**MT**) were combined with *Campinas* (**SP**) and *Rio de Janeiro* (**RJ**) to form a cluster (**Fig 3b**). In a similar way, the cluster formed by *Rio de Janeiro* (**RJ**), *Santa Maria* (**RS**) and *Campinas* (**SP**) (**Fig. 3a**) stressed out the terms *weird*, *exotic*, *China* and *protein*. Thus, when *Santa Maria* (**RS**) was separated and stood as a distinct cluster (**Fig. 3b**), the same terms were the most evoked. *Macapá* (**AP**), *Manaus* (**AM**) and *Porto Velho* (**RO**) had the highest frequency for *culture* among the clusters defined by the Euclidean distances (**Fig. 3a**), while among the Ellegård's index clusters (**Fig. 3b**) the terms *bad*, *culture*, *curious*, *in need*, *nutritive* and *healthy* were the most salient and *I would not eat*, *protein*, *novelty* and *fear* had a low frequency of mentions.

3.4. Polarity index by cluster

Polarity indexes indicate the connotation of the terms produced by participants and can be a predictor of their attitude concerning diverse objects. In our case, the relative prediction to "*food made with edible insects*". The **Fig. 4** shows the polarity index disclosed by Euclidean distances (**Fig. 4a**) and Ellegård index clusters (**Fig. 4b**). According to De Rosa (2002), a positive attitude is indicated by values between +0.05 and +1, while a negative attitude is denoted between –1 and –0.05. Values between –0.04 and +0.04 indicate neutral attitude. The participants from the cluster formed by *Rio de Janeiro* (**RJ**), *Santa Maria* (**RS**) and *Campinas* (**SP**) (**Fig. 4a**) exhibited 46% of positive and 39% of negative attitude. However, in the detachment of *Santa Maria* (**RS**) from this cluster, the most positive group (63%) was created (**Fig. 4b**). *Macapá* (**AP**), *Manaus* (**AM**) and *Porto Velho* (**RO**) composed the second most positive cluster, with 55% of positive associations. When we compare the results of the polarity index with the most mentioned terms (**Fig. 3**), *Santa Maria* (**RS**) presented the highest number of mentions of terms with general positive associations, such as *protein*, *exotic* and *China*. In the same trend, people from *Macapá* (**AP**), *Manaus* (**AM**) and *Porto Velho* (**RO**) associated with *culture*, *nutritive* and *healthy*. On the other hand, participants from *Cuiabá* (**MT**) showed the most negative attitude (50%) followed by *Aracaju* (**SE**) (43%) (**Fig. 4a**). When these cities were grouped together with *Campinas* (**SP**) and *Rio de Janeiro* (**RJ**) (**Fig. 4b**), they formed most negative cluster using Ellegård index, containing more negative (47%) than positive associations (42%). These negative associations were due to the high frequency of evocations of generally negative terms (**Fig. 3**), like *disgust*, *cockroach* and *I would not eat*.

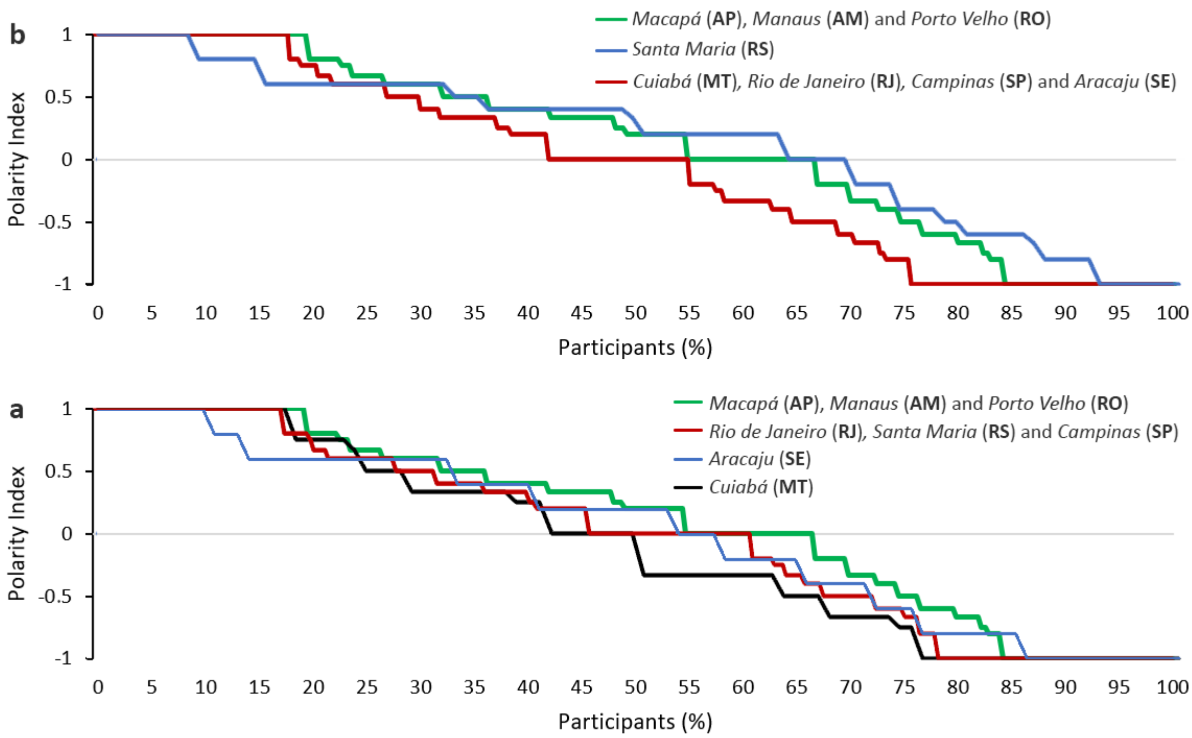

Fig. 4. Polarity index of the terms of the disclosed clusters from the dendrogram of Euclidean distances (**a**) and Ellegård indexes (**b**).

3.5. Categorization of terms: Interpreting consumers representation

In consumers representation studies (Andrade et al., 2016; Rodrigues et al., 2015; Son et al., 2014; Guerrero et al., 2010), the step of categorization of the evoked terms is indispensable, enabling the organization of ideas and preparing the interpretation of one or more dictionaries by the researchers. In this study, a total of 851 different terms were mentioned and previously grouped into categories by triangulation process. **Table 3** shows some examples of the terms grouped into the same category. A total of 72 final categories were obtained by consensus among three researchers.

Table 3. The most relevant categories and examples of mentioned terms.

Categories	Terms
Rejection	Bad, I would not eat, do not like, inedible.
Disgust	Disgust, disgusting, nausea, to throw up.
Asia	China, Asia, Japan, Thailand.
Weird	Weird, bizarre, strange, surreal.
Curiosity	Curiosity, curious, interesting, intriguing.
Different	Different, unusual, difference, out of the ordinary.
Acceptance	I would eat, I would try, I would taste, tasty.
Exotic	Exotic, exotic food, peculiar, delicacy.
Nutrition	Nutrition, nutritious, nutrients, source of nutrients.
Cricket	Cricket, grasshopper.
Innovative	Novelty, new, innovative, something new.
Culture	Culture, cultural, regionalism, ethnicity.
Fear	Fear, dread, shiver, horror.
Survival	In need, hunger, survival, poverty.
Ant	Ant, *saúva*, *tanajura*, *içá*.

The 23 categories that were elicited by more than 5% of the participants from each investigated city are shown in **Table 4**. The most frequently mentioned categories were *Rejection* (n=390), *Disgust* (n=373), *Asia* (n=128), *Weird* (n=102), *Curiosity* (n=99), *Different* (n=92) and *Acceptance* (n=92). Chi-square test showed significant differences (P<0.05) between the cities in 15 categories. Cultural differences were most evident for *Rejection* and *Disgust* being more salient in *Aracaju* (**SE**), *Macapá* (**AP**) and *Rio de Janeiro* (**RJ**). On the other hand, *Acceptance* stood out in *Manaus* (**AM**), *Porto Velho* (**RO**) and *Campinas* (**SP**); *Curiosity* category was more often cited in *Manaus* (**AM**), *Aracaju* (**SE**) and *Campinas* (**SP**), while *Fear* only in *Aracaju* (**SE**). *Food made with edible insects* was linked to sensory aspects (*Flavor* category) by *Santa Maria* (**RS**) and *Aracaju* (**SE**) participants. It was more linked to *Nutrition* in *Manaus* (**AM**) and *Macapá* (**AP**). In the Northern cities of Brazil (*Manaus, Porto Velho* and *Macapá*) *Culture*, *Survival* and *Sustainability* stood out as relevant categories, while in *Campinas* (**SP**) and *Santa Maria* (**RS**) participants mentioned most of the *Protein* category, revealing a better knowledge about the composition of edible insects.

Table 4. Frequency of categories mentioned by at least 5% of the participants in the word association task and results from the chi-square test.

	Total	*Manaus* (**AM**)	*Porto Velho* (**RO**)	*Macapá* (**AP**)	*Cuiabá* (**MT**)	*Aracaju* (**SE**)	*Rio de Janeiro* (**RJ**)	*Campinas* (**SP**)	*Santa Maria* (**RS**)	p-value
Rejection	390	61	46	**62**	47	**63**	**49**	17	45	*
Disgust	373	49	37	**55**	36	**72**	**36**	40	48	*
Asia	128	16	**19**	**28**	5	10	**18**	9	**23**	**
Weird	102	9	8	**15**	7	15	**16**	**11**	21	*
Curiosity	99	**18**	14	11	12	**16**	4	**16**	8	*
Different	92	8	9	10	7	19	7	10	22	n.s.
Acceptance	92	**22**	**18**	12	7	9	8	**8**	8	*
Exotic	82	10	14	8	7	7	8	8	20	n.s.
Nutrition	81	**28**	9	**13**	8	9	4	2	8	***
Cricket	77	11	9	8	18	13	5	4	9	n.s.
Innovative	75	**15**	0	5	**14**	**15**	1	4	**21**	***
Culture	72	8	**17**	**18**	9	6	2	4	10	**
Fear	70	5	5	8	10	**28**	1	4	9	***
Survival	69	**17**	**10**	**12**	**15**	6	3	3	3	**
Ant	67	16	10	7	6	12	6	3	7	n.s.
Cockroach	63	10	7	8	13	7	6	6	6	n.s.
Flavor	57	9	4	6	1	**13**	2	0	**22**	***
Health	55	12	7	5	10	10	0	3	8	n.s.
Worm-like	55	11	8	6	11	5	2	2	10	n.s.
Sustainability	53	**14**	**10**	**9**	7	2	2	0	9	*
Unhealthy	43	3	7	4	9	8	6	0	6	n.s.
Protein	42	3	2	5	0	5	4	**9**	**14**	***
Unfamiliar	38	0	4	2	**7**	2	4	**6**	**13**	***

*n.s. not significant; * p<0.05; ** p<0.01; *** p<0.001.*
The bold numbers indicate that the observed value is higher than the expected theoretical value under the assumption of row-/column independence.

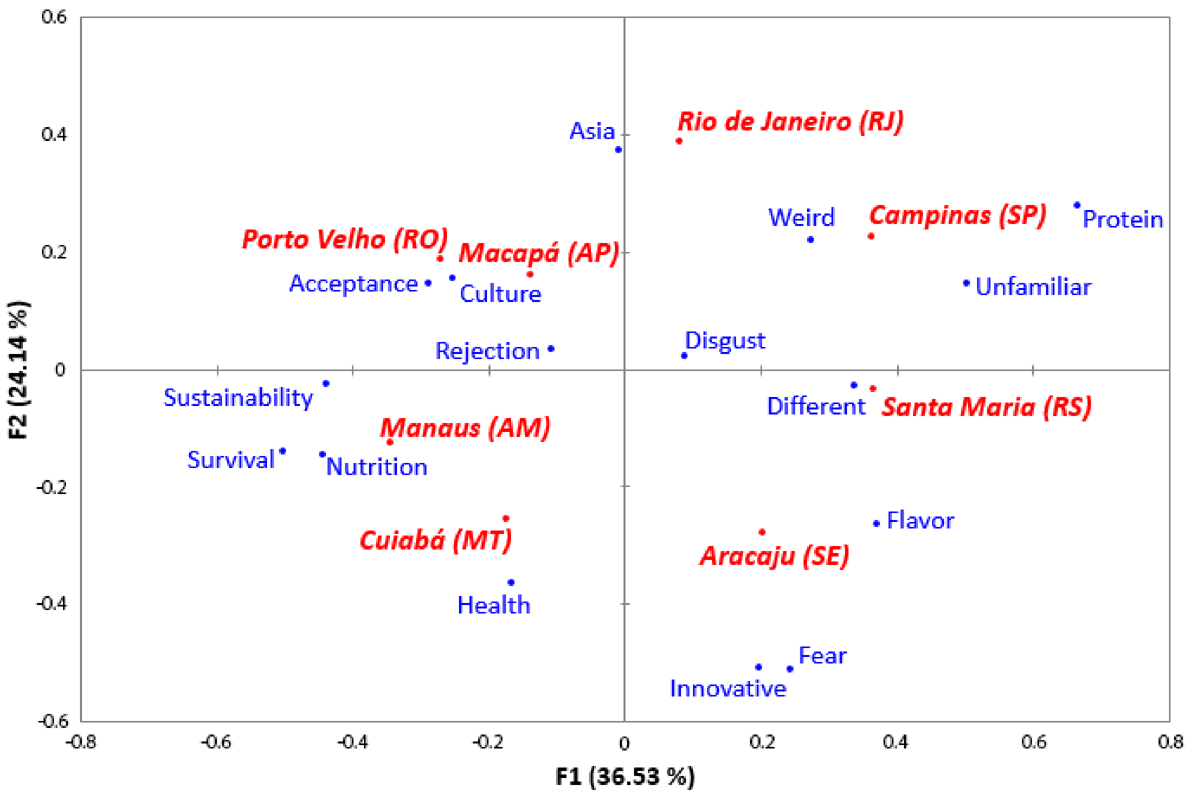

Fig. 5. Perceptual map obtained from the correspondence analysis performed on the frequency of the categories mentioned by at least 5% of the participants in the word association task: categories are depicted in blue and Brazilian cities in red.

Correspondence analysis was performed to help visualize the main differences between the cities (**Fig. 5**). For that purpose, a correspondence analysis was initially done with the categories mentioned by at least 5% of the respondents. Then, the categories closer to the center of the graph and with lower frequency (Ant, Cockroach, Cricket, Curiosity, Exotic, Unhealthy and Worm-like) were removed to improve the chart explanation. The first and second dimensions explained 61% of the variance. This low explanation occurred mainly because we used an unfamiliar food (edible insects) as induction term to the word association task, and concepts that are not well established to the respondents tend to cause diffuse answers. Even so, an explanation of 61% is sufficient to demonstrate that there are differences in representation among the cities about an unfamiliar food. The first dimension, which explains 37% of the variance, separates the cities in two major groups: (a) *Manaus* (**AM**), *Cuiabá* (**MT**), *Porto Velho* (**RO**) and *Macapá* (**AP**); and (b) *Santa Maria* (**RS**), *Campinas* (**SP**), *Rio de Janeiro* (**RJ**) and *Aracaju* (**SE**). The first group comprehends the cities from the North and Central-west regions of Brazil, which are located in the center of continent, while the second group includes the cities from the South, Southeast and Northeast regions, located near the shore of the Atlantic Ocean. Participants from *Manaus* (**AM**) were located closer to *nutrition*, *survival* and *sustainability* categories, while *Macapá* (**AP**) and *Porto Velho* (**RO**) grouped closely together and were associated with categories of *acceptance* and *culture*. *Cuiabá* (**MT**) projected closer to *health*. *Innovative* and *fear* categories situated near *Aracaju* (**SE**). *Campinas* (**SP**) was located closer to *protein, unfamiliar* and *weird*, while *Rio de Janeiro* (**RJ**) grouped near to *Asia*. The category *different* was associated with *Santa Maria* (**RS**).

4. Discussion

The present exploratory experiment investigated the effect of the regional-culture and geographical distances among regions on unfamiliar food representation. For this purpose, the participants of this study were interviewed in different cities of different states of Brazil, a country with a continental size and different constructions of ethnic identity in its regional culture. Also, the restricted continued association procedure was employed wherein participants were invited to produce words or expressions about edible insects, which are uncommon in the Brazilian diet. In this way, a variety of representations about edible insects were identified along the Brazilian cities. As our study was exploratory, in order to facilitate the understanding of this complex subject, our discussion will be focused on two research questions, previously addressed at the end of the introduction.

In relation to our first question, the participants from each region expressed themselves in distinct ways resulting in different representations, despite this study has been performed in a country where Portuguese is the only official language. For example, the group *Macapá* (**AP**), *Manaus* (**AM**) and *Porto Velho* (**RO**), composed by the cities from Northern Brazil, stressed out terms from *culture*, *acceptance*, *nutrition*, *survival* and *sustainability* categories (**Fig. 5**). These elicitations seem to be the expression of their regional culture, formed primarily by the Amerindians, i.e., considering that the habit of eating insects was part of their culture and entomophagy was mainly practiced for survival during the rainy season when hunting was more difficult (Costa Neto & Ramos-Elorduy, 2006). Despite the consumption of insects by autochthon Amerindians from the North region of Brazil in ancient times, this food habit was not perpetuated on the contemporary Brazilian society. Besides, the sustainability concept was perceived because the rearing of edible insects is globally recognized as an alternative source of protein of animal origin competing with cattle ranching, which is an important economic activity and was the main cause of deforestation in Northern Brazil (Bowman, et al., 2012). In contrast, the categories *protein*, *Asia*, *weird, different* and *unfamiliar* were associated with the cities of *Rio de Janeiro* (**RJ**), *Santa Maria* (**RS**) and *Campinas* (**SP**), from South and Southeast regions of Brazil. The elicitation of terms from the *protein* category shown that the participants from these cities have a better knowledge about the composition of edible insects; while the mentions of *Asia* reflected the perception that insects are usually eaten in countries from this continent; likewise, the categories of *weird, different* and *unfamiliar* support that entomophagy is out of the food habits in southern and southeastern Brazil. The dissociation of entomophagy from these regions is mainly due to the cultural formation, composed mostly from European ethnicities, such as Portuguese, Italian, Spanish and German. Even though insect consumption is deeply rooted in human evolutionary history and nowadays Western countries are taking interest towards entomophagy, Europeans did not have the habit of eating insects (Mlcek et al., 2014) during the migratory period in Brazil between 19th and 20th centuries (Instituto Brasileiro de Geografia e Estatística, 2007).

Studies comparing the perception of consumers about food or beverage in different regions of the same country are scarce in the literature. Regarding this issue, Campbell, Mhlanga and Lesschaeve (2013) investigated the knowledge and beliefs of Canadians about local and organic food. Similar to our study, consumers from different regions exhibited distinct perceptions about the subject. For instance, British Columbia state consumers are more prone to confuse and equate the concepts of local and organic food compared to people from the states of Alberta, Northwest and Yukon Territory. While Quebecers tend to associate local food with products with fewer pesticide residues, which may or may not be the case. In the same line Soares et al. (2017), evaluated the effects of regional aspects into the Brazilians' perceptions of *coalho* cheese. The Brazilian Northeast and Southeast consumers were congruent for the “way of preparing” and “sensory characteristics” concepts. In opposition, consumers from the Southeast region associated *coalho* cheese with social and leisure situations, while those of the Northeast related with family aspects. It is important to notice that, unlike our work, both mentioned studies (Campbell, Mhlanga &

Lesschaeve, 2013; Soares et al., 2017) used a familiar food in order to spotlight the differences in perception between distinct regions in the same country.

Concerning the second question, i.e. the clusters formed by geographical distances compared with those formed by the similarity of the mentioned terms (**Fig. 2**), some similarities and differences were observed. The group formed by *Macapá* (**AP**), *Manaus* (**AM**) and *Porto Velho* (**RO**) remained the same in the two forms of classification, in this case, the geographical proximity corresponded to a similar representation among these cities. Moreover, as previously mentioned, the cities from the North region of Brazil have a similar ethnic foundation based on the first inhabitants of the region, the Amerindians. This all contributed to form a region with the cities having similar cultural identity and unfamiliar food representation.

In opposition, the same trend was not observed for the other cities. *Cuiabá* (**MT**) and *Aracaju* (**SE**), which were classified as isolated clusters by their Euclidean distances, were grouped together with *Campinas* (**SP**) and *Rio de Janeiro* (**RJ**) due to their similarity of mentioned terms. The shared Portuguese colonization between these cities might explain the resembling perception of a novel food. *Santa Maria* (**RS**), which at first was grouped with *Campinas* (**SP**) and *Rio de Janeiro* (**RJ**) by their geographical proximity, then stood alone as a separate cluster by its unique form of expression due to the strong and distinct cultural identity of Rio Grande do Sul State. These results demonstrated that physical distance is not the conclusive factor to delineate the representation of a novel food by a group of people because the regional culture has a stronger impact on how people represent about food. This becomes more evident when we observe the differences in expression between each Ellegård's index clusters. The most mentioned terms (**Fig. 3b**) and the participants' attitude (**Fig. 4b**) about edible insects, showed pronounced differences between these groups. Other studies also demonstrated differences on the perception of consumers from the same country and with different ethnicity or culture background (Campbell, Mhlanga, & Lesschaeve, 2013; Priven et al., 2015; Chang, Thach, & Olsen, 2016). Priven et al. (2015) evaluated the perceptions of healthfulness by American consumers in "free-from" products. Two picture-based food product questions were used to evaluate which products were perceived healthier by 256 consumers. Participants were shown two pictures of cracker boxes that were identical, except for the presence of a free-from label ("MUI free" or "gluten-free") on one of the boxes, then, they were asked to choose which product was the healthiest one. The reported results suggested that certain ethnic groups might have been more strongly influenced by “free-from” claims. Hispanics were significantly more likely than Caucasians to recognize the free-from product as healthier, while African Americans were more prone to perceive the conventional product as healthier. In another study, Chang, Thach & Olsen (2016) explored the wine and health representation of American consumers. After an online survey with more than 1000 U.S. wine consumers, they found that African Americans and Asians are more concerned about their health compared to Hispanics and Caucasians. Moreover, African Americans tend to perceive sparkling wine as healthier than people from any other ethnic groups. These studies are particularly interesting since the authors contrasted perceptions of different ethnicities within the same country, demonstrating that even when people are born in the same country, cultural background formed by the ethnic origin may play a key role in people's food representation.

5. Concluding comment, perspectives and limitations

The current study has demonstrated that there are different representations of edible insects in the same country. Participants from *Campinas* (**SP**) and *Cuiabá* (**MT**) were more succinct, while those from *Rio de Janeiro* (**RJ**), *Santa Maria* (**RS**) and *Porto Velho* (**RO**) produced a higher diversity of terms to express the perception about edible insects. Besides, people who were born and live in the states close to each other did not always have a similar representation. The cultural formation similarity was more determinant to explain the analogous representations among the cities than their geographical proximity. Moreover, the cities were classified in groups according to their distinct ways of expression and attitude. Essentially, Brazil was divided into two main groups: one formed by the cities from the continental region (North and Central-West), which has a strong cultural influence from the Amerindians; and the other composed by the cities located near the shore of the Atlantic Ocean (Northeast, Southeast and South regions) that present a cultural formation influenced by the Europeans immigrants from the 19th and 20th centuries.

As far as we are concerned, this is the first study on consumer science focusing on food to use the Ellegård’s index to identify the proximity among different dictionaries of elicited terms. As a result, it was possible to group the mentioned dictionaries of the cities according to their similarity and to evaluate the elements of distinction between those groups. Thus, the regional culture formed by the ethnic colonization of each city disclosed as the main element to explain the clustering through Ellegård's index.

As a practical matter, our results could help marketing managers to effectively introduce a novel food in a country with varied regional culture. Consumers from different regions of Brazil had distinct representations of edible insects, in this way, the marketing strategy should be focused on the values and beliefs of their regional culture subgroups instead of a homogeneous strategy for the whole country.

This work was exploratory and presents some limitations. Firstly, we worked in cities from eight of the 26 states of Brazil, which were chosen by their diversity of regional culture and ethnic foundation. Therefore, we should be careful about generalizing these results for other Brazilian cities. Further studies should focus on a quantitative methodological approach and a research design with time series, which would lead to stronger conclusions about the Brazilian consumer perceptions of edible insects.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. We also acknowledge the National Council for Scientific and Technological Development (CNPq) for the master Fellowship awarded to Lorena Januário. We want also warmly thank the participants from all eight cities for their voluntary participation in this experiment. Heber Rodrigues acknowledges the CAPES Foundation for his Postdoctoral Fellowship.

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CAPÍTULO IV

"Food made with edible insects": Exploring the social representation of entomophagy where it is unfamiliar

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Este artigo foi publicado na *Appetite*:
Bisconsin-Junior, A., Rodrigues, H., Behrens, J. H., Silva, M. A. A. P., & Mariutti, L. R. B. (2022). "Food made with edible insects": Exploring the social representation of entomophagy where it is unfamiliar. *Appetite*, *173*, 106001. https://doi.org/10.1016/j.appet.2022.106001

Abstract

Insects have attracted much attention as a novel food source because of their environmental and nutritional advantages. In Latin America, some traditional groups consume insects; but the urban areas of Brazil resist adopting insects as food, like most Western countries. Thus, this study investigated the social representation of edible insects to understand the barrier behind this avoidance and to identify their segmentation in the Brazilian population. Seven hundred and eighty individuals were interviewed in all the Brazilian regions. The interview was composed of a word association task, a risk perception evaluation and two open-ended questions about the subject. According to the structural approach of social representation, *Disgust* was the primary association with edible insects. Three segments of consumers were identified using the risk perception of eating insects. The first segment, composed mainly of young men with higher educational levels, had the lowest risk perception, and the most positive attitude about entomophagy, their associations were also positive, such as *Acceptance* and *Sustainability*; the second had average risk perception and neutral attitude towards edible insects, with neutral and positive associations (e.g., *Flavor* and *Culture*); and the third, formed mostly by older women with lower educational level, presented the highest risk perception and the most negative attitude, and exhibited negative associations (*Disgust,* and *Fear*). The insect consumption in Brazil would be favored by using species of crickets, grasshoppers, ants, and their fried and roasted styles of preparations. Also, participants would eat insects mainly because of survival and curiosity.

Keywords: entomophagy; consumer segmentation; word association; risk perception; unfamiliar food.

1. Introduction

Insect consumption is one of the most studied and promoted alternatives to support the increasing protein demand caused by the world population growth (Mariutti et al., 2021). Nevertheless, besides proteins, insects have much more to offer. They can provide a significant amount of energy, lipids, fibers, minerals, and vitamins (Nowak et al., 2016). In addition, their production requires less water and land, has a better feed conversion, and generates fewer greenhouse gases and ammonia than conventional livestock (van Huis & Oonincx, 2017).

With so many benefits, the interest in using insects as food grows in Western countries. Consequently, studies have been done in recent years with diverse population like in the USA (Woolf et al., 2019), Germany (Lammers et al., 2019), Italy (Tucillo et al., 2020), and Australia (Sogari et al., 2019). However, all the nutritive and environmental advantages of eating insects do not seem to be enough to convince most Western consumers to adopt insects into their diet. In a recent review about consumer views on edible insects, Dagevos (2021) pointed out that it is widely recognized that insects do not belong to the current traditional Western diet, making it difficult for entomophagy to be incorporated as a normal eating behavior by Western societies. As a matter of fact, alternative protein sources like insects or cultured meat might trigger neophobic responses among segments of consumers, whereas planted-based proteins are more likely to be accepted (Onwezen et al., 2021). Therefore, most studies have shown that consumers still respond with a natural aversion to insects as food.

Even though the Western world is considered unwilling to adopt entomophagy, treating it as one uniform food culture is inaccurate. Most consumer research about insects as food in Western countries was done in Europe and North America (Ardoin & Prinyawiwatkul, 2021). However, detailed evaluations of consumer studies expose different attitudes, drivers, and inhibitors of entomophagy among countries and cultures (Ardoin & Prinyawiwatkul, 2021). Mancini et al. (2019) reviewed the acceptability of edible insects in Europe. They found that Northern countries had higher acceptability than the Central, Mediterranean, or Western countries, primarily due to the northern region being the first to introduce edible insects in their market. The study conducted by Piha et al. (2018) demonstrated that consumers from Finland and Sweden (Scandinavian countries) were more willing to buy and positive towards insects as food than Germans and Czechs (Central Europe). Even within the same country, regional culture seems to influence the way people approach entomophagy majorly. Woolf et al. (2019), for example, exposed significant differences in the willingness to consume insect-incorporated food between different states in the USA. Bisconsin-Júnior et al. (2020) found that people from the North and Central-West regions of Brazil, with a solid Amerindian cultural influence, held more positive views on eating insects than people in the Northeast, Southeast, and South regions, which were notably influenced by the European colonization.

Latin America, which is considered part of the Western world, has an old tradition of entomophagy. The Aztecs in Mesoamerica (Mexico) consumed insects and insect eggs, recognized as one of their prime protein sources in diet (van Huis et al., 2013). In Brazil, entomophagy has been documented among 39 indigenous groups from all over the country (Costa-Neto, 2016). Therefore, Latin America is a compelling region to investigate how people understand and identify the use of insects as food.

Brazil is the largest and most populated country in Latin America, with more than 200 million inhabitants (IBGE, 2018). Despite entomophagy being a standard component in the food culture among some ancestral groups in Latin America (Costa-Neto, 2015), most of Brazilians live in urban areas and share the Western food pattern. Moreover, Brazil is one of the world's major producers, exporters, and consumers of meat (FAO, 2021). Thus, understanding how Brazilians perceive edible insects could play an important role in implementing insects as food in Latin America, working as a model prototype for launching strategies to the progress of meat substitution by its alternatives, such as insects.

To evaluate the Brazilian consumer's understanding of edible insects, we employed the social representation theory. This theory has already been widely used in consumer studies on different kinds of food products such as craft beer (Gómez-Corona et al., 2016), edible flowers (Rodrigues et al., 2017), seafood (Bostic et al., 2018), pulses (Melendrez-Ruiz et al., 2020), wines (Urdapilleta et al., 2021), and hamburgers (Patinho et al., 2021). According to Lo Monaco & Bonetto (2019), social representation is "*a structured set of ideas, opinions, knowledge, and beliefs shared by a social group* [such as Brazilians] *about specific social objects* [such as edible insects]". Thus, this theory focuses on forming of a common view of a social object (representation) by the interactions between members within a social group. Social representations have an important role in studying the cultural acceptance of new products, indicating the transition from "something disturbing and unknown" into "something familiar and known" (Bäckström et al., 2003). Therefore, a social representation study might allow us to discover whether Brazilians share common beliefs, opinions, and attitudes concerning edible insects.

The structural approach, proposed by Abric (1976), has been used for decades in social psychology to investigate social representations and has demonstrated great applicability in the food domain (Gómez-Corona et al., 2016; Rodrigues et al., 2017; Melendrez-Ruiz et al., 2020; Patinho et al., 2021). In this type of analysis, the meaning that a social group assigns to the object of a social representation is formulated as a central core surrounded by peripheral elements (Moliner & Lo Monaco, 2017). The central core is composed of highly consensual and stable elements about the object, and its main role is to give structure and meaning to the representation. Around the central core, the peripheral elements gravitate to represent particular and contextualized experiences, which individuals associated with the object of representation (Abric, 2003).

Regarding consumer segmentation, this approach involves identifying and reducing a heterogeneous population into smaller, homogeneous groups of consumers who share similar needs, wishes, or motives (Wedel & Kamakura, 2012). The segmentation of Brazilians regarding edible insects could help to improve our understanding of the diverse groups that might consume or avoid insects, including their characteristics and motivations. That kind of information is helpful as a marketing tool for determining pre-product launch positioning (Gengler & Mulvey, 2017) and could be employed by the insect food industry to increase their products success rate.

In this context, the objective of this study was to examine the social representation of edible insects for Brazilians and to identify consumer segments, revealing their profile in terms of socio-demographics and main associations with the subject. Further, the study explored the styles of preparation and drives that would favor Brazilians to consume insects.

2. Methods

The methods concerning the participants, the interviewers' training, the interview procedure, and lemmatization, categorization, and translation of the evoked terms were described in detail in Bisconsin-Junior et al. (2020).

The research was approved by the Research Ethics Committee of the University of Campinas under the protocol CAAE 80665117.5.0000.5404. All the respondents signed a consent form prior to the interview.

2.1. Participants

A total of 780 individuals participated in the study, all of them living in the urban area of eight different cities covering all the regions of Brazil: Manaus (AM), Porto Velho (RO), and Macapá (AP) in the North; Cuiabá (MT) in Center-West; Aracaju (SE) in the Northeast; Rio de Janeiro (RJ) and Campinas (SP) in the Southeast; and Santa Maria (RS) in the South. A convenience sample of about 100 individuals was recruited in each city by intercepting people in central areas such as markets, squares, and bus stations. The interviewers stopped any possible participants and invited them to participate in the study. Individuals who stated being vegetarian or vegan were excluded to avoid the bias of considering that all animals, including insects, are inedible, and those who had eaten insects before, to eliminate the effect of previous experience with entomophagy. Interviews were conducted between January and June 2018. The socio-demographic characteristics of the participants are shown in **Table 1**.

Table 1. Characteristics of the participants (n=780).

	Participants (%)
Sex	
Women	52.3
Men	47.7
Age	
18-30 years	44.6
31-40 years	18.6
41-50 years	22.9
51 and older	13.9
Highest educational level	
Elementary	12.7
High school	57.9
Bachelor and graduate	29.4

2.2. Procedure

2.2.1. Interviewers' training

Before starting the experiment, the interviewers were trained by an experienced researcher to ensure that the procedure would be applied in the same way in the eight cities. The training session lasted 2 h and comprised: (i) explanation of the word association technique; (ii) how to approach people in public spaces; (iii) how to explain to the participant the procedure before starting and (iv) how to enter the data on the Excel program. Finally, the interviewers practiced the technique with each other and then with students on the campus of the University of Campinas. Seven interviewers have performed the interviews in different locations.

2.2.2. Interview procedure

The interviews were conducted individually, face-to-face, registered in paper forms by the interviewer, and lasted about 15 min. They included a brief socio-demographic questionnaire, the free word association task, ranking and rating the evoked terms, a risk perception rating, and two open-ended questions. To familiarize the participants with the word association task, the interviewer started with a warm-up: participants were asked to mention the first five words or expressions (terms) that came to their minds when the interviewer said the word ‘‘sky” and then the word ‘‘car”. Once the participants felt comfortable with the procedure, the task started with the following inductor expression: “When I say *food made with edible insect*, what comes to your mind?”. The first five evoked terms were registered. Then, they were asked to rank those terms according to the relative importance, from (1) least important to (10) most important. Afterward, participants evaluated their positive or negative attitude towards each term related to the inductor expression on a seven-point scale, going from −3 (completely negative) to +3 (completely positive). Next, they were requested to evaluate the risk perception of eating insects, from (0) safe to (6) very dangerous. Finally, participants responded to two open-ended questions: “Why would you eat insects?” and “How would you eat insects?”.

2.3. Data analysis

Before conducting any analysis, the evoked terms of the word association task were formatted and grouped into categories. Initially, typing or spelling mistakes were verified in the original Portuguese language. Then, the words were converted to their standardized form (Bécue-Bertaut et al., 2008), known as a lemma, by: deleting all connectors, auxiliary terms, and adverbs from each comment, and standardizing the evoked words in infinitive for the verbs, singular for the nouns, and masculine-singular for the adjectives. Next, a triangulation process was carried out (Apostolidis, 2003). Three experienced researchers independently grouped all the terms into categories by their synonyms and semantic context; then, these categories were compared, and the final list of categories was consensually validated. The terms with the highest frequencies of elicitation were used to group all its synonyms. The ambiguous terms, which were difficult to group, were carefully analyzed by the researchers who decided whether they could be regrouped or left as an independent term. After triangulation, the categories were translated into English by two bilingual researchers using a two-way translation process: one person translates the words from Portuguese into English; then the other person translates the English words into the Portuguese language. The translation was approved if a perfect match was found, otherwise, the translators repeated the two-way translation process until an agreement. Following the same procedure, the answers to the two open-ended questions were formatted and arranged into groups.

2.3.1. Polarity index

The polarity index (P) was calculated by category to define the positive or negative connotation of each category, and by the participant to evaluate the positive or negative attitude associated with the representations, according to Eq. (1):

$$(P) = \frac{Number\ of\ positive\ terms - Number\ of\ negative\ terms}{Number\ of\ total\ elicited\ terms} \tag{1}$$

The polarity index of each category or participant was calculated as the number of times that each category or participant had positive connotations minus the frequency of the negative connotations, divided by the total frequency of elicitation of the terms. This index ranges between −1 and +1; a value between −1 and −0.05 indicates that the category had a negative connotation or that the participant had a negative attitude about the subject; a value between −0.04 and +0.04 indicates a similar number of positive and negative connotations, whereas a value between +0.05

and +1 indicates that the category or the participant had a positive connotation or attitude about the subject (de Rosa, 2002).

2.3.2. Structural approach of social representation

To find the central core and peripheral areas of the social representation about food made with edible insects, we used the structural approach method proposed by Abric (2003). First, for each category two criteria were calculated: the frequency of evocations of all terms belonging to the category and the average importance of the terms. The categories mentioned by less than 5% of the participants in the word association task were discarded from the analysis. Then, frequency and importance cutoff points were determined. The frequency cutoff point was obtained following Wachelke and Wolter (2011), defining half the frequency of the most frequent category as the cutoff. Following Abric (2003), the importance cutoff point was obtained by averaging the value of importance across categories. A social representation map was built with four zones (2 × 2) delimited by the cutoff points. The first zone regroups the categories with high frequency that is considered very important, which is the central core. Zone 2 (low importance and high frequency) regroups the more important peripheral categories, named the first periphery. Zone 3 (high importance and low frequency) clusters the contrasting elements. This zone usually reveals the existence of minority subgroups with a different representation. Zone 4, named the second periphery, provides the elements with low frequency and low importance in the representation.

2.3.3. Consumer segmentation by risk perception

Cluster analysis was applied to segment the participants to picture better their perceptions, beliefs, and attitudes. First, to obtain distinctive homogenous groups, Hierarchical Cluster Analysis was conducted using the complete linkage method on the Euclidian distances of the scores from the risk perception. Three clusters were determined as the optimum number, and then, k-means clustering was applied to refine the clusters. The silhouette coefficient of cohesion and separation was evaluated to validate the number and participant distribution between clusters (Rousseeuw, 1987). The silhouette coefficient considers the average distance of all elements within a cluster (cohesion) and the average distance between clusters (separation). The coefficient ranges between -1 and +1, and in a good solution, the within-cluster distances are small, and the between-cluster distances are large, resulting in a silhouette coefficient higher than +0.5. The obtained clusters and participant distribution between clusters reached a +0.7 silhouette coefficient. Upon defining the participants' clusters, the profiles of the resulting groups were compared based on the participants' risk perception, attitude (polarity index), age, education, and sex. To evaluate the differences between clusters, chi-square was performed on sex, while for the other characteristics, the Welch's analysis of variance (Welch's ANOVA) followed by Games–Howell post hoc test was used because the sample size between clusters was different and the variances were not homogeneous.

To examine the differences of perceptions between clusters, the frequencies of category were calculated for each cluster, then, chi-square tests were conducted on the frequencies of each category mentioned by at least 5% of participants. Additionally, to better visualize the main differences among clusters, correspondence analysis was performed on the categories with uneven distribution between clusters (chi-square, $p<0.05$).

Finally, the answers to the two open-ended questions between clusters, "Why would you eat insects?" and "How would you eat insects?" were compared. To understand and interpret their representations the frequencies of answer groups were calculated for each cluster, and chi-square tests were conducted. Only answer groups elicited by at least 5% of the participants were considered.

Statistical analyses were performed using XLStat® software, version 2013.04.02 (Addinsoft, 2013).

3. Results

In total, 2,864 terms were produced by the participants in the word association task, being 851 different terms (types) and 744 terms elicited just once during the interviews (hapax). The triangulation process grouped the 851 different terms into categories, resulting in a total of 72 categories. Finally, only 23 categories elicited by at least 5% of participants remained.

For each of the two open-ended questions, a total of 780 answers was obtained. For the question "Why would you eat insects?" 19 categories of responses were defined by triangulation, but only nine categories mentioned by at least 5% of the participants remained. Similarly, the question "How would you eat insects?" produced

45 categories, but only seven were further examined in the study, following the same criteria.

3.1. Social representation and polarity index

The polarity index of categories reveals the connotations of the elicited terms used to form the respective category. In other words, the polarity index reflects how positive or negative are the respondents' associations to food made of insects. Considering the 23 categories (Fig. 1), most (14) were positive, seven were negative, and two were neutral. The positive categories that stood out with polarity indexes above +0.5 were associated with nutrition (*Protein*, *Nutrition*, and *Health*), exoticism (*Exotic* and *Asia*), willingness to try (*Curiosity* and *Acceptance*), and *Culture*, *Innovative,* and *Sustainability*. On the other side, the negative categories with polarity less than -0.5 were *Rejection*, *Disgust,* and *Unhealthy*. The polarity indexes of the elicited insect categories also revealed marked differences: the categories *Cricket* (+0.34), which grouped the words cricket and grasshopper, and *Ant* (+0.25) had positive connotations regarding edible insects, whereas *Worm-like*, which gathered larva, worm, and caterpillar words, was considered neutral (-0.02), and *Cockroach* had a negative appeal (-0.44).

Fig. 1 summarizes the significant categories of words that emerged in the word association task and their polarity denoting positive or negative connotations.

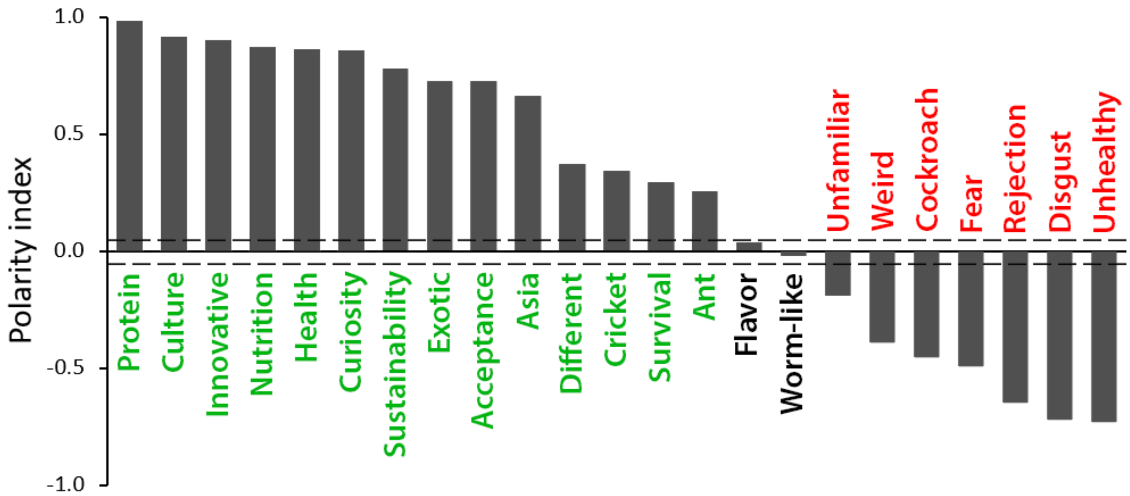

Fig. 1. Polarity index of categories mentioned by at least 5% of participants in the word association task: positive categories are shown in green, neutral in black, and negative in red; dashed lines represent the category connotation cutoff points.

The structural approach of social representation for *food made with edible insects* is presented in **Fig. 2.** The cutoff citation frequency for the inductor expression is 195, so all categories with a frequency above 195 were classified as having a high frequency, while those with lower values were classified as having a low frequency. For the importance, the cutoff point is 6.9; thus, all categories with an importance score higher than this value were classified as having a high importance and those with an importance score below that were considered as having a low importance.

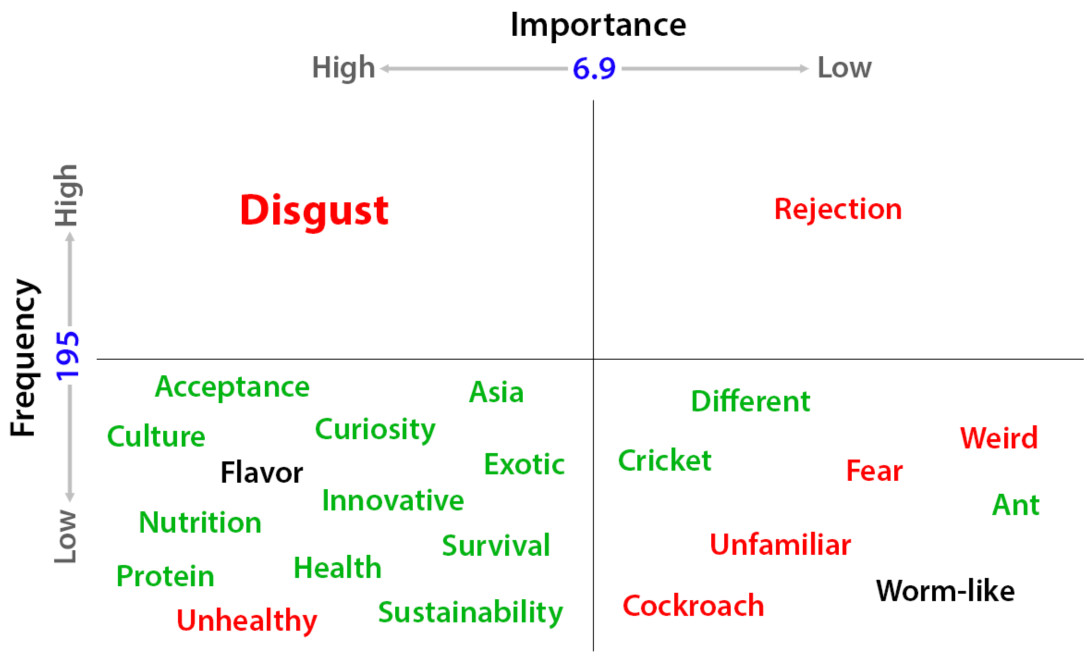

Fig. 2. The four representation zones of *food made with edible insects* for Brazilians (positive categories are green, neutral black, and negative red).

The top-left cell of **Fig. 2** represents the central core zone of the social representations where the categories with high frequency and high importance are located. The central core contains stable, shared, and consensual elements among the participants, and therefore, *Disgust* was the only central element with a strong negative connotation (polarity index of -0.71). The top-right cell constitutes the first periphery and includes secondary elements of the representation, with high frequency and low importance categories. Only *Rejection* is in the peripheric zone, supporting the *Disgust* in the central core with also a negative connotation (polarity of -0.64).

The bottom left cell corresponds to the contrasting zone representing low frequency and high importance categories. This zone reveals the existence of subgroups of respondents mostly demonstrating positive representations. For those subgroups, the elements of the contrasting zone might compose the central core. The categories located in this zone were those associated with health and nutrition concerns, such as *Protein*, *Nutrition*, and *Health*; categories related to the exotic and cultural aspects of edible insects for Brazilians, such as *Exotic*, *Asia* and *Culture*; categories representing the willingness to try insects such as *Curiosity* and *Acceptance*; elements that evoke sensory properties, combined in the *Flavor* category; elements associating the consumption of insects with hunger and means to survive (*Survival*); elements that denote the low environmental cost of producing edible insects (*Sustainability*); and finally, elements which show the perception of novelty about food made with edible insects (*Innovative*). All the categories in the contrasting zone had positive connotations, except for *Flavor* (+0.04) and *Unhealthy* (-0.72).

The bottom right cell represents the second periphery where the categories with low frequency and low importance are placed, and more idiosyncratic are included. This zone includes the insect types categories, *Cricket* (+0.34), *Ant* (+0.25), *Worm-like* (-0.02), and *Cockroach* (-0.44); categories that denote that edible insects are unknown or not usual for Brazilians, such as *Different* (+0.37), *Unfamiliar* (-0.18), *Weird* (-0.38); and finally, a negative emotion expressed by some participants when thinking about *food made with edible insects*: *Fear (*-0.49).

3.2. Consumer segmentation

The numerous and mostly positive categories found in the contrasting elements zone of social representation motivated the evaluation of consumers' segmentation. Cluster analysis revealed three distinct consumer groups. The characteristics of each cluster are reported in **Table 2**.

Cluster 1 (n=249) comprised mostly men exhibiting the lowest risk perception about eating insects and the most positive attitude to *food made with edible insects*. They were younger and more educated. Cluster 2 was the largest (n=287) and composed of similar proportions of male and female individuals. This segment displayed a slightly positive attitude to edible insects but an average risk perception. Finally, Cluster 3 (n=244) were mostly women presenting the highest risk perception and negative attitude. Besides, these individuals were older and with fewer years of formal education compared to the other clusters.

Table 2. Characteristics (mean ± standard deviation) of Brazilian clusters grouped by risk perception.

	All participants (n=780)	Cluster 1 (n=249)	Cluster 2 (n=287)	Cluster 3 (n=244)
Risk perception	3.28 ± 1.97	0.92[a] ± 0.87	3.36[b] ± 0.48	5.61[c] ± 0.49
Attitude (polarity index)	+0.04 ± 0.71	+0.33[a] ± 0.63	+0.07[b] ± 0.65	-0.29[c] ± 0.71
Age (years)	36.1 ± 12.8	34.3[a] ± 11.9	33.6[a] ± 11.8	40.8[b] ± 13.6
Education (years)	13.9 ± 2.8	14.4[a] ± 2.7	14.2[a] ± 2.7	11.9[b] ± 2.9
Sex (n)*				
Women	408	111 (-)	140	157 (+)
Men	372	138 (+)	147	87 (-)

Means with different superscript letters within the same row differ significantly (Welch's ANOVA, Games Howell test p<0.05).
*(+) or (−) indicate that the observed value is higher or lower than the expected theoretical value (chi-square test, p<0.05).

Table 3. Frequency of categories mentioned by at least 5% of participants in the word association task and results from chi-square test by cluster.

	Cluster 1	Cluster 2	Cluster 3
Rejection	72	138	**182** ***
Disgust	64	141	**165** ***
Asia	**54** ***	52	22
Weird	29	45	28
Curiosity	**42** **	**39** **	18
Different	27	43	22
Acceptance	**43** **	26	23
Exotic	**33** **	**35** **	13
Nutrition	30	26	25
Cricket	30	27	20
Innovative	30	26	18
Culture	**30** ***	**36** ***	9
Fear	15	23	**32** *
Survival	20	26	23
Ant	28	24	15
Cockroach	23	24	16
Flavor	14	**30** *	12
Health	20	18	17
Worm-like	22	22	11
Sustainability	**24** *	18	11
Protein	**18** **	**20** **	4
Unhealthy	2	9	**31** ***
Unfamiliar	16	12	10

* p<0.05; ** p<0.01; *** p<0.001.
The bold numbers indicate that the observed value is higher than the expected theoretical value under the assumption of row-/column independence.

Table 3 shows the associations of the terms' categories with the clusters. The Chi-square test revealed significant differences (p<0.05) among the clusters in 12 out of the 23 categories, *Rejection, Disgust, Fear,* and *Unhealthy* were more often mentioned by participants from Cluster 3, corroborating the negative views on eating insects in this group. *Food made with edible insects* was linked to sensory aspects (*Flavor* category) mainly in Cluster 2, while *Asia*, *Acceptance,* and *Sustainability* were significant associations characterizing Cluster 1 – corroborating more positive and favorable perception of eating insects. *Curiosity*, *Exotic*, *Culture,* and *Protein* categories were shared as relevant for both Cluster 1 and Cluster 2 participants. *Weird*, *Different*, *Innovative,* and *Unfamiliar*, which suggests that the participants are unfamiliar with edible insects, and *Cricket*, *Ant*, *Cockroach*, *Worm-like Nutrition*, *Health,* and *Survival* had a homogenous distribution between clusters.

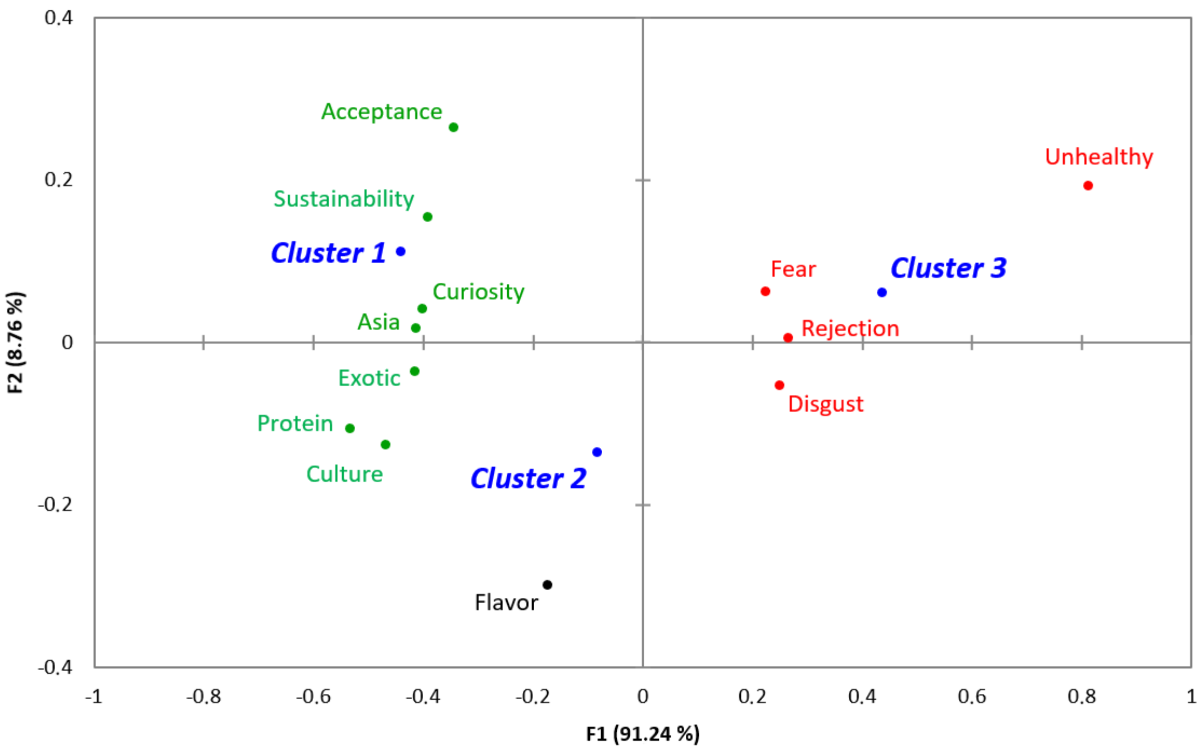

Fig. 3. Correspondence analysis of the categories (green words for positive categories; black for neutral; and red for negative) by cluster (blue color) was performed on the frequency of the categories mentioned by at least 5% of participants in the word association task.

Correspondence analysis was performed to help visualize the associations of categories and clusters (**Fig. 3**). Only those categories mentioned by at least 5% of the participants were included in the analysis. The first dimension was responsible for

91% of the variance and provided a noticeable separation between the three clusters. Cluster 1, located on top-left, comprehended the participants with the lowest risk perception about eating insects and was placed closer to all the positive categories (*Acceptance*, *Sustainability*, *Curiosity*, *Asia*, *Exotic*, *Protein,* and *Culture*). Whereas Cluster 2, on the bottom left, includes the respondents with moderate risk perception, which projected closer to the neutral category of *Flavor*. On the other hand, participants from Cluster 3 (top right), with the highest risk perception, were associated with the negative categories of *Unhealthy*, *Fear*, *Rejection,* and *Disgust*.

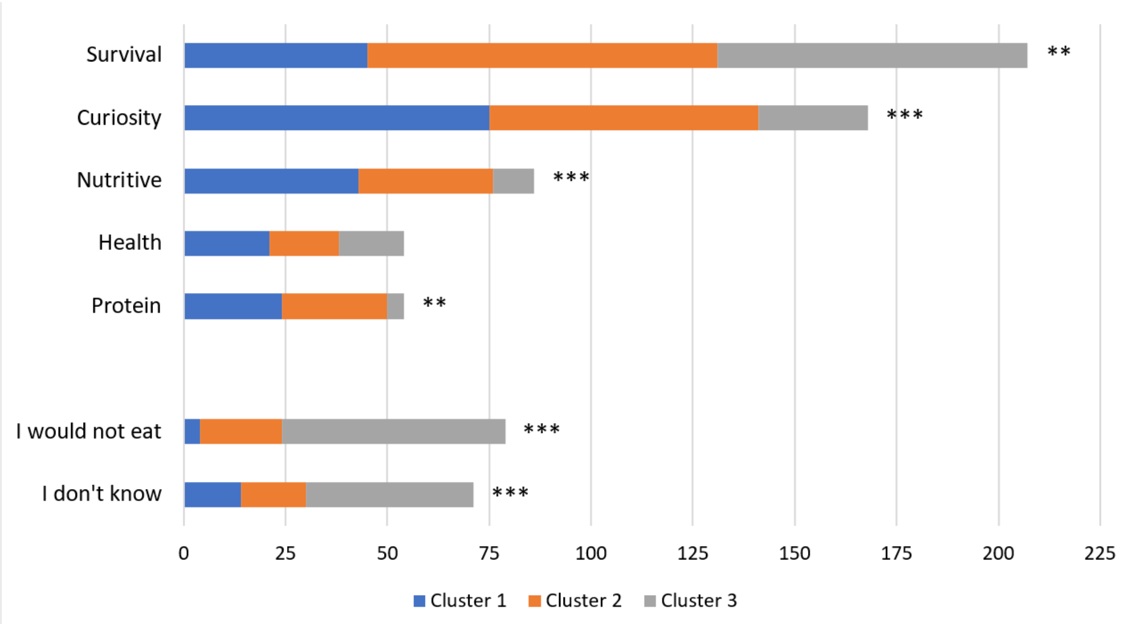

Fig. 4. Frequency of answers for the question "Why would you eat insects?" by at least 5% of participants. Chi-square test: * p<0.05; ** p<0.01; *** p<0.001.

The open-ended question "Why would you eat insects?" aimed to understand which motivations would lead Brazilians to consume insects. The answers were grouped and compared between clusters. Chi-square tests were conducted on the frequencies of answer groups elicited by at least 5% of the participants (**Fig. 4**) and revealed that the primary motivation for insect consumption was *Survival* (207 responses), which grouped answers related to the circumstances of necessity, such as "in need", "hunger", "survival", and "lack of food", most often elicited by respondents from Clusters 2 and 3. The second, third and fifth most replied motivations were *Curiosity*, *Nutritive,* and *Protein*, respectively, which were evoked mainly by the youngest and more educated participants (Clusters 1 and 2). *Health* did not associate with a specific cluster. Interestingly, 79 participants replied, "I would not eat", reinforcing rejection on insect consumption, and 71 participants answered, “I do not know”. The majority of these respondents were in Cluster 3.

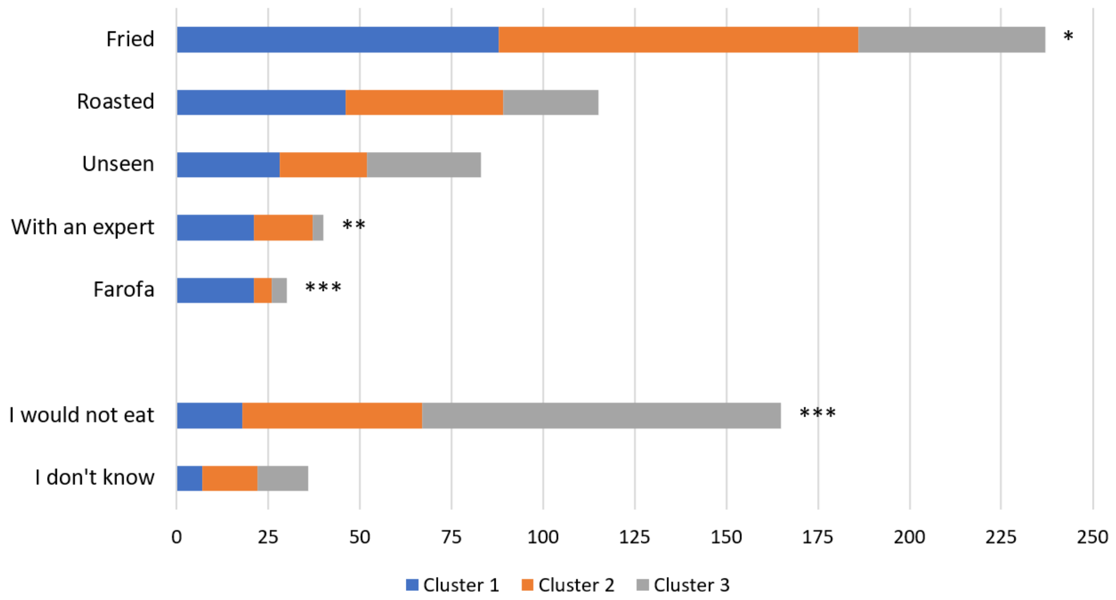

Fig. 5. Frequency of answers for the question “How would you eat insects?” by at least 5% of participants. Chi-square test: * p<0.05; ** p<0.01; *** p<0.001.

When the participants were asked, “How would you eat insects?” (**Fig. 5**), *Fried* was the most elicited style of preparation (237 responses), mainly by Clusters 1 and 2. Likewise, those participants replied mostly on the *With an expert* group, which comprehends the responses that rely on the preparation by someone who has background knowledge on insect preparation, such as “in a restaurant”, “with someone who knows how to prepare it”, “in a country where it is normal to eat them”, and “if it is ready-to-eat on a food market”. The second most mentioned answer was *Roasted*, which gathered answers like “roasted”, “barbecue”, and “insect skewers”, and the third most elicited was *Unseen*, which grouped replies related to not perceiving the insect, such as “as powder”, “as flour”, “ground”, “as an ingredient in a regular food (bread, cookie, cake, hamburger)”, “without seen the whole insect”, “without felling the insect texture or flavor”, and “without knowing there are insects inside the food”. The *Roasted* and *Unseen* did not associate with a specific cluster. The fifth most mentioned preparation was *Farofa*, a traditional Brazilian dish of cassava or corn flour toasted with other ingredients, in this case, insects, and is typically eaten as a side dish to the main meal. *Farofa* was prompted mainly by participants from Cluster 1. In addition, 165 respondents answered “I would not eat”, most of them from Cluster 3, while 36 participants replied, “I do not know”.

4. Discussion

Disgust was the central core of the social representation of *food made with edible insects* for Brazilians (**Fig. 2**) and evoked a strong negative connotation (**Fig. 1**). Shared by all humans, disgust is a basic emotion, believed as a mechanism of the behavioral immune system designed to protect us from contact with pathogens (Terrizzi et al., 2013). Because insects are considered a pathogen carrier by most Western populations, insect-incorporated food generally elicits disgust, which leads to the rejection of the food to prevent the ingestion of pathogenic substances (Rozin, Haidt, & McCauley, 2017). Like our study, several works identified disgust as the main barrier for insect consumption in Western’s countries, such as Woolf et al. (2019) in the USA; Lammers et al. (2019) in Germany; Tucillo et al. (2020) in Italy; and Sogari et al. (2019) in Australia. Even though disgust is a universal human emotion, it is important to note that the population’s cultural background dramatically affects what is recognized as disgusting (Amman et al., 2020; Bisconsin-Junior et al., 2020). This is evident with insect-eating behavior, considered acceptable and pleasant in many places in Africa and Asia, whereas it is perceived as disgusting in most Western countries (van Huis et al., 2013). Also, the introduction of insect-based food becomes especially challenging in cultures with a strong culinary tradition, such as those living in the Mediterranean (Toti et al., 2020).

Several strategies have been proposed to overcome the disgust associated with edible insects. The most promoted strategy relates to insect visibility, making them not visible, as a flour, for example. The degree of insect processing, and therefore, the insect visibility, affects the emotional expectations about an insect-incorporated food. Gmuer et al. (2019) assessed the emotional evaluations, willingness to eat, and expected liking of 428 Swiss participants on snacks with and without insects. The snacks consisting of tortillas with insect flour or insect “bits” (crushed, particles bigger than flour) had higher willingness to eat, expected liking, positive emotional evaluation, and lower negative emotional evaluation than the snacks with visible insect bodies. The same trend was observed in our results, which *Unseen* was the third most elicited answer about styles of preparation for insect consumption (**Fig. 5**). Moreover, the use of processed insects as an ingredient in well-known products reduces the disgust connotation. Familiar foods and preparations, such as cookies (Hartmann et al., 2015), meatballs (Tan et al., 2017), and hamburgers (van Thielen et al., 2018), rather than the reproduction of existing insect-based dishes from a different culture, tend to have higher acceptance by the consumers. Besides, the disgust associations tend to be passed between generations, reinforcing cultural differences in what evokes disgust and rejection. Thus, as proposed by Jensen and Lieberoth (2019), the availability of insect-incorporated foods in the market along with the diffusion of positive social models, for example, broadcasting insect-eating behavior by persuasive role models, could help shape the culture around insects, therefore reducing the Westerners' insect food disgust over time with repeated positive exposure.

Even though disgust was the central core of social representation, several positive associations were elicited and evaluated as important by segments of the participants, revealing groups of Brazilians with distinct representations. Therefore, two main aspects of these associations with edible insects should be highlighted: the healthiness and the willingness to try novel food.

The healthy properties were recognized by Brazilians as indicated by the categories of *Health*, *Nutrition*, and *Protein* (**Fig. 2**) and by the 194 replies on the question, "Why would you eat insects?" stating that the *Nutritive*, *Health*, and *Protein* aspects would lead the respondents to consume insects (**Fig. 4**). A similar trend was observed in some studies with Western populations, in which insects were recognized as being healthy and nutritive (Schlup & Brunner, 2018; Menozzi et al., 2017; Hartmann et al., 2018), while the insect consumers perceived themselves as health-conscious (Hartmann et al., 2018). Therefore, the healthy features of insect-eating led to a higher acceptance of insect-incorporated foods (Schlup & Brunner, 2018; Mancini et al., 2019). For this reason, the advertisement of the healthy and nutritional aspects of insect food products can be used to increase their acceptance and willingness to eat by consumers.

Another important way to enhance the acceptance and establish regular consumption of insects as food is a positive first eating experience. Many participants elicited *Curiosity* and *Acceptance* (**Fig. 2**) and stated that *Curiosity* drives them to try insects (**Fig. 4**). However, if their first experience eating insects is unpleasant, they probably would not eat it again and become regular consumers. Participants expressed this concern about the insect-eating experience by *Flavor*, which was considered as an important feature (**Fig. 2**); however, they exhibited a neutral polarity (**Fig. 1**), most of the respondents were uncertain if the insects' flavor would be something good or bad, suggesting some degree of neophobia. Mishyna et al. (2020) explored the contributions of flavor and texture on Western consumers' attitudes toward edible insects and recognized that insect-based dishes that adapted their sensory characteristics and aesthetic features to the Western style are more attractive to consumers. Thus, insect food products should have flavor and texture that meet consumers' expectations similar to their familiar food to elicit positive emotions.

In Western countries, the most favorable texture characteristics for insect consumption are firmness and crunchiness rather than softness and juiciness. This fact became evident observing the results for the question, "How would you eat insects?", in which *Fried* and *Roast* were the most elicited answers (**Fig. 5**), while just a few participants answered *Stew* (18), *Raw* (13), and *Boiled* (7) - not exhibited on Figure 5 because of the few answers. Studies with whole insects tasting session presented a similar trend: Caparros Megido et al. (2014) provided to 189 people oven-baked and boiled mealworms and house crickets in a Belgium insectarium, and hedonic testing showed that crispy baked insects were more appreciated than the boiled ones; while Grabowski et al. (2017) evaluated the consumption pattern of 175 participants who ate deep-fried insects in German events and concluded that insects with a relatively firm exterior and a soft interior were considered unpleasant compared to hard and crunchy insects.

The elicited insect categories (*Cricket*, *Ant*, *Worm-like,* and *Cockroach*) had pronounced differences on the polarity indexes (**Fig. 1**): while *Cricket* and *Ant* were positively evaluated, *Worm-like* appeared neutral and *Cockroach* negative. Therefore, to obtain an accurate understanding of consumer representations, edible insects should be studied at the species level, avoiding the assumption that all insects produce the same level of disgust and rejection. Likewise, mammals that are considered edible, such as cattle, pigs, sheep, and rabbits, are distinctively preferred by consumers due to their representations and sensory characteristics of their meat. Thus, if two different inductor questions, one about *food made with crickets* and *food made with cockroaches,* had been used in this study, the emerged representations would certainly be completely different from each other. Schäufele et al. (2019) verified this difference between two insect species on the willingness-to-try and appetizing appeal, being grasshoppers more liked than mealworms by German consumers. However, it is also relevant to consider the regional culture on consumers' preferences of the insect species, as pointed out in the study of Hartmann et al. (2015). Chineses demonstrated a higher willingness to eat and more positive attitudes towards silkworms than crickets. In contrast, Germans had no substantial preference between the two species. Our results in Brazil followed a similar trend to those observed in the USA and India (Ruby et al., 2015), in which ant, cricket, and grasshopper had the highest scores in willingness to eat, whereas mealworm and caterpillar had average scores, and cockroach, the lowest.

Risk perception is an individual judgment of the likelihood that a consequent loss could happen and the significance of the expected outcomes (Sheeran et al., 2014). Consumer choices usually involve some form of risk, and when it is perceived as high, it dampens expectations and attitudes towards the choice, such as expected liking or the intention to consume a product (Ha, Shakur & Do, 2020). Our study found three groups of participants with distinct levels of risk perceptions about eating insects, and these groups presented differences in attitude, age, educational level, and sex (**Table 2**). This identification of consumer characteristics provides important information for the insect-food industry to accurately segment, target, and market consumers.

Younger and highly educated participants in Clusters 1 and 2 (**Table 2**) linked insect consumption mostly to positive associations like *Curiosity*, *Protein*, and *Culture* (**Fig. 3**). Younger people are more open to considering insects as food because they are inclined to have thrilling novel eating experiences (Tan et al., 2016) and are more aware of sustainability and environmental issues related to food production and consumption (Sogari et al., 2019). Besides, further years of formal education tend to promote access to more information about insects as food and their positive features, e.g., nutritional aspects, which favor consumers' attitude towards eating insects and reduce insect food rejection (Mancini et al., 2019; Lombardi et al., 2019). When this younger and well-educated group was composed mostly of men, Cluster 1 (**Table 2**), the risk perception decreased, the positive attitude increased, and other positive associations became more evident (*Acceptance* and *Sustainability*). This could be partially explained because men, in general, are less sensitive to disgust and have a lower animal reminder disgust sensitivity compared to women (Hamerman, 2016). Also, men tend to have a more adventurous and explorative taste orientation than women, being more likely to try unfamiliar foods (de Boer et al., 2013). On the other hand, women usually show a higher insect aversion than men (Landová et al., 2021). However, as Dagevos (2021) reviewed, gender only influences the willingness to eat whole and unprocessed insects, while for processed food containing invisible insects, no significant difference between genders was evident in the literature.

Most of the aspects influencing the acceptance or rejection of edible insects reported in this study corroborate those found in other Western countries. The main spotted difference is the association with *Survival,* an important positive association with edible insects (**Fig. 2**). At the same time, *Survival* was the most evoked answer to the open-ended question “Why would you eat insects?” (**Fig. 4**). At both procedures, the *Survival* grouped terms and answers related to means to survive, hunger, and circumstances of necessity. This specific driver found in our study is because entomophagy is part of the food habits among some non-urban Brazilian populations, mostly indigenous tribes, traditional communities, and underprivileged rural people (Costa-Neto, 2015). Thus, even if the urban areas – where the study took place – do not consume insects, the recognition that they can be used as food for survival seems intuitive for most Brazilians living in urban areas. In the same line, the previously published study Bisconsin-Júnior et al. (2020) identified that Brazilian regions with markedly indigenous cultural influence exhibited stronger associations between edible insects and survival than regions with pronounced European colonization culture.

5. Limitations

An expressive number of individuals were interviewed in all regions of Brazil, a country with a continental dimension and profound cultural and socio-demographic differences across its territory. Nonetheless, this work was exploratory and presented some limitations. Even though statistical procedures were performed in the qualitative data, our results may not be conclusive about the Brazilians intention to consume insects. Thus, further studies should use a quantitative methodological approach, which would lead to more robust conclusions about the Brazilian consumer intentions about edible insects.

We excluded individuals with previous consumption of insects to avoid previous experiences with entomophagy, which could somewhat bias the research findings. However, an assessment of participants' level of knowledge about entomophagy was not performed during data collection. Prior exposure to the idea of eating insects can be used as an indicator of entomophagy cultural appropriateness and imply a greater willingness to consume them. As Verbeke (2015) reported, people who claimed familiarity with the idea of entomophagy were 2.6 times more likely to adopt insects as food than those who said they had never heard about the eating of insects.

Finally, this study was conducted before the COVID-19 pandemic crisis. The pandemic context significantly impacted on consumer food habits as it increased people's awareness of health risks. For example, recent research conducted by Khalil et al. (2021) pointed out that the willingness to consume insect-based food had a significant decrease in Spain during the COVID-19 lockdown (May and June 2020). However, it is still uncertain if the COVID-19 pandemic will have a significant and enduring effect on the willingness to try non-conventional foods like edible insects.

6. Concluding comments

This study has demonstrated the social representation of edible insects among Brazilian consumers and highlighted segmentation, exposing diverse consumer characteristics and major differences regarding edible insects.

Brazilian consumers generally show *Disgust* as the main association with edible insects. However, consumers segmentation revealed that younger men with higher educational levels have a lower risk perception of eating insects and could accept them as a new food source, while older women with less formal education strongly reject edible insects.

The survival aspect was identified as a major association with entomophagy in Brazilian society. This uncommon feature relates to the evidence that insect consumption is a traditional component in the food culture among some ancestral groups. Thus, most Brazilians acknowledge insects as food in survival circumstances, even in urban areas.

As a practical output, our findings could help the food industry develop and introduce insect-based foods into the Brazilian market. Some of the key aspects regarding insect food products observed in our work: insects like cricket, grasshopper, and ant were preferred over larva, caterpillar, and cockroach; associations with the cultural and/or exotic origins, the environmentally friendly aspect, and the nutritional qualities were considered positive and should support consumers acceptance; the curiosity element should be explored, especially to promote the first contact with this type of food; the flavor was considered an aspect of concern and should be communicated and guaranteed by the food industry as having an attractive profile to favor a good eating experience; the marketing should target mostly young and highly educated men, as they were identified as the most potential consumers; the fried and roasted styles of preparations were preferred, also process that would prevent the perception of the whole insect in the food, such as pasta, flour, or powder, would favor its consumption; and finally, associations of disgust, unhealthy, and fear lead to the rejection of insect-based foods, thus the food industry should avoid or reduce these negative associations.

Author contributions

ABJ, HR, JHB and LRBM contributed to the design of the study. ABJ and HR oversaw acquisition of the data from all interviewers. ABJ, HR and MAAPS contributed to data collection. Before data analysis, ABJ, MAAPS and JHB formatted and categorized all raw data. HR and JHB supervised statistical analyses. ABJ was responsible for data analyses. ABJ and LRBM wrote the first draft of the manuscript. All authors provided relevant contributions to the manuscript. All authors read and approved the final version of the manuscript.

Ethics statement

This study was approved by the Research Ethics Committee of the University of Campinas (UNICAMP) under the protocol CAAE 80665117.5.0000.5404. Written informed consent was obtained from all participants.

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available, but are available under request to the corresponding author.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgments

The authors want to express their gratitude to the following interviewers, who helped us gather the data from eight cities across Brazil: Lorena Aurora Januário, Mari Silvia Rodrigues de Oliveira from Federal University of Santa Maria (UFSM), Rosires Deliza from Embrapa Food Technology, and Verena Silva Lima from Federal University of Amazonas (UFAM).

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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CAPÍTULO V

Comparing ultrasound, pulsed electric fields, and high pressure on cricket protein: extraction, color, structure, and functional properties

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Artigo em preparação para ser submetido ao *Innovative Food Science and Emerging Technologies*

Abstract

Edible insects are a source of proteins typically processed using high temperatures, which can cause undesired effects on the protein's properties. Non-thermal technologies, like ultrasound (US), pulsed electric fields (PEF), and high pressure (HP), can assist in the extraction and improve protein properties. This study compared the effects of US, PEF, and HP on the extraction yield, color, and functional and structural properties of cricket (*Gryllus assimilis*) protein concentrate. All treatments increased the protein solubility (US was the most effective) while reducing the time and the chemicals required for the extraction. US produced a protein concentrate with a lighter color, fewer aggregates, and a stronger negative charge, resulting in increased solubility, water retention, and foaming properties. HP increased oil retention, emulsion properties, and gel formation, while PEF improved foaming properties. These non-thermal technologies offer a promising way to enhance the production of cricket protein concentrate for use in the food industry.

Keywords: edible insects; non-thermal technologies; emerging technologies; sustainability; protein concentrate; enzymatic browning.

1. Introduction

The United Nations' Agenda 2030 aims to foster sustainable development worldwide by pursuing seventeen distinct goals (United Nations, 2023). Embracing edible insects as an alternative food source represents a promising approach to advancing at least five goals: zero hunger, good health and well-being, responsible consumption and production, climate action, and life on land (Mariutti et al., 2021). Edible insects offer a range of benefits, including high nutritional value, sustainable production practices, and potential economic viability.

However, despite their potential advantages in nutrition, sustainability, and economics, edible insects have not gained widespread acceptance for consumption in most countries. Recent studies have identified two strategies to increase the acceptance of this food source: using cricket species, which are considered more acceptable than other insect species, and processing the insects into forms that prevent the consumer from recognizing them in the food (Tzompa-Sosa et al., 2023; Bisconsin-Júnior et al., 2022; Rovai et al., 2021).

Crickets are highly valuable in terms of nutritional value. During the adult phase of their life cycle, crickets exhibit a protein content that is much higher than other nutrients, with an average variation of 55 to 65% protein in dry weight (Magara et al., 2021). In addition to proteins, crickets are rich in lipids, certain minerals, and vitamins (Ververis et al., 2022). Several species of crickets, such as *Acheta domesticus*, *Gryllus assimilis*, and *Gryllus bimaculatus*, are already being raised in commercial farms for human consumption in various regions across Asia, Africa, America, and Europe. Cricket farming is a relatively simple process that can be conducted on a small scale in rural environments (Caparros-Megido et al., 2017) or on a larger, automated scale (Tanga et al., 2021).

Insect processing is necessary to ensure the safety and quality of insect-based food. The most adopted treatments in insect processing are blanching and drying (Melgar-Lallane et al., 2019). These treatments are highly effective in reducing the microbiota that can lead to the development of foodborne diseases or product deterioration, as well as extending the shelf life of the food product. For instance, Vandeweyer et al. (2017) evaluated the impact of blanching for 40 s in boiling water on *Tenebrio molitor* larvae. They found a significant reduction in the total aerobic count (from 8.0 to 1.5 log cfu/g), yeasts and molds (from 5.0 to 2.8 log cfu/g), and

Enterobacteriaceae (from 7.4 to <1.0 cfu/g). Meanwhile, Kröncke et al. (2018) evaluated various drying methods in *T. molitor* larvae. These authors had excellent results in freeze-drying, vacuum-drying, and rack oven-drying treatments. These processes did not negatively affect the insect's nutrients and reduced water activity to below 0.3, thus preventing the multiplication of microorganisms, enzymatic activity, and non-enzymatic browning reactions.

However, it is worth noting that traditional processing technologies involving elevated temperatures can adversely affect the final product's quality, leading to color browning, lipid oxidation, and other undesired changes. For example, Khatun et al. (2021) observed browning and lipid oxidation after blanching and oven-drying adult crickets of the *A. domesticus* and *G. assimilis* species. At the same time, Mishyna et al. (2020) examined the impact of different drying methods on the volatile and sensory characteristics of edible locusts and silkworms. Notably, the locusts and silkworms subjected to microwave-drying exhibited Maillard reaction and lipid oxidation and received low sensory scores from the panelists, indicating undesirable appearance, flavor, and aroma development.

In addition to these deleterious effects, thermal technologies can significantly impact proteins' structure and functional properties. The most observed effect is protein denaturation, which can result in profound modifications of the protein molecular structure. Furthermore, various active compounds, such as reducing sugars and active carbonyl compounds, can react with proteins causing structural changes upon heating. Any changes to protein structure will accordingly alter its properties (Zhang et al., 2021a). Azagoh et al. (2016) conducted a study to examine the impact of blanching (90 ^{o}C for 10 min), followed by oven drying (75 ^{o}C for 6 h), on the proteins of *T. molitor*. The results showed that the thermal treatment caused a significant reduction in both solubility and surface hydrophobicity. Similarly, Bußler et al. (2016) observed that thermal treatment of *T. molitor* above 100 ^{o}C caused a significant decrease in protein solubility, water binding capacity, and intrinsic fluorescence intensity. Likewise, Kröncke et al. (2018) found that high temperatures during drying treatments significantly reduced *T. molitor* protein solubility. The observed changes in protein solubility, surface hydrophobicity, water binding capacity, and fluorescence intensity are likely attributed to protein denaturation caused by the applied heat. The denaturation led to the unfolding of protein structures and the exposure of hydrophobic groups. These groups then interacted, forming aggregates with lower solubility, thus causing the hydrophobic regions to be buried inside the structure.

Non-thermal food technologies, namely ultrasound (US), pulsed electric fields (PEF), and high pressure (HP), are viable alternatives to traditional thermal processing to avoid the adverse effects of heat on proteins. In addition, non-thermal technologies provide shorter treatment times and sustainable, eco-friendly processing with negligible environmental impacts (Chakka et al., 2021). A comprehensive review conducted by Ojha et al. (2021) investigated the processing pathways for edible insects and how non-thermal technologies can be employed. As an example, US can be used to enhance protein extraction and modify its functional properties. Choi et al. (2017) found that using US on *Bombyx mori*, *T. molitor*, and *G. bimaculatus* increased the protein extraction yield, while Mishyna et al. (2019) observed that US-assisted extraction could increase the solubility, coagulability, and foam stability of *Schistocerca gregaria* protein extracts. In contrast, the same treatment reduced solubility and coagulability in *Apis mellifera* extracts. Regarding PEF, Psarianos et al. (2022) conducted a study in which they applied PEF directly to ground *A. domesticus*, and then evaluated the freeze-dried samples. Results showed that more intense PEF treatments improved the oil binding capacity, protein solubility, and emulsification capacity. As for HP, Boukil et al. (2022) applied treatments ranging from 70 to 600 MPa for 7 min on *T. molitor* soluble proteins and confirmed the protein unfolding and the formation of large aggregates of mealworm proteins by the decrease in fluorescence intensity, shift in the maximum emission wavelength, increase in surface hydrophobicity, and the molecular weight profile.

Our study aimed to investigate the potential of non-thermal technologies, namely US, PEF, and HP, to produce a cricket protein concentrate with desirable characteristics for the food industry. To our knowledge, no previous studies have compared the efficacy of these technologies for extracting protein from crickets. Thus, we produced protein concentrates from *Gryllus assimilis* using these non-thermal technologies and evaluated their impact on protein extraction efficiency, molecular structure, functional properties, color, and enzymatic browning of the final product.

2. Material and Methods

2.1. Raw Material

Adult-age frozen Jamaican field crickets (*Gryllus assimilis*) were purchased from a German producer (Fauna Topics GmbH, Marbach am Neckar, Germany) and kept frozen at -40 ^oC. Before any experiment, the insects were washed with cold water and allowed to drain to remove impurities. The water content was determined after placing fresh ground crickets in an oven at 105 ^oC for 48 h and calculating the weight difference. The crude protein content of the crickets was determined by the Kjeldahl method (Bradstreet, 1954), using 5.33 as the nitrogen-to-protein conversion factor, as suggested by Boulos et al. (2020) and Janssen et al. (2017).

2.2. Kinetics of cricket protein solubility assisted by non-thermal technologies

Eight experiments were designed for each technology and conducted in triplicate to investigate the impact of non-thermal technologies on the transfer kinetics of cricket protein to an alkaline solvent. Initially, the crickets were homogenized using a T-25 Ultra Turrax (IKA, Staufen, Germany) with a 0.025 M NaOH aqueous alkaline solution, in a ratio of 1:6, at 10,000 rpm for 10 s, resulting in a slurry. Subsequently, this slurry was subjected to one of the planned extraction experiments; then, the protein solubility was evaluated. The increase in solubility was plotted against the treatment time, and regression analyses were performed to determine the mathematical model that best fit the experimental data. The coefficient of correlation (R^2) and root mean square error (RMSE) were calculated to select the most appropriate model. The model with the highest R^2 and lowest RMSE was considered to provide the best fit for the experimental data.

2.2.1. Ultrasound (US)

The 200 mL of cricket slurry (cricket in 0.025 M NaOH, ratio of 1:6) was placed in a 250 mL beaker, then positioned in an ice water bath to prevent overheating. The treatment was performed using the UIP1000hdT ultrasonic device (Hielscher, Germany), equipped with a BS4d22 sonotrode (tip diameter of 22 mm). The probe was immersed to a depth of half the slurry height. The duty cycle was set at 100%, the applied frequency was 20 kHz, and the output power was 200 W, resulting in a power density of 1 W/mL. Sonication was carried out intermittently (30 s on and 30 s off), and the sonication time varied from 1 to 20 min. The US untreated sample was collected just before the start of sonification. The temperature was monitored during the treatments using a digital thermocouple (General Tools & Instruments, NJ). The initial temperature was approximately 7 °C and never exceeded 30 °C during treatment.

2.2.2. Pulsed electric fields (PEF)

The treatments were carried out on the cricket slurry (cricket in 0.025 M NaOH, 1:6) using the ELCRACK HVP-5 PEF system (DIL, Quackenbrück, Germany) within a batch chamber with an electrode gap width of 80 mm. During treatment, the pulse was monitored using a Tektronix TDS 1012 oscilloscope (Beaverton, OR, USA) with two channels. One channel displayed a positive pulse, corresponding to the applied peak voltage, while the other showed a negative pulse, corresponding to the current. The oscilloscope displayed the measured values as peak-to-peak readings. The pulse was bipolar and nearly rectangular. The treatments were carried out at 2.15 kV/cm. The nominal pulse width and frequency were kept constant at 18 μs and 10 Hz, respectively. The number of applied pulses varied from 50 to 800. The PEF untreated sample was collected just before the start of electric pulses. A digital thermometer (General Tools & Instruments, NJ) measured the sample temperature before and after treatment. The initial temperature was ca. 10 °C, which never exceeded 30 °C.

2.2.3. High pressure (HP)

The selection of the HP treatment conditions was based on preliminary tests to evaluate the effect of different pressure levels on the solubility of cricket protein (data not shown). Based on these preliminary tests, a pressure level of 200 MPa was chosen as causing the most significant increase in cricket protein solubility, which is consistent with the results reported by Kim et al. (2021) for *P. brevitarsis* larva.

Cricket slurry (crickets in 0.025 M NaOH, 1:6) was deaerated and sealed in sterile plastic bags. The bags were then placed in a 100 mL pressure chamber (U33, Unipress, Warsaw, Poland) filled with distilled water as a pressure-transmitting medium. HP treatment was conducted at 200 MPa for 1 to 20 min. The compression rate was 8 MPa/s, and decompression occurred in less than 8 s. The HP untreated sample was collected before the start of pressurization. A digital thermocouple (General Tools & Instruments, NJ) monitored the temperature within the pressure chamber. The initial temperature was ca. 19 °C, which never exceeded 25 °C.

2.2.4. Protein solubility increase

Immediately after the treatment, the protein solubility of the slurry was evaluated. The slurry was centrifugated at 3,240 g for 30 min at 4 °C. The protein content in the supernatant was determined using the Bradford assay (1976). The protein solubility increase was calculated using the following formula:

$$\text{Protein solubility } (\%) = [\left(\frac{\mathrm{PS_t}}{\mathrm{PS_c}}\right) - \left(\frac{\mathrm{PS_u}}{\mathrm{PS_c}}\right)] \times 100 \quad (1)$$

Where: $\mathrm{PS_t}$ - protein content in the US, PEF, or HP-treated sample supernatant, $\mathrm{PS_c}$ - total protein content of the crickets, $\mathrm{PS_u}$ - protein content in the supernatant of the respective untreated samples.

2.2.5. Validation of kinetic models

The kinetic models were validated by comparing experimental and predicted data for protein solubility increase. For each non-thermal technology, the extraction conditions were selected based on the model parameters that resulted in the highest cricket protein solubility. Three experiments with the chosen conditions were performed for validation purposes.

2.3. Production of cricket protein concentrates assisted by non-thermal technologies

Protein concentrates assisted by non-thermal technologies were produced using the extraction conditions that resulted in the highest solubility of cricket protein. These conditions were chosen to maximize the proportion of soluble proteins extracted from the cricket in each concentrate. For US, the cricket slurry was treated using the conditions described in section 2.2.1 Ultrasound (US) for 14 min. For PEF, the conditions presented in section 2.2.2 Pulsed Electric Fields (PEF) were used, with 800 pulses of 18 µs. For HP, the same conditions presented in section 2.2.3 High Pressure (HP) were used for 9 min.

In addition to the non-thermal technology-assisted concentrates, a control concentrate was produced for comparison, using a standard alkaline protein extraction method. The cricket was homogenized (T-25 Ultra Turrax IKA, Staufen, Germany) with distilled water (1:6), and the pH of the slurry was adjusted to 10 using 6 or 1 M NaOH. The slurry was stirred at 400 rpm for 1 h at room temperature. During the extraction period, the pH was monitored and maintained at 10.

Following the control alkaline extraction process or extraction assisted by a non-thermal technology, the resulting slurry was centrifugated at 3,240 g for 30 min at 4°C. The supernatant was carefully collected, and its pH was adjusted to 7.00 (±0.02) using 6 M or 1 M HCl. After the pH adjustment, the neutralized supernatant was frozen overnight at -40°C and subsequently freeze-dried for 48 h at -20°C, using a Christ Alpha 1–4 LD Plus freeze-dryer (Osterode, Germany). The amounts of NaOH and HCl reagents used for the concentrates production were recorded to compare the reagent consumption among the different treatments.

2.4. Cricket protein concentrates characteristics

2.4.1. Protein and water content

The crude protein content of protein concentrates was determined by the Kjeldahl method. The conversion factor of 5.60 was used to convert nitrogen-to-protein content, as Boulos et al. (2020) and Janssen et al. (2017) proposed. The water content was determined after placing the protein concentrates in an oven at 105 ^{o}C for 48 h and calculating the weight difference.

2.4.2. Extraction yields

The dry matter yield of the protein concentrates from fresh crickets was calculated using the following formula:

$$\text{Dry matter yield (\%)} = (\mathrm{P_{DM}}/\mathrm{C_{DM}}) \times 100 \quad (2)$$

Where: $\mathrm{P_{DM}}$ - weight of protein concentrate, dry matter, $\mathrm{C_{DM}}$ - weight of crickets used to obtain the protein concentrate, dry matter.

The protein extraction yield of the protein concentrates from the crickets was calculated using the following formula:

$$\text{Protein extraction yield (\%)} = [\frac{(\mathrm{P_{DM}} \times \%\mathrm{ProtPC_{DM}})}{(\mathrm{C_{DM}} \times \%\mathrm{ProtFC_{DM}})}] \times 100 \quad (3)$$

Where: $\mathrm{P_{DM}}$ - weight of protein concentrate, dry matter, $\%\mathrm{ProtP_{DM}}$ - protein content (w/w) of the protein concentrate, dry matter, $\mathrm{C_{DM}}$ - weight of crickets used to obtain the protein concentrate, dry matter, $\%\mathrm{ProtC_{DM}}$ - protein content (w/w) of the cricket, dry matter.

2.5. Color and browning of cricket protein concentrates

2.5.1. Color and solution browning

To determine the color of the protein concentrates, the powder was placed in Petri dishes, and the color was measured at five different locations using a Minolta Spectrophotometer (CM-2600D, Konica Minolta Inc., Japan) with a CIELab system, D65 illuminant (daylight), SCI mode (specular component included), and 10° viewing angle. The total color difference (ΔE) of the non-thermal treated concentrate compared to the control concentrate was calculated using the following formula:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad (4)$$

Where: L^*, a^*, and b^* - color values of the non-thermal treated protein concentrate, L_0^*, a_0^*, and b_0^* - color values of the control protein concentrate.

The powder was diluted (1% w/v) in 0.1 M sodium phosphate buffer (pH 7.0) and kept under agitation at 300 rpm to record the enzymatic browning of the protein concentrate solutions. At time intervals, a sample was taken from the solution, placed in a 3.5 mL quartz cuvette, and the lightness (L* value) was read using a Minolta Spectrophotometer (CM-2600D, Konica Minolta Inc., Japan) with a CIELab system, D65 illuminant (daylight), SCI mode (specular component included), and 10° viewing angle. The decrease in lightness was monitored throughout 48 h.

2.5.2. Polyphenol oxidase (PPO) activity

The polyphenol oxidase (PPO) activity of the protein concentrates was determined according to Reinkensmeier et al. (2016). The 400 mg of protein concentrate was homogenized (T-25 Ultra Turrax, IKA, Staufen, Germany) with 10 mL of 0.1 M sodium phosphate buffer (pH 6.5) at 10,000 rpm for 1 min, followed by 30 min incubation on ice and centrifugation at 3,214 g, 8 °C for 30 min. The collected supernatant was used as the enzyme extract. The reaction mixture comprised 1.5 mL of reaction buffer [0.5 mM of sodium dodecyl sulfate in (30% 0.1 M citric acid and 70% 0.2 M disodium phosphate, pH 6.5)], 0.2 mL of 0.5 M L-proline in reaction buffer, and 0.1 mL of enzyme extract. The reaction was initiated by adding 0.2 mL of 25 mM catechol dissolved in the buffer. The formation of the pink proline-catechol adduct was recorded at 525 nm every 15 s for 6 min at 25 °C. The PPO activity was calculated from the initial linear slope of the absorption-time curve. For blank correction, the slopes of a sample blank (0.1 mL water instead of extract) and a reagent blank (0.2 mL reaction buffer instead of 25 mM catechol) were subtracted. The PPO activity was expressed relative to the PPO activity of the control concentrate.

2.5.3. Polyphenolic content

The polyphenolic content of the protein concentrates was measured using the Folin-Ciocalteu phenol reagent (Lowry et al., 1951). An aqueous dilution of the protein concentrates (10 mg/ml) was mixed with a solution of methanol and HCl (1.48%) in a ratio of 4:6 (v/v) and vortexed for 2 min. After centrifugation (3,214 g for 2 min), 100 µl of the supernatant was added to 2 ml of sodium carbonate solution (2%, w/v), vortexed for 3 min, and incubated with 50 µl of Folin-Ciocalteu reagent for 2 min at room temperature. The absorbance was measured at 765 nm. The polyphenolic content was expressed in mg of gallic acid equivalents per gram of protein concentrate.

2.6. Protein structural characteristics of cricket protein concentrates

2.6.1. Molecular weight distribution (SDS-PAGE)

The extraction was performed according to Sagu et al. (2021), wherein 100 mg of each protein concentrate was mixed with 1.5 mL of polyvinylpolypyrrolidone (PVPP) extraction buffer (0.2 M 3-(N-morpholino) propane sulfonic acid (MOPS) pH 7.0, 5% PVPP, 1% Triton X-100, 10% glycerol, and 2 mM DTT) at 95 rpm for 60 min. The mixtures were centrifuged at 10,000 g, 4 °C for 5 min. The supernatants were collected, placed at -20 °C for 20 min, thawed at 4 °C for 20 min, and briefly vortexed before being centrifuged at 10,000 g, 4 °C for 5 min. The clear supernatants were used in SDS-PAGE.

The molecular weight distribution of protein concentrates was determined using polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE), according to Laemmli (1970), with some modifications. Briefly, the protein concentrate extracts were mixed in a 1:1 (v/v) ratio with sample buffer (125 mM Tris buffer, pH 6.8, 20% (v/v) glycerol, 2% (w/v) SDS, 0.1% (w/v) bromophenol blue). For reduced proteins, 2% (v/v) 2-mercaptoethanol was added to the mixture. The mixtures were then heated for 5 min at 95 °C. After cooling to room temperature, 10 µL of each sample was loaded onto gels (Invitrogen NuPAGE 10% Bis-Tris protein gel, 12 wells, Thermo Fisher Scientific, Carlsbad, CA, USA). A Spectra™ Multicolor Broad Range Protein Ladder with molecular weight proteins of 10, 15, 25, 35, 55, 70, 100, 130, and 250 kDa

(Thermo Fisher Scientific) was used for calibration purposes. The gels were initially run at 20 mA for 30 min, and then the current was increased to 50 mA until the end of separation. The gels were stained overnight with a Coomassie blue solution and then destained with a 10% acetic acid solution. The destaining solution was changed regularly for about 3 h. The gels were finally scanned (Bio-5000 Professional VIS Gel Scanner, SERVA Electrophoresis GmbH, Heidelberg, Germany) and analyzed using Image Lab software (Bio-Rad Laboratories Ltd., Hemel Hempstead, United Kingdom).

2.6.2. Intrinsic fluorescence

Each protein concentrate was dissolved in 0.1 M phosphate buffer (pH 7.0) to achieve a uniform concentration of 0.5 mg protein/mL. Fluorescence emission spectra were measured using a PerkinElmer LS55 fluorescence spectrometer (Rodgau-Jügesheim, Germany) equipped with a pulsed xenon lamp and a red-sensitive photomultiplier (R928) at an excitation wavelength of 280 nm. The fluorescence spectra were scanned in a wavelength range of 300-400 nm, with a 290 nm cut-off filter placed in front of the emission monochromator (slit width 5). The phosphate buffer was used as a blank (Bußler et al., 2015).

2.6.3. Surface hydrophobicity

The surface hydrophobicity of cricket protein concentrates was determined using the hydrophobic fluorescence probe ANS according to Mintah et al. (2019) with some modifications. Protein concentrate suspensions (0.03 - 0.12 mg protein/mL) in phosphate buffer (0.01 M, pH 7.0) were used. The suspensions were vortexed and allowed to stand (10 min, 25 °C), then centrifuged at 4,000g for 10 min. The supernatant protein solution (4 mL) was mixed with 8.0 mM ANS (20 µL) in 0.01 M phosphate buffer (pH 7.0), kept in the dark (14 min), and subsequently measured for fluorescence (relative intensity). The Shimadzu RF-1501 spectrofluorometer (Kyoto, Japan) was used with excitation at 390 nm and emission read from 400 to 600 nm (slit width of 5 nm and scan speed of 120 nm/min). Using linear regression, the surface hydrophobicity was calculated as the initial slope (Is) of the relative fluorescence intensity against mg of protein/mL.

2.6.4. Particle size and zeta potential

A Zetasizer Ultra (Malvern Instruments, Worcestershire, UK) measured particle size and zeta potential. Protein concentrate dispersions were diluted to 0.1% w/v in 10 mM phosphate buffer (pH 7).

For particle size, dispersions were filtered through 0.45 μm syringe filters (Pall, New York, USA). Measurements were taken at 25°C, 633 nm, with a 90° scattering angle using a refractive index of 1.45 and a solvent refractive index of 1.33.

For zeta potential, dispersions were measured at 25°C in a folded capillary cell after 1 min of equilibration. The refractive index, dielectric constant, and viscosity of the dispersant were 1.33, 78.5, and 0.887 mPa s, respectively.

2.7. Functional properties of cricket protein concentrates

2.7.1. Effect of pH on protein solubility

The protein concentrate (50 mg) was dispersed in distilled water (50 mL), and the pH of the mixture was adjusted to different values ranging from 2 to 10 using either 1 or 6 M HCl or 1 or 6 M NaOH. After stirring for 90 min, the mixture was centrifuged at 3,240 g for 30 min at 4 °C. The protein content in the resulting supernatant was determined using the Bradford method (1976). The total protein content of the concentrate was determined using the Kjeldahl method and a conversion factor of 5.60 to convert nitrogen content to protein content (Boulos et al., 2020; Janssen et al., 2017). The solubility of the protein was then calculated using the formula:

$$\text{Protein solubility (\%)} = (\mathrm{P_s}/\mathrm{P_c}) \times 100 \qquad (5)$$

Where: $\mathrm{P_s}$ - protein content in the supernatant, $\mathrm{P_c}$ - total protein content in the concentrate.

2.7.2. Water holding capacity (WHC) and oil holding capacity (OHC)

Water holding capacity (WHC) and oil holding capacity (OHC) were determined following the methods described by Bußler et al. (2015). Briefly, 0.5 g of protein concentrate was added to pre-weighted 15 mL centrifuge tubes and mixed with 2.5 mL of either water (for WHC) or canola oil (for OHC), which was also weighed. The mixtures were vortexed for 60 s and centrifuged at 4,000 g for 20 min. After discarding the supernatant, the pellet was weighed. WHC and OHC were calculated using the established formulas:

$$\text{Water holding capacity (WHC) (g water/g d.m.)} = (\text{P} - \text{M}) / \text{M}_{\text{DM}} \tag{6}$$

$$\text{Water holding capacity (OHC) (g oil/g d.m.)} = (\text{P} - \text{M}) / \text{M}_{\text{DM}} \tag{7}$$

Where: P - pellet weight, M - sample weight, M_{DM} - sample weight, dry matter.

2.7.3. Foaming capacity (FC) and foam stability (FS)

To evaluate the FC and FS, the protein concentrate was dispersed in distilled water at 1% w/v, and the pH was adjusted to 7.0. The mixture was stirred on a shaker for 1 h at 25°C and then transferred to a 50 mL graduated cylinder. The suspension was homogenized (T-25 Ultra Turrax, IKA, Staufen, Germany) at 12,000 rpm for 2 min. The volume of the foam layer was measured at 10 s, 5 min, 10 min, 30 min, 60 min, 120 min, and 180 min after homogenization (Mishyna et al., 2019). FC and FS were calculated using the following formulas:

$$\text{Foaming capacity (FC) (\%)} = [(\text{V}_\text{t} - \text{V}_0)/\text{V}_0)] \times 100 \tag{8}$$

$$\text{Foam stability (FS) (\%)} = (\text{FC}/\text{FC}_0) \times 100 \tag{9}$$

Where: V_t – volume after homogenization at different times, V_0 – volume before homogenization, FC_0 – foaming capacity at 10 s.

2.7.4. Emulsion capacity (EC) and emulsion stability index (ESI)

The evaluation of EC and ESI followed the method of Zielinska et al. (2018). Protein concentrate was dispersed in distilled water (1% w/v), pH was adjusted to 7.0, and 15 mL of the dispersion was homogenized (T-25 Ultra Turrax, IKA, Staufen, Germany) with 15 mL of canola oil at 20,000 rpm for 1 min. For EC, the emulsion was centrifuged at 3,000 g for 5 min, and the volume of each layer was recorded. For ESI, the emulsion was heated for 30 min at 80 °C, followed by centrifugation at 3,000 g for 5 min, and the volume of each layer was recorded. EC and ESI were calculated using the following formulas:

$$\text{Emulsion capacity (EC) (\%)} = (\text{V}_\text{e}/\text{V}) \times 100 \tag{10}$$

$$\text{Emulsion stability index (ESI) (\%)} = (\text{V}_{30}/\text{V}_\text{e}) \times 100 \tag{11}$$

Where: V – the total volume of tube contents, V_e – the volume of the emulsified layer, V_{30} – the volume of the emulsified layer after heating.

2.7.5. Least gelling concentration (LGC)

The LGC was determined following the method described by Santiago et al. (2021). The protein concentrates were suspended in distilled water (pH 7) at varying concentrations (4-9% w/w), then heated in a water bath at 90 ^{o}C for 15 min. Subsequently, the tubes containing the dispersions were allowed to cool at room temperature for 2 h. The cooled dispersions were evaluated for gelation by inverting the tubes and recording the concentration at which the gel remained intact without sliding or falling.

2.8. Data analysis

All measurements were performed at least in triplicate, and the results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to analyze the significance of the data ($p < 0.05$), and the mean values were compared using Tukey's post hoc test ($p < 0.05$). OriginPro software (version 9.0) was used for statistical analysis and graph plotting.

3. Results and discussion

3.1. Effects of non-thermal technologies on cricket protein solubility kinetics

The solubility kinetics of cricket protein was evaluated for each non-thermal technology to establish the optimal treatment conditions for obtaining protein concentrates with a high content of soluble proteins. All treatments were performed at a temperature range from 5 to 30 °C to reduce potential thermal effects.

3.1.1. Ultrasound (US)

The effect of US treatment for 1 to 20 min on the solubilization of cricket protein into an alkaline solution is shown in **Fig. 1A**. The US untreated sample presented 45.65 ± 2.72 mg of solubilized protein per g of fresh cricket. Results demonstrated a positive effect up to the 12 min assay point, after that, US treatment led to insignificant increased protein solubilization. The main reason is the physical, mechanical, and chemical impacts of US-induced cavitation. Cavitation causes an increase in protein solubility by diffusing through cricket cells and then disrupting the membranes to expose cellular content, such as proteins, to the extraction medium (Rahman & Lamsal, 2021). However, after the 12 min assay point, no significant difference in protein solubility was observed. Zhang et al. (2023a) reported a similar effect when evaluating the US treatment time (10 to 50 min) in *Tenebrio molitor* protein. After 20 min of US treatment, there was just a minor increase in the protein solubilization, and when the treatment time was longer than 30 min, the solubility was slightly lower. The stabilization and decrease in protein solubility can be explained by the reinforcement of the ultrasonic cavitation effects that take place during more extended treatments, which may cause the formation of insoluble protein aggregates (Suchintita Das et al., 2022).

The experimental data were employed to identify the most suitable kinetic model. The second-order model was confirmed as having the best fit to the data of protein solubilization assisted by US. The high coefficient of determination ($R^2 > 0.991$) and low value of root mean squared error (RMSE < 0.675) indicate that the proposed kinetic model exhibits excellent fitting to the experimental results, reflecting a strong agreement with the observed data. The analysis of the proposed model revealed that the maximum increase in protein solubility (51.43%) would be achieved with 14 min of US treatment, with a 95% confidence interval ranging from 49.13% to 53.72%. Validation assays conducted with 14 min of US treatment further confirmed the model's accuracy, as an average increase in protein solubility of 50.42 ± 3.04% was obtained. These results confirm the reliability and consistency of the proposed kinetic model in predicting the observed increase in protein solubility through the application of US.

3.1.2. Pulsed electric fields (PEF)

Fig. 1B depicts the impact of 18 µs PEF pulses number, ranging from 50 to 800, on the increase of cricket protein solubility. PEF untreated sample had 47.42 ± 3.01 mg soluble proteins per g fresh cricket. The increase in the number of PEF pulses had a positive linear effect on cricket protein solubilization, reaching a maximum of 13.67 ± 0.72% with 800 PEF pulses of 18 µs. During PEF treatment, the high voltage may change the permeability of cricket cell membranes or disrupt cells irreversibly, leading to the rapid diffusion of intracellular substances, like proteins, to the extraction medium (Zhang et al., 2021b). In the study by Psarianos et al. (2022), an increase in protein solubility from *Acheta domesticus* was observed after PEF treatment.

Specifically, the combination of PEF treatment and a 60 min alkaline extraction procedure enhanced 16.55 to 22.76% in protein solubilization.

A linear model was confirmed as having the best fit using the experimental data of protein solubilization assisted by PEF. The low value of RMSE (< 0.628) and high value of R^2 (> 0.993) showed a good fit for the results, demonstrating that the first-order kinetic model strongly reflects the process. Using the established model, the maximum increase in protein solubility (14.15%) would be obtained with 800 PEF pulses treatment, with a 95% confidence interval ranging from 13.50% to 14.79%. The model's reliability was further confirmed by validation assays, in which 800 PEF pulses treatment caused an increase in protein solubility of 13.84 ± 1.03%, thus, corroborating the proposed kinetic model for its predictive capacity on the increase of cricket protein solubility by using PEF treatment.

3.1.3. High pressure (HP)

The effect of 1 to 20 min of HP treatment (200 MPa) on cricket protein solubilization is displayed in **Fig. 1C**. The HP untreated sample had 49.98 ± 4.63 mg soluble proteins per g fresh cricket. HP treatment caused a positive effect on protein solubilization up to the 8 min assay. There were no significant changes from 8 to 12 min, and after 12 min, HP reduced the cricket protein solubility. HP can increase protein solubility by causing the breakage of noncovalent bonds, such as hydrophobic and electrostatic interactions. However, longer treatments or higher pressure levels could cause the formation of new bonds and interactions, resulting in insoluble protein aggregation (Queiroz et al., 2023a; Boukil et al., 2022). Kim et al. (2021) described a similar effect of HP on *Protaetia brevitarsis seulensis* proteins. HP-treated samples had higher protein solubility than untreated ones; notably, the 200 MPa pressure level promoted the highest increase in solubilization (15.29%), while higher pressure levels tended to decrease protein solubility.

The second-order model was identified as best fitting for the HP-assisted protein solubilization experimental data. The suitability of the proposed kinetic model is confirmed by the high R^2 value (> 0.963) and the low RMSE (< 0.530), which indicate a strong concordance between the model predictions and the observational data. According to the proposed model, an HP treatment of 9 min would achieve the maximum increase in cricket protein solubility (18.36%), with a 95% confidence interval ranging from 16.35% to 20.37%. Validation tests were performed to assess the reliability of the proposed model, in which a 9 min HP treatment increased protein solubility by 17.05 ± 1.25%. Thus, the proposed kinetic model was accurate and consistent in the prediction of the effect of HP treatment on cricket protein solubility.

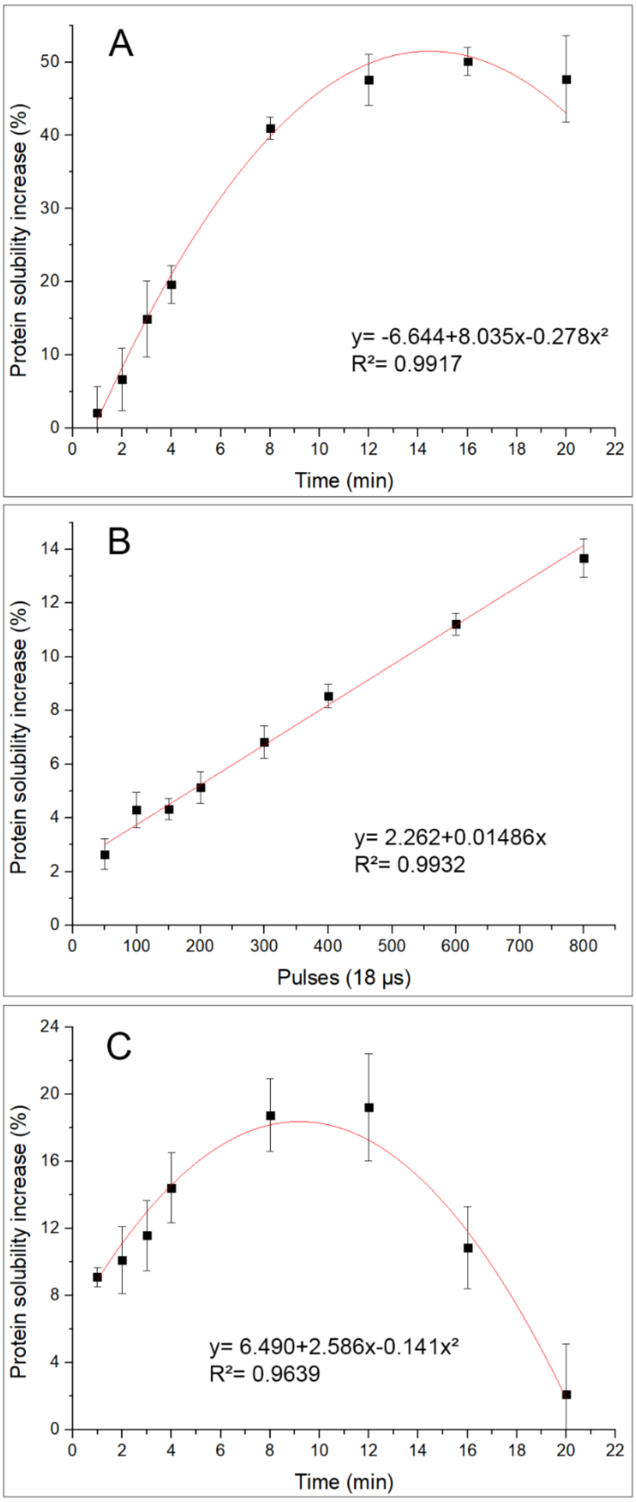

Figure 1. Effect of treatment time during the assisted protein extraction for **A**) ultrasound, **B**) pulsed electric fields, and **C**) high-pressure (200 MPa) on the increase of cricket protein solubility. Values are given as means ± SD from triplicate experiments.

3.2. Cricket protein concentrates characteristics

The protein concentrates were produced using the extraction conditions defined in each kinetic model that resulted in the highest solubility of cricket protein. These conditions were chosen to maximize the protein yield in each concentrate.

During protein concentrate production, several parameters were monitored throughout the extraction process (**Table 1**). Special consideration was given to maintaining the maximum temperature below 30 °C to mitigate potential heating effects. The control concentrate production was found to be the most time-consuming. It required nearly twice the amount of NaOH and HCl compared to the non-thermal technology-assisted protein concentrate production. Therefore, using non-thermal technologies to produce protein concentrates may enhance process efficiency while rendering it more environmentally friendly than conventional methods, which usually take 1 h and use a stronger alkaline solution for protein extraction (Ojha et al., 2021). In addition to the non-thermal technology-assisted concentrates, a control concentrate was produced for comparison, using a standard alkaline protein extraction method. The cricket was homogenized with distilled water (1:6), the slurry was stirred at 400 rpm for 1 hour, at room temperature, and the pH was monitored and maintained at 10.

Table 1. Extraction parameters of control, US, PEF, and HP protein concentrates.

Protein concentrates	Time	NaOH (g/kg of cricket)	HCl (g/kg of cricket)	Temperature (ºC)		pH	
				Initial*	Max.	Initial*	Final
Control	60 min	12.2 ± 0.2	7.8 ± 0.3	17.3 ± 2.4	21.2 ± 0.8	6.8 ± 0.1	10.0 ± 0.0
US	14 min	6.0 ± 0.0	3.9 ± 0.4	7.0 ± 0.5	29.4 ± 0.3	9.5 ± 0.1	8.4 ± 0.2
PEF	14,4 ms**	6.0 ± 0.0	3.6 ± 0.4	9.5 ± 1.7	28.3 ± 0.6	9.5 ± 0.1	8.7 ± 0.1
HP	9 min	6.0 ± 0.0	3.7 ± 0.2	18.9 ± 0.8	23.5 ± 0.3	9.6 ± 0.1	8.6 ± 0.2

Values are means ± SD from four replicate experiments.
* Just after blending crickets with water for control or NaOH 0.025 M for non-thermal treatments.
** 800 pulses of 18 µs.

The protein content and yields of each protein concentrate are shown in **Table 2**. Among the applied treatments, US demonstrated the highest efficiency in protein extraction, with a yield of 63.6%, statistically comparable to the control (64.3%). However, it also resulted in the highest extraction of dry matter from the cricket (61.9%), leading to a protein concentrate with the lowest protein content (55.3%) and higher levels of other substances. This phenomenon can be attributed to the disruptive effect of acoustic cavitation caused by US, which ruptures the cells of the cricket, resulting in the release of proteins and other intracellular substances. Furthermore, US can promote emulsification, which further enhances the extraction of lipids during the process (Rahman & Lamsal, 2021). After US treatment, Mishyna et al. (2019) also observed an increase in dry matter yield in an alkaline extraction procedure, 26% for *Schistocerca gregaria* and 12% for *Apis mellifera*. PEF and HP protein concentrates presented the lowest dry matter yield (ca. 46.5%). Still, HP showed a higher protein extraction yield than PEF, producing a protein concentrate with the highest protein content (61.7%).

Table 2. Dry matter yield, protein extraction yield, and protein content of control, US, PEF, and HP protein concentrates.

Protein concentrates	Dry matter yield (%)	Protein extraction yield (%)	Protein content (%)
Control	$57.90^{b} \pm 2.36$	$64.33^{a} \pm 2.62$	$59.78^{b} \pm 0.60$
US	$61.94^{a} \pm 3.22$	$63.61^{a} \pm 2.02$	$55.26^{d} \pm 0.19$
PEF	$46.79^{c} \pm 2.23$	$49.96^{c} \pm 2.09$	$57.96^{c} \pm 0.69$
HP	$46.38^{c} \pm 2.22$	$53.63^{b} \pm 2.14$	$61.68^{a} \pm 0.40$

Values are mean ± SD from four replicate experiments.
Different letters in the same column indicate significant differences ($p < 0.05$).

3.3. Color and browning of cricket protein concentrates

Protein concentrates are shown in **Fig. 2,** and their instrumental color in **Table 3**. The US protein concentrate exhibited the most different color compared to the control (ΔE), a light brown color showing the highest values for lightness (L*), redness (a*), and yellowness (b*). In contrast, HP and control concentrates had a darker brown color, with the lowest lightness (L*) and redness (a*). The characteristic brown coloration observed in adult *Gryllus assimilis* is due to the melanin pigment production in the insect's cuticles (Futahashi & Osanai-Futahashi, 2021). Therefore, the color of the protein concentrates is directly related to the ability to extract this pigment from the raw material. In addition, according to Mishyna et al. (2019), the alkaline conditions from the extraction procedure could lead to the auto-oxidation of phenolic compounds in insect cuticles, and cause protein–phenolic interaction, causing a decrease in L* and b* and an increase in a* value.

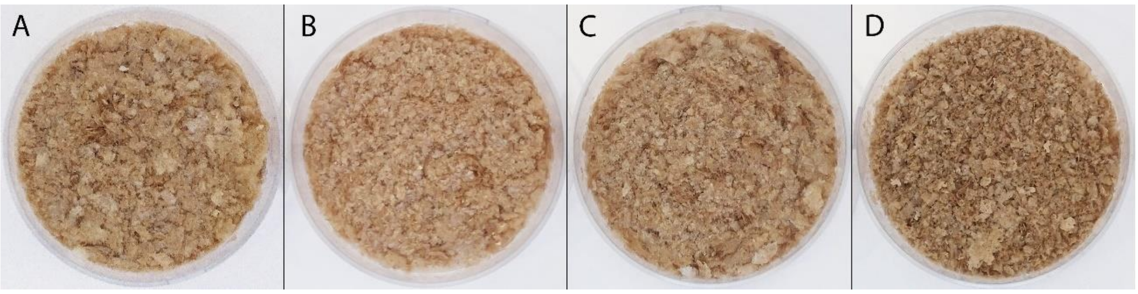

Figure 2. Protein concentrates from cricket: **A**) control, **B**) US, **C**) PEF, and **D**) HP.

Table 3. Instrumental color of the control, US, PEF, and HP protein concentrates.

Protein concentrates	L*	a*	b*	ΔE
Control	$59.66^{c} \pm 0.55$	$3.22^{c} \pm 0.09$	$6.69^{c} \pm 0.14$	-
US	$63.54^{a} \pm 0.90$	$4.25^{a} \pm 0.11$	$9.71^{a} \pm 0.15$	$5.06^{a} \pm 0.9$
PEF	$61.55^{b} \pm 0.13$	$3.70^{b} \pm 0.17$	$8.88^{b} \pm 0.35$	$2.94^{b} \pm 0.9$
HP	$60.70^{b,c} \pm 0.47$	$3.04^{c} \pm 0.09$	$8.55^{b} \pm 0.11$	$2.17^{b} \pm 0.9$

Values are mean ± SD from five replicate determinations.
Different letters in the same column indicate significant differences ($p < 0.05$).

To investigate the browning of protein concentrate solutions, the decrease in luminosity (L* value) was monitored for 48 h (**Fig. 3**). Upon dilution of the protein concentrates (1% w/v) in 0.1 M sodium phosphate buffer solution (pH 7.0), the luminosity of the solutions exhibited a similar trend to that of the powders concentrates. The US solution displayed a lighter color (L* = 31.6), while the other samples showed darker colors with lower L* values, ranging from 29.4 to 30.5. Over time, all solutions underwent a progressive browning process. Up to 240 min, the control solution exhibited the slightest variation in luminosity (ΔL* = -1.2), while the US solution had the highest variation (ΔL* = -2.5), resulting in a darker color compared to the control. After 48 h, the control and US solutions reached similar L* values (28.7), while PEF had 28.0 and HP 27.2. Throughout the entire experiment, the HP solution consistently exhibited the darkest color.

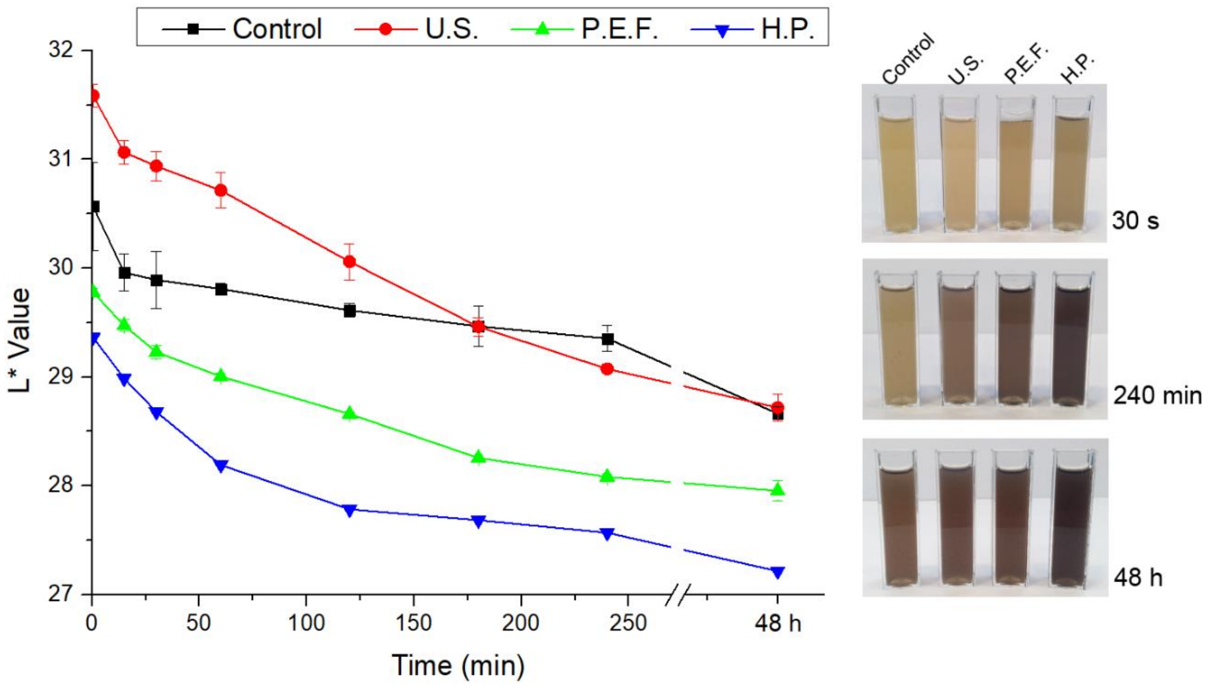

Figure 3. Effect of time on reducing L* value color for control, US, PEF, and HP protein concentrate solutions. Values are given as means ± SD from triplicate determinations.

The formation of brown color in the solutions is likely attributed to the activity of oxidative enzymes, mostly polyphenol oxidase (PPO), which catalyzes the production of melanins and benzoquinones from the polyphenolic compounds in the solution (Janssen et al., 2019a). The relative PPO enzyme activity and polyphenolic content of the protein concentrates are shown in **Fig. 4**. The relative enzyme activity compared to the control varied from 57 to 240% (**Fig. 4A**), while the content of the polyphenolic compounds ranged from 13.9 to 17.6 mg/g (**Fig. 4B**). Notably, the HP concentrate exhibited the highest PPO activity and polyphenolic compound content, which elucidates the rapid and pronounced brown color development observed in the HP solution (**Fig. 3**). In a recent investigation by Zhou et al. (2022), the impact of HP treatment on the structural and molecular dynamics of the PPO enzyme was carefully examined. Their findings revealed that PPO exposed to 200 MPa, the same pressure level used in our study, led to increased exposure of the active site and expansion of the substrate channel, thereby facilitating substrate access to the enzyme active site. These structural changes enhanced the affinity of PPO towards the substrate, resulting in increased enzyme activity.

In contrast to the HP concentrate, the lowest values for enzymatic activity and polyphenolic content were found for the US concentrate (**Fig. 4**). This might be attributed to two main reasons. Firstly, the US treatment caused the greatest dry matter extraction from the raw material (**Table 2**), leading to a more significant dilution of phenolic compounds and PPO enzymes in the US protein concentrate. Secondly, US can reduce PPO activity through cavitation effects. As reported by Iqbal et al. (2019), the application of US can partially disrupt the protein complex of PPO by breaking noncovalent bonds, such as electrostatic and hydrophobic interactions, and it also can enhance the synergy among protein molecules and drive the PPO molecules to dissociate and form larger protein aggregates.

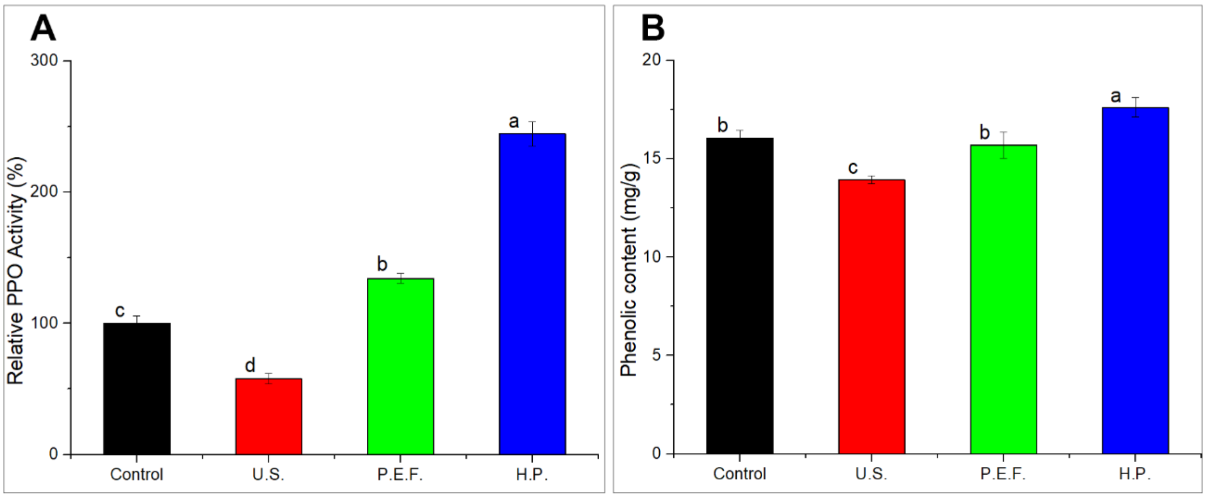

Figure 4. A) Relative polyphenol oxidase (PPO) activity and **B**) polyphenolic content of control, US, PEF, and HP protein concentrates. Values are mean ± SD from triplicate determinations. Different letters indicate significant differences ($p < 0.05$)

The browning of insect-based products is undesirable and can negatively affect consumers' perception of their quality. Therefore, developing effective methods to prevent this unwanted reaction is crucial. The most common process involves high temperatures, which are efficient in the inactivation of PPO enzymes. For instance, Janssen et al. (2019b) reported a reduction of over 90% in PPO activity when *T. molitor*, *H. illucens*, or *A. diaperinus* larvae were blanched for 50 s at 90 ^{o}C. However, it is important to note that the treatment time must be carefully considered as prolonged exposure to high temperatures, such as during oven drying, may trigger non-enzymatic darkening reactions, such as the Maillard reaction (Brishti et al., 2020). Other effective methods of reducing PPO activity and the consequent browning of insect products include the addition of sulfites or EDTA during insect protein extraction and lowering the pH to values below 4 (Janssen et al., 2019a; Janssen et al., 2019b; Yi et al., 2017).

3.4. Structural characteristics of protein concentrates

3.4.1. Molecular weight distribution (SDS-PAGE)

The impact of non-thermal technologies on the molecular weight profile of cricket protein concentrates is presented in **Fig. 5**. Under non-reducing conditions (**Fig. 5A**), the distribution of bands and molecular weight profiles were similar for control, PEF, and HP protein concentrates: an average of 42% of the proteins had a molecular weight above 100 kDa, 33% between 35-100 kDa, 17% between 15-35 kDa, and 9% had a molecular weight less than 15 kDa. The US treatment reduced the intensity of protein aggregates visible at the top of the gel, with molecular weight above 250 kDa, and decreased the band strength at 110 kDa, reducing the relative volume of proteins with molecular weight higher than 100 kDa from 42 to 34%. Moreover, the intensity of bands between 35-55 kDa was enhanced, increasing the relative volume of 35-100 kDa from 33 to 39%.

Under reducing conditions (**Fig. 5B**), the aggregates visible at the top of the gel disappeared, indicating that disulfide bonds are responsible for forming high molecular weight protein aggregates in the cricket concentrates. Similar to the non-reducing gel (**Fig. 5A**), the US concentrate was the only one that displayed a different molecular weight distribution profile, characterized by reduced intensities in bands above 100 kDa and increased intensities in bands between 35-55 kDa.

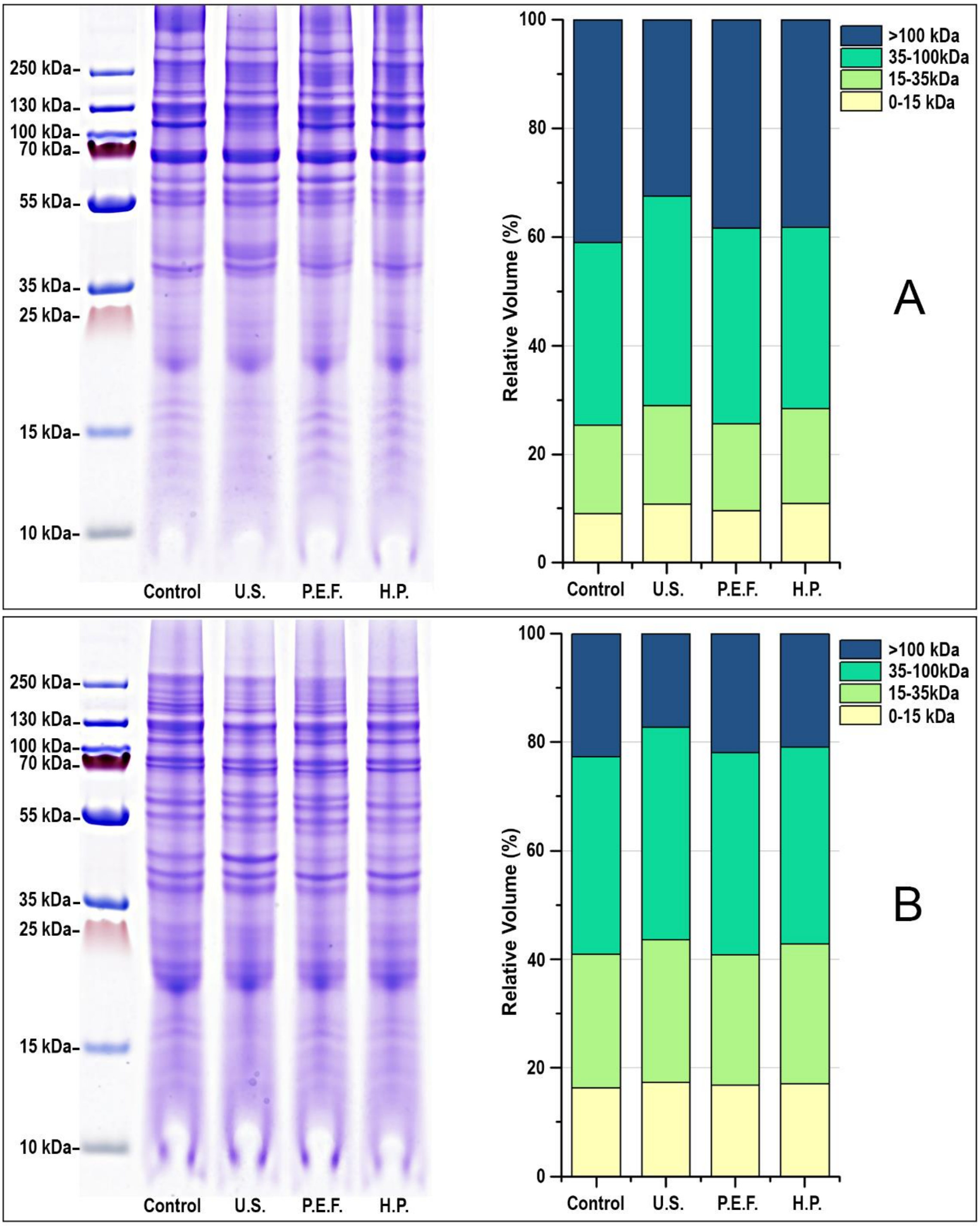

Figure 5. SDS-Page gel profile and molecular weight relative volume from control, US, PEF, and HP protein concentrates in **A**) non-reducing and **B**) reducing conditions (with β-mercaptoethanol).

The applied US treatment was able to reduce the proportion of molecules with high molecular weight (> 100 kDa) due to cavitation effects, which can disrupt the disulfide interactions responsible for the formation of protein aggregates (Ding et al., 2022). This treatment decreased molecular weight, with the affected molecules likely falling within the 35-55 kDa range.

3.4.2. Intrinsic fluorescence

Some hydrophobic amino acids, such as tryptophan and tyrosine, can emit fluorescence upon excitation. Among these, tryptophan residues' fluorescence is commonly employed as an indicator of conformational changes in the tertiary structure of proteins, as they are sensitive to the polarity of their microenvironments (Wang et al., 2017). The use of non-thermal technologies during extraction induced changes in the tertiary structure of cricket protein, as verified by the decrease in relative fluorescence intensity (**Fig. 6A**) and the shift in the maximum emission wavelength (λmax) compared to the control (**Fig. 6B**).

Among the non-thermal technologies evaluated, US caused the greatest reduction in fluorescence intensity, followed by HP and PEF. This reduction may be attributed to the different efficiency of energy transfer between tryptophan and tyrosine or to the partial unfolding of the protein structure and subsequent exposure of tryptophan residues to the aqueous environment, resulting in quenching of tryptophan fluorescence (Zhang et al., 2023b; Wang et al., 2017). Other studies also reported a reduction in fluorescence intensity after their protein sample was treated by PEF (Li et al., 2022), HP (Boukil et al., 2022), and US (Kang et al., 2022). The maximum emission wavelength (λmax) showed a similar pattern to fluorescence intensity. US and HP caused a shift in λmax towards longer wavelengths (red shift), indicating that tryptophan residues had moved to a more polar environment (Malik & Saini, 2018). This likely resulted from the disruption of hydrophobic interactions during the treatments, which caused the hydrophobic groups to move from the core toward the surface of the protein and become exposed to the aqueous solvent. Similarly, proteins from *T. molitor* exhibited a red shift in wavelength after being subjected to treatments with HP (Boukil et al., 2022) and US (Zhang et al., 2023b).

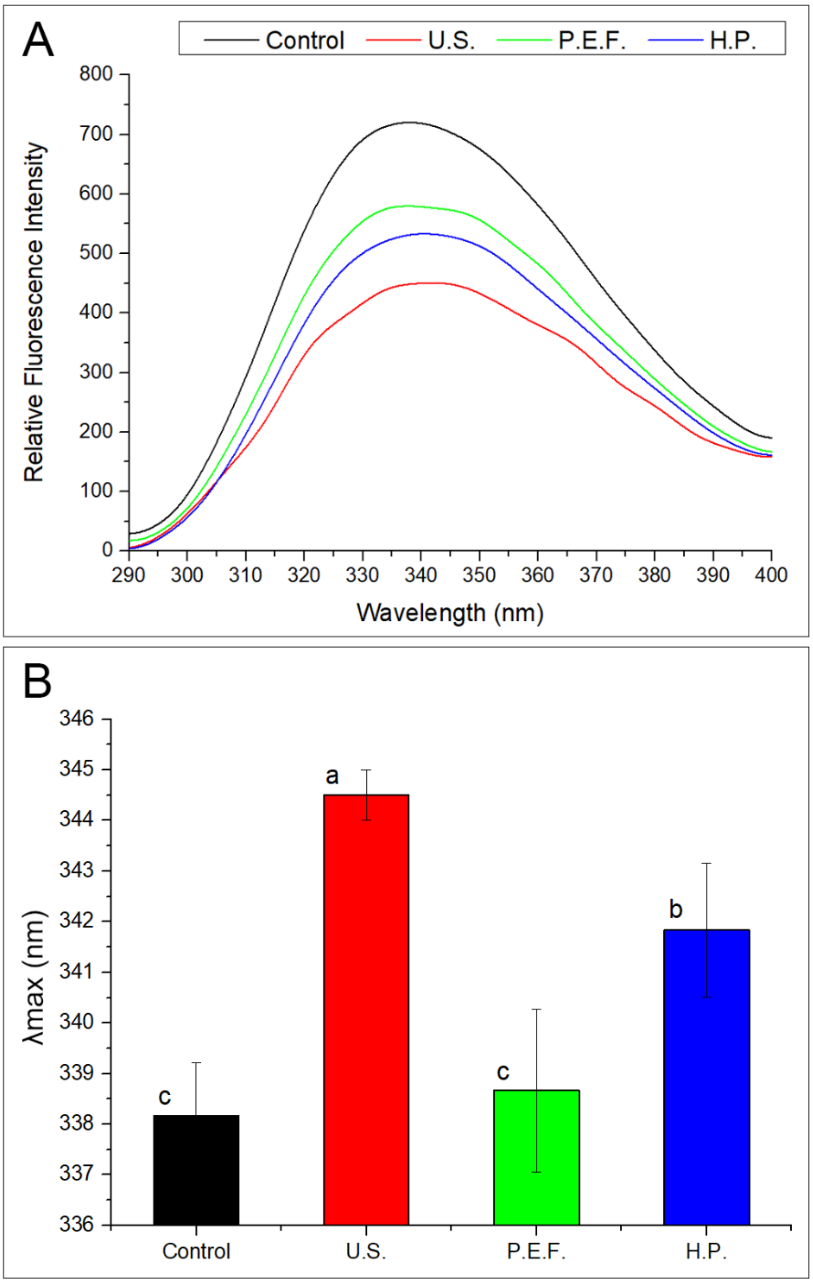

Figure 6. A) Intrinsic fluorescence spectrum, and **B)** maximum emission wavelength (λmax) of control, US, PEF, and HP protein concentrates. Values are mean ± SD from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

3.4.3. Surface hydrophobicity

Surface hydrophobicity is a significant characteristic of proteins that can be used to reveal the content of hydrophobic groups exposed on their surface. It is often employed to assess conformational changes in the protein, which may affect its functional properties (Liu et al., 2022). The changes in surface hydrophobicity of cricket protein concentrates are shown in **Table 4**. The surface hydrophobicity of the control and PEF-treated concentrates was similar. However, HP and US concentrates exhibited higher surface hydrophobicity than the control, with the US showing a more significant increase (+29%). Proteins subjected to HP treatment up to 400 MPa undergo a gradual exposure to hydrophobic regions buried inside the molecules, increasing the surface hydrophobicity (Mula et al., 2022). The observed increase in surface hydrophobicity following US treatment can be attributed to the phenomenon of cavitation, which induces a degree of the molecular unfolding of proteins, leading to the exposure of hydrophobic groups and regions that are originally inside the protein molecules (Bezerra et al., 2022). Additionally, US treatment led to a reduction in particle size (**Table 4**), as well as a decrease in protein aggregates in the molecular weight profile (**Fig. 5**). Thus, the resultant increase in surface hydrophobicity can also be attributed to the dissociation of larger protein aggregates, which increased the exposure of hydrophobic regions.

Table 4. Average particle size, zeta potential, and surface hydrophobicity of control, US, PEF, and HP protein concentrates.

Protein concentrates	Avg. particle size (nm)	Zeta potential (mV)	Surface hydrophobicity (slope x 10^3)
Control	$45.09^b \pm 0.47$	$-16.04^b \pm 1.54$	$4.41^c \pm 0.23$
US	$30.02^c \pm 0.48$	$-25.48^a \pm 1.63$	$5.70^a \pm 0.12$
PEF	$56.83^a \pm 0.77$	$-18.11^b \pm 0.81$	$4.59^c \pm 0.09$
HP	$44.67^b \pm 0.12$	$-18.87^b \pm 1.08$	$4.83^b \pm 0.07$

Values are mean ± SD from triplicate determinations.
Different letters in the same column indicate significant differences ($p < 0.05$).

3.4.4. Particle size

The protein particle size is a relevant factor closely related to various functional properties, such as solubility, foamability, and emulsification (Rahman & Lamsal, 2021). The effects of US, PEF, and HP treatments on the average particle size are presented in **Table 4**. Treatments exhibited varying effects on particle size. While the control and HP concentrates showed similar average particle sizes (ca. 45 nm), PEF caused an increase of 28%, and US led to a reduction of 33% in particle size compared to the control. Perez and Pilosof (2004) investigated the mechanism by which PEF causes particle aggregation in β-lactoglobulin and egg white, increasing protein particle size. PEF was found to induce polarization of the protein molecule, disrupt the quaternary structure by dissociating noncovalent bonds between protein subunits, and alter protein conformation by exposing internal hydrophobic and sulfhydryl groups. Furthermore, in solutions with high protein concentration, PEF was found to promote protein aggregation through hydrophobic interactions and the formation of disulfide bonds. In our study, although an increase in the average particle size was confirmed (**Table 4**), we did not observe an increase in protein aggregates in the non-reduced SDS-PAGE gel (**Fig. 5A**), indicating the absence of covalent bonds, such as disulfide bonds, in the formation of these aggregates. In contrast, US was able to cause the dissociation of high molecular weight protein aggregates (**Fig. 5A**), reducing the average particle size. These observations are consistent with previous studies that reported a protein size reduction following US treatment (Huang et al., 2023; Mintah et al., 2020; Ren et al., 2020). Such size reduction is attributed to the phenomenon of cavitation and micro-streaming, which generate strong collisions and lead to the formation of smaller particles (Rahman & Lamsal, 2021).

3.4.5. Zeta potential

The zeta potential is a crucial parameter for understanding the surface charge characteristics of proteins and the electrostatic interactions between protein particles and the surrounding solvent. This feature affects proteins' solubility and functional properties, such as emulsification and foaming (Amiri et al., 2021). In the present study, we observed negative zeta potential values for all cricket protein concentrates (**Table 4**), which indicated that the number of negative charges on the protein surface was greater than the number of positive charges. This can be attributed to the evaluation of cricket protein at pH 7.0, which is higher than its isoelectric point (pH 5.0), as indicated in **Fig. 7**. The zeta potential of control, PEF, and HP concentrates, which were not significantly different from each other, ranged from -10 to -20 mV, indicating that their dispersions were relatively stable (Cano-Sarmiento, 2018). Meanwhile, US treatment caused an increase in the absolute value of zeta potential, resulting in negative charges ranging from -20 to -30 mV, indicating that its dispersion is moderately stable. The observed increase in negative charge on the protein surface may be attributed to the reduced particle size (**Table 4**), which can facilitate the exposure of internal amino acid groups to the surrounding solvent, thereby enhancing the number of amino acids with negative charges on the protein surface (Kumar et al., 2022; Zhao et al., 2022).

5.3.5. Functional properties of protein concentrates

3.5.1. Protein solubility

The interactions between proteins and water largely influence the functionality of protein-based ingredients in food production. In this context, high solubility is a desirable property for such ingredients. **Fig. 7** displays the protein solubility of concentrates at different pH levels. Protein concentrates had higher solubility (>70%) at more acidic (≤3) and more alkaline (≥9) pH values, resulting in a typical U-shaped solubility profile. The pH range between 4.5 and 5.5 exhibited low solubility (<10%), where pH 5.0 had the lowest solubility, which could be attributed to the isoelectric point of the cricket proteins. Other studies corroborate that the isoelectric point of crickets' proteins falls within this range (Santiago et al., 2021; Brogan et al., 2021; Kim et al., 2017). Among the protein concentrates, the one treated with US exhibited higher solubility at almost all evaluated pH values. Various intrinsic and extrinsic factors determine protein solubility. Although surface hydrophobicity generally negatively impacts protein solubility, the US concentrate demonstrated higher protein solubility due to the small particle size and high zeta potential value of its proteins (**Table 4**). An improved pH-solubility pattern has also been observed in other insect proteins treated with US, such as *H. illucens* (Mintah et al., 2019), *T. molitor* (Huang et al., 2023), and *S. purpurascens* (Cruz-López et al., 2022).

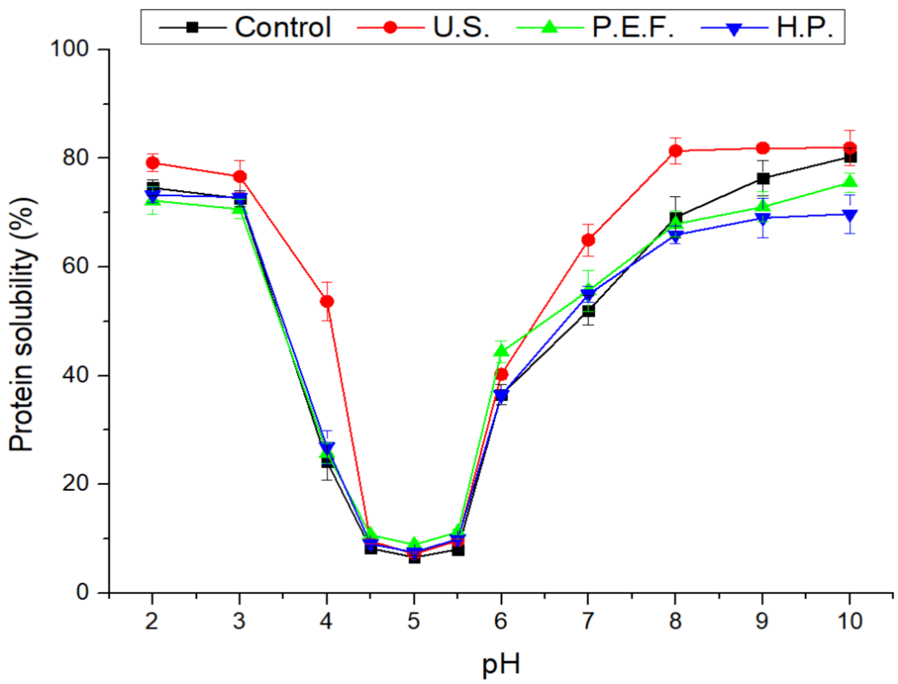

Figure 7. Protein solubility of control, US, PEF, and HP protein concentrates. Values are mean ± SD from triplicate determinations.

3.5.2. Water holding capacity (WHC) and oil holding capacity (OHC)

WHC refers to the ability of a protein's three-dimensional structure to absorb and avoid the release of water. Meanwhile, OHC is the capacity of proteins to absorb and retain oil and interact with lipid molecules. These two properties are vital for developing desirable food textures, as they also affect other functional properties such as solubility, emulsification, foamability, and gelation (Zhang et al., 2021c). The application of non-thermal technologies caused significant changes in the WHC and OHC of protein concentrates (**Fig. 8**). The WHC values ranged from 4.0 to 6.3 g/g of protein concentrate. Despite statistical differences, the control, PEF, and HP concentrates showed adjacent WHC values (4.0 - 5.0 g/g). In contrast, the US-treated concentrate showed a noticeable 40% increase in WHC compared to the control. The increase in the WHC of protein concentrates caused by US treatment can be attributed to various factors. One potential mechanism is the effect of cavitation, which reduces the particle size (**Table 4**) and enhances the surface area for interaction between proteins and water molecules. Another possible contributing factor is the formation of a sponge-like structure within the protein's peptide backbone and some ionized polar groups, which can further increase the contact area and the force of interaction between proteins and water (Zou et al., 2017). Regarding OHC, the values of the concentrates ranged from 4.5 to 5.4 g/g, with HP showing 10% more OHC than the control. Hydrophobic interactions mainly determine the stability of lipid-protein complexes and proteins with more hydrophobic regions tend to exhibit better OHC. In a recent study by Bolat et al. (2021), powders of *T. molitor* and *A. domesticus* were subjected to HP treatment at 500 MPa. Interestingly, the authors reported a reduction of 13% in OHC for mealworms, whereas cricket exhibited an increase of 9% in OHC, which is compatible with the results of our study. Thus, different insects may yield opposing outcomes following similar HP treatments, as functionality depends not only on the treatment applied but also on the protein profile that varies according to the insect species, life stage, and feeding, among other factors (Queiroz et al., 2023a).

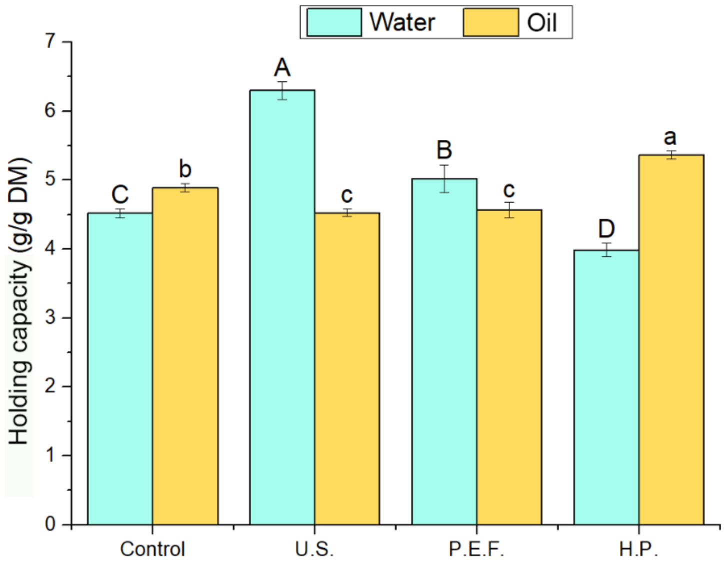

Figure 8. Water and oil holding capacities of control, US, PEF, and HP protein concentrates. Values are means ± SD from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

3.5.3. Foaming capacity (FC) and foam stability (FS)

A foam is a dispersion system in which the gaseous phase is surrounded by a continuous phase (liquid or solid). However, foam is an unstable system and will readily collapse unless it is stabilized. The process of protein foam formation is described in three stages. The protein must rapidly adsorb at the air-water interface. Then, the proteins rapidly unfold and rearrange themselves at the interface with an orientation of hydrophobic groups towards the air phase. Finally, the proteins interact to form a viscous and continuous film (Queiroz et al., 2023b).

The FC is related to the amount of interfacial area a protein can create. FS indicates the protein's capacity to prevent foam collapse against opposing forces like gravity.

The non-thermal treatments used during the production of protein concentrates have significantly altered their FC (**Fig. 9A**), and FS (**Fig. 9B**). US, PEF, and HP protein concentrates had higher FC than control, with US and PEF showing the most significant increases reaching ca. 220%. Similarly, US and PEF showed superior performance in FS, producing the most stable foams. Notably, US maintained a foam with over 50% of its initial volume even after 180 min. Protein solubility, surface flexibility, and hydrophobicity are the main determinants of efficient FC. Once the foam is formed, FS depends on the protein film's physical properties, protein-protein interactions, and some environmental factors (Zhang et al., 2021c). The perceived structural changes can explain the superior performance of US in FC and FS. Partial protein unfolding (**Fig. 6**) may have contributed to the rapid adsorption at the air-water interface and protein flexibility, allowing for rapid structural change and adaptation to the interface. Additionally, the smaller particle size and higher hydrophobicity (**Table 4**) favored the foam properties of the US concentrate.

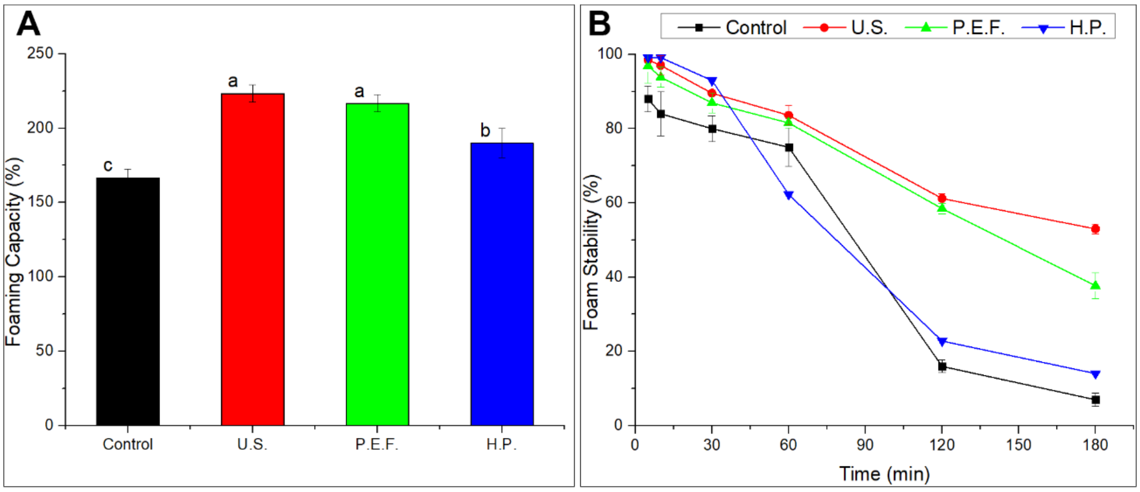

Figure 9. A) Foaming capacity, and **B**) foaming stability of control, US, PEF, and HP protein concentrates. Values are means ± SD from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

3.5.4. Emulsion capacity (EC) and emulsion stability index (ESI)

Emulsions are homogeneous mixtures of two immiscible liquids, with one liquid dispersed in small droplets within the other continuous phase. In the food industry, emulsions are commonly classified as oil-in-water or water-in-oil. However, emulsions are inherently unstable due to the interfacial tension between the two liquids, and this instability increases with a larger contact area. Amphiphilic molecules reduce interfacial tension and stabilize the emulsion to address this issue. Among the various emulsifiers available, proteins are extensively used in food systems due to their ability to migrate to the interface and position their polar and nonpolar amino acid residues towards the aqueous and lipid phase, respectively. This orientation forms a stable coating around the droplets and enhances the stability of the emulsion (Zhang et al., 2021b; Queiroz et al., 2023b).

EC is the proportion of grams of oil per gram of protein that can be emulsified, and ESI is related to the resistance of the emulsion over a specific time. Overall, the applied treatments caused minimal changes in EC (**Table 5**). Specifically, HP treatment resulted in a slight increase in EC compared to the control, while the other treatments did not exhibit any significant statistical differences, with an average of 52% EC. During HP treatment, water molecules can permeate protein molecules and interact with hydrophobic residues, thereby exposing hydrophobic groups on the surface. This structural modification can promote interactions between proteins and lipids (Xue et al., 2018). Similar to our results, Bai et al. (2021) observed a modest enhancement in the EC protein after exposing myosin to HP at 150 MPa for 2 min. Regarding ESI (**Table 5**), all cricket protein concentrates had similar results, with an average of 58% ESI.

Table 5. Emulsion capacity (EC) and emulsion stability index (ESI) of control, US, PEF, and HP protein concentrates.

Protein concentrates	EC (%)	ESI (%)
Control	$52.5^{b} \pm 1.1$	$60.0^{a} \pm 0.0$
US	$51.3^{b} \pm 0.8$	$55.8^{a} \pm 1.4$
PEF	$52.3^{b} \pm 0.6$	$58.3^{a} \pm 2.9$
HP	$54.4^{a} \pm 0.5$	$59.2^{a} \pm 1.4$

Values are mean ± SD from triplicate determinations.
Different letters in the same column indicate significant differences ($p < 0.05$).

3.5.5. Least gelling concentration (LGC)

Gelation capacity measures a protein's ability to aggregate and form a gel, primarily due to disulfide bridges and hydrophobic interactions between protein molecules. Most studies on the gelation of food proteins describe the process of heat-induced gel formation. This process is based on protein denaturation with subsequent conformational changes, followed by interactions, aggregation, and eventual gelation (Villaseñor et al., 2022). LGC was used as an index of the gelation capacity of cricket protein concentrates, and it is considered the lowest concentration at which a protein solution does not flow after inversion. As shown in **Fig. 10**, the solutions prepared from the control and PEF concentrates had the same LGC value (6.0%), while US exhibited a higher value (7.0%) and HP a lower one (5.0%). These findings are consistent with a recent study by Santiago et al. (2021), where the LGC of a protein isolate from the same cricket species (*G. assimilis*) was reported to be 6.5%. The gelation capacity of proteins is a complex phenomenon influenced by several factors. Among them, protein concentration, pH, ionic strength, processing conditions, and protein denaturation are the most significant (Queiroz et al., 2023b). It is worth noting that the impact of non-thermal technologies on LGC is closely linked to the protein content of each concentrate, with HP having the highest and US having the lowest protein content levels (**Table 2**). Furthermore, the reduction of the average particle size (**Table 4**) and the high molecular weight protein aggregates (**Fig. 5**) may have contributed to the decrease in the gel-forming ability of the US concentrate.

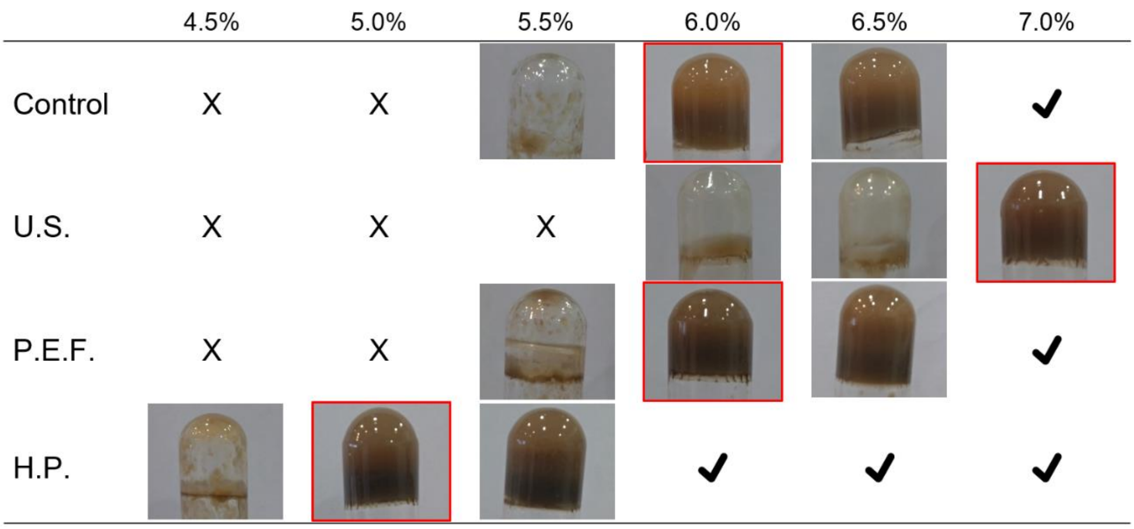

Figure 10. Least gelling concentration (LGC) of control, US, PEF, and HP protein concentrate.

4. Conclusions

This study demonstrated the efficacy of using high pressure, pulsed electric fields, and particularly ultrasound technologies to extract proteins from cricket.

PEF, HP, and US increased the solubility of cricket protein, with the maximum increase being 14% for PEF, 17% for HP, and 50% for US. The protein concentrates produced with the assistance of US and with a conventional alkaline extraction (control) showed similar protein extraction yields (ca. 64%), while those produced using HP and PEF had a lower extraction yield (ca. 52%). Nevertheless, using non-thermal technologies reduced the time (at least four times) and reagents (about half) consumed during extraction.

The protein concentrates exhibited low color variation, except for the US protein concentrate, which had a lighter color than the others. Enzymatic browning of the concentrates was observed in aqueous solution, with the HP and PEF concentrates showing faster enzymatic browning and reaching a darker color. In contrast, the control concentrate solution exhibited greater color stability.

The technologies used to assist in the extraction caused distinct structural changes in the cricket proteins, resulting in functional properties alterations. US induced degradation of larger proteins aggregates into smaller fragments, which reduced the particle size, intensified negative surface charge, and slightly increased the protein surface hydrophobicity. As a result, protein concentrate treated with US exhibited higher solubility, water retention capacity, foam formation, and stability but lower gel-forming capacity compared to the other treatments. HP and PEF subtly altered the protein structure, with PEF causing slight protein aggregation and a good foam capacity and stability. In addition, HP increased oil holding capacity, emulsion, and gel formation.

Therefore, these non-thermal technologies can be employed to assist the extraction of insect proteins in a much faster and more sustainable way, while inducing changes in both structural characteristics and functional properties. Thus, these technologies have the potential to facilitate the production of protein concentrates with tailored properties adapted to the requirements of the food industry.

Declaration of interests

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

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Antônio Bisconsin-Júnior: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft. **Lilian Regina B. Mariutti**: Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition. **Sorel Tchewonpi Sagu**: Investigation, Writing - Review & Editing. **Harshadrai M. Rawel**: Methodology, Resources, Writing - Review & Editing. **Oliver K. Schlüter**: Conceptualization, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

Acknowledgments

The work of the authors was partially supported by different funding sources: Deutscher Akademischer Austauschdienst (DAAD) individual scholarship (grant number: 91793370), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and CNPq (305778/2022-6).

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CAPÍTULO VI
DISCUSSÃO E CONCLUSÃO GERAIS

1. Discussão geral

Esta tese foi desenvolvida a fim de compreender as percepções dos brasileiros em relação ao uso de insetos como fonte alimentar e desenvolver um produto alimentício à base de insetos utilizando as informações obtidas a partir do estudo com os consumidores.

Os trabalhos já publicados sobre insetos comestíveis demonstram de maneira clara as diversas vantagens do seu uso como alternativa alimentar para suprir a crescente demanda de proteína animal. Esse potencial é justificado pela elevada concentração de proteínas de alta qualidade e pelo baixo impacto ambiental na sua criação. Entretanto, uma das principais barreiras para difundir o consumo de insetos é a baixa aceitação da população ocidental em aderir a essa prática alimentar.

Ainda assim, populações com experiências e influências culturais diferentes podem ter percepções distintas sobre a entomofagia. Para auxiliar a indústria alimentícia na introdução de produtos à base de insetos no mercado brasileiro, é fundamental estudar as percepções dos consumidores do país e identificar as associações que poderiam levar ao consumo ou à rejeição desses produtos. Nesse sentido, a primeira etapa deste estudo consistiu em investigar a representação social dos insetos comestíveis para a população brasileira, a partir da realização de 780 entrevistas pessoais em 8 cidades distribuídas pelas 5 regiões do país. Empregou-se a metodologia de associação de palavras, além de avaliar a percepção de risco associada ao consumo de insetos, explorar duas perguntas abertas sobre os motivos e as formas de consumo de insetos, e por fim, coletar dados sociodemográficos dos participantes.

Inicialmente, investigamos os resultados obtidos a fim de compreender como os participantes de diferentes locais manifestaram suas percepções sobre o consumo de insetos. Os resultados indicaram que existem representações distintas sobre o assunto dentro de um mesmo país. Além disso, pessoas que nasceram e residiam em cidades próximas nem sempre tinham representações similares. Como é possível observar na **Figura 2** do **capítulo III**, os participantes das cidades de Rio de Janeiro (RJ) e Santa Maria (RS), mesmo sendo geograficamente próximas, apresentaram percepções distintas sobre insetos comestíveis, enquanto os participantes de Aracaju (SE), Cuiabá (MT) e Campinas (SP) tiveram representações similares. Na verdade, verificou-se que a formação cultural foi um fator mais determinante para explicar as similaridades de representações entre as cidades do que a proximidade geográfica. Dessa forma, as cidades foram classificadas em grupos de acordo com suas representações e atitudes em relação ao consumo de insetos (**Figura 5**, **capítulo III**). Em suma, foi possível identificar dois grupos principais de cidades no Brasil: um composto pelas localidades da região continental (Norte e Centro-Oeste), que possuem forte influência cultural dos povos indígenas originários; e outro formado pelas cidades próximas ao litoral do Oceano Atlântico (Nordeste, Sudeste e Sul), as quais apresentam uma formação cultural influenciada pelos imigrantes europeus.

Posteriormente, estruturamos a representação social e identificamos os grupos de consumidores brasileiros, destacando suas características e diferenças em relação aos insetos comestíveis. O *Nojo* foi revelado como a associação principal dos brasileiros com insetos comestíveis, sendo a única associação presente no núcleo central da representação social (**Figura 2**, **capítulo IV**). Também, foram encontradas diversas associações positivas com o tema (**Figura 1**, **capítulo IV**), como *Proteína*, *Cultura*, *Curiosidade*, *Sustentabilidade* e *Exótico*, dentre outras relevantes. A partir da percepção de risco associada ao consumo de insetos, identificamos grupos de consumidores brasileiros distintos (**Tabela 2**, **capítulo IV**). Homens jovens com alto nível educacional demonstraram ter uma percepção de risco menor em relação ao consumo de insetos e mostraram-se mais propensos a aceitá-los como fonte alimentar, enquanto mulheres mais velhas com baixa escolaridade rejeitaram fortemente os insetos comestíveis. Por fim, os motivos mais citados pelos brasileiros para consumir insetos foram *sobrevivência* e *curiosidade* (**Figura 4**, **capítulo IV**), sendo que a preparação mais mencionada foi a *fritura* (**Figura 5**, **capítulo IV**).

Além disso, o estudo de consumidores revelou uma preferência pelo uso de grilos na produção de alimentos em detrimento de larvas, lagartas e baratas. Ainda, é importante considerar que a visão do inseto inteiro no alimento foi considerada um fator de repulsa para os consumidores, portanto, métodos de processamento que descaracterizem a aparência do inseto, como a moagem, são desejáveis. Nesse sentido, selecionamos uma espécie de grilo nativo brasileiro (*Gryllus assimilis*), criado comercialmente e com alto teor de proteína, para a produção de um concentrado proteico em pó. Para auxiliar na produção, foram usadas tecnologias não térmicas de ultrassom, campos elétricos pulsados e alta pressão, a fim de evitar os efeitos negativos das altas temperaturas e reduzir o tempo de processamento. Foram avaliados os efeitos destas tecnologias na eficiência de extração das proteínas, na estrutura molecular proteica e nas propriedades funcionais dos concentrados produzidos.

Durante o processo de extração de proteínas dos grilos, a tecnologia de ultrassom foi capaz de aumentar a solubilidade proteica em 50%, enquanto as outras tecnologias causaram um aumento de aproximadamente 15% (**Figura 1**, **capítulo V**). Como resultado, o rendimento na extração das proteínas foi superior com o auxílio do ultrassom em comparação com alta pressão e campos elétricos pulsados (**Tabela 2**, **capítulo V**). Além disso, o uso de tecnologias não térmicas reduziu o tempo em pelo menos quatro vezes e o consumo de reagentes pela metade durante a extração (**Tabela 1**, **capítulo V**).

Os concentrados proteicos de grilo produzidos com o auxílio das tecnologias não térmicas apresentaram modificações estruturais nas proteínas, ocasionando alterações nas suas propriedades funcionais. Em particular, o ultrassom promoveu a quebra dos agregados proteicos em fragmentos menores, resultando em uma redução no tamanho médio das partículas e um aumento na carga superficial negativa. Assim, o concentrado proteico obtido com o auxílio do ultrassom apresentou maiores valores de solubilidade, capacidade de retenção de água, formação e estabilidade de espuma. Em contrapartida, os campos elétricos pulsados tiveram poucos efeitos sobre a estrutura proteica e, consequentemente, nas propriedades funcionais do concentrado proteico. Por fim, a aplicação de alta pressão foi capaz de produzir um concentrado com um teor de proteínas mais elevado, apresentando maior capacidade de retenção de óleo, formação de emulsão e de gel.

2. Conclusão geral

Nesta tese foi possível explorar a representação social dos insetos comestíveis para os brasileiros, além do desenvolvimento de concentrados proteicos de grilo produzidos com o auxílio de tecnologias não térmicas.

A partir da representação social, concluímos que os consumidores de diferentes regiões do Brasil possuem representações distintas em relação aos insetos comestíveis. Sendo que as regiões Norte e Centro-Oeste demonstraram uma maior aceitação e familiaridade com o consumo de insetos, em comparação com as regiões Nordeste, Sudeste e Sul. Esses resultados são essenciais para orientar a indústria de alimentos na efetiva introdução de produtos à base de insetos no contexto brasileiro, que se caracteriza por uma marcante diversidade cultural regional. Dessa forma, estratégias de marketing devem ser desenvolvidas levando em consideração os valores e crenças dos subgrupos culturais regionais, em vez de adotar uma abordagem idêntica para todo o país.

Além disso, é importante ressaltar a identificação de outros aspectos fundamentais na representação social dos insetos comestíveis. A escolha da espécie de inseto utilizada nos alimentos tem impacto determinante na sua aceitação, destacando-se grilos, gafanhotos e formigas como as preferidas. A associação desses alimentos à base de insetos com elementos culturais e/ou exóticos, aliados ao seu baixo impacto ambiental e excelente qualidade nutricional, favorece o consumo entre os brasileiros. O fator de curiosidade em experimentar deve ser explorado pelas indústrias, a fim de promover o primeiro contato com este tipo de alimento. Neste primeiro contato, é crucial que a indústria estabeleça uma comunicação clara e assegure um perfil sensorial agradável do produto, a fim de proporcionar uma boa experiência alimentar, que promova a adoção dos insetos comestíveis na cultura alimentar dos consumidores. Em termos de estratégias de marketing, é recomendado direcionar esses produtos para homens jovens com alto nível de educação formal, uma vez que foram identificados como os consumidores com maior potencial. Os métodos de preparo preferidos foram a fritura e o assamento, além da utilização de processos que ocultem a presença visual do inseto inteiro no alimento.

Durante o processo de desenvolvimento dos concentrados proteicos de grilo, verificamos que a aplicação da tecnologia de ultrassom se destacou ao promover uma melhor extração das proteínas, resultando em um concentrado proteico com elevada solubilidade, capacidade de retenção de água e excelentes propriedades de formação e estabilidade de espuma. Enquanto, a tecnologia de alta pressão foi responsável pela obtenção de um concentrado com elevado teor proteico, maior capacidade de retenção de óleo, e formação de gel e emulsão. De forma geral, o uso das tecnologias não térmicas proporcionou uma redução significativa no tempo e na quantidade de reagentes necessários para o processo de extração. Essas tecnologias, portanto, mostram um potencial promissor para serem utilizadas pela indústria de alimentos ao facilitar a obtenção de concentrados proteicos de grilo, contribuindo para uma produção mais rápida e sustentável, e permitindo a customização das suas propriedades funcionais.

Os resultados obtidos nesta tese são importantes para a comunidade científica e para a indústria de alimentos, fornecendo informações inéditas e essenciais para orientar a introdução de produtos alimentícios à base de insetos no mercado brasileiro, além de informações sobre a produção de um ingrediente rico em proteínas, derivado de insetos, que poderá ser usado na formulação de novos alimentos.

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ANEXOS

Anexo I. Parecer consubstanciado do CEP

CEPUNICAMP
comitê de ética em pesquisa

UNICAMP - CAMPUS CAMPINAS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Representação social dos consumidores brasileiros sobre o uso de insetos comestíveis em alimentos

Pesquisador: Antonio Bisconsin Junior

Área Temática:

Versão: 2

CAAE: 80665117.5.0000.5404

Instituição Proponente: Faculdade de Engenharia de Alimentos

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.482.583

Apresentação do Projeto:

Introdução:

A Organização das Nações Unidas prevê que a população mundial irá atingir 9,7 bilhões de pessoas em 2050, exigindo maior produção de alimentos.

A compreensão das intenções e atitudes dos consumidores com relação aos alimentos não familiares é fundamental para definir estratégias capazes de alterar o comportamento alimentar.

Alimentos desconhecidos chamam a atenção de consumidores pela indução da curiosidade, graças a aparência exótica e o caráter desconhecido. Como exemplo, o uso de flores e insetos na dieta de algumas sociedades.

Em algumas culturas, insetos e flores fazem parte da alimentação diária em países como México ou China, enquanto em outras culturas são completamente estranhos e desconhecidos.

O consumo de insetos, se mostra como uma alternativa, pois a combinação de características como alto valor nutricional, menor impacto ambiental e baixos custos de produção dão suporte para promover o uso de insetos como uma fonte sustentável de proteína animal.

Ainda que diversos insetos sejam considerados como excelente alternativa à proteína animal convencional, poucos são os ocidentais que se aventuram em comê-los.

A compreensão das intenções e atitudes dos consumidores com relação aos alimentos não

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familiares é fundamental para definir estratégias capazes de alterar o comportamento alimentar.
Para verificar o entendimento que o consumidor brasileiro tem sobre alimentos produzidos com insetos comestíveis, iremos utilizar a teoria da representação social. Representações sociais são uma maneira específica de entender e comunicar o que já sabemos, ligando cada imagem a uma ideia e cada ideia a uma imagem. Esta teoria tem sido usada para analisar o conhecimento do consumidor sobre a percepção de alimentos.

Em forma de entrevista individual, será aplicado o teste de associação livre de palavras seguido de uma classificação e rankeamento das palavras evocadas.
O estudo iniciará com a expressão: “alimentos produzidos com insetos comestíveis”.
Em seguida, os participantes classificarão as palavras evocadas de 1 (menos importante) a 10 (mais importante).
Depois, os participantes irão avaliar a atitude positiva ou negativa com relação a cada palavra, usando uma escala de -3 (completamente negativo) a +3 (completamente positiva).
Após obter os dados das entrevistas e fazer o tratamento estatístico, espera-se obter a representação social dos consumidores brasileiros sobre o uso de insetos comestíveis para produzir alimentos.
Esta informação será utilizada para a escrita de textos científicos (resumo para congresso, artigo) e também para dar suporte ao desenvolvimento de um alimento feito a partir de insetos comestíveis.

Metodologia Proposta:

Para avaliar a representação social dos consumidores brasileiros sobre o uso de insetos comestíveis em alimentos serão recrutados 800 participantes de diversas regiões do país, sendo 100 de cada uma das seguintes cidades: Porto Velho-RO, Manaus-AM, Cuiabá-MT, Brasília-DF, Aracaju-SE, Rio de JaneiroRJ, Campinas-SP e Porto Alegre-RS.
Inicialmente, será solicitado a cada participante para ler e assinar o termo de consentimento livre e esclarecido sobre a participação voluntária no estudo.
Depois, em forma de entrevista individual, será aplicado o teste de associação livre de palavras seguido de uma classificação e rankeamento das palavras evocadas. Como uma etapa de familiarização com o teste, os participantes serão solicitados a falar todas as palavras ou expressões que vierem a mente após o condutor da entrevista falar as palavras "céu" e "carro".

No momento em que os participantes estiverem confortáveis com o procedimento, o estudo iniciará com a expressão: "alimentos produzidos com insetos comestíveis".
Em seguida, os participantes classificarão as palavras evocadas de 1 (menos importante) a 10 (mais importante). Finalmente, os participantes irão avaliar a atitude positiva ou negativa com relação a cada palavra, usando uma escala de -3 (completamente negativo) a +3 (completamente positiva).
Para avaliar a atitude implícita, positiva ou negativa, associada com as representações sociais, um índice de polaridade será calculado pela equação: [(número de palavras positivas) – (número de palavras negativas)] / (número de palavras evocadas).

Critério de Inclusão:
A coleta de dados será realizada em ambientes abertos e públicos, como praças e calçadas de pedestres. As pessoas serão abordadas e questionadas se estão disponíveis para um questionário de cinco minutos, aquelas que se mostrarem disponíveis serão os participantes do estudo.

Critério de Exclusão:
Para análise de dados, não será considerada a pessoa que informar ter alguma dieta específica ou restrição alimentar, como vegetarianismo ou alergias. Contudo, mesmo para estas pessoas a entrevista será aplicada na sua totalidade.

Hipótese:
A entrevista que será aplicada aos participantes deste estudo irá demonstrar a representação social dos consumidores brasileiros sobre o uso de insetos comestíveis na produção de alimentos, sendo possível identificar e comparar características por região, sexo, idade e escolaridade.

Número total de participantes da pesquisa: 800 pessoas

Objetivo da Pesquisa:
Objetivo Primário:

Investigar as três dimensões da representação social (a atitude, a informação e o campo de representação) de alimentos produzidos com insetos comestíveis em consumidores brasileiros.

Avaliação dos Riscos e Benefícios:
(conforme apresentado pelo pesquisador)

Riscos:
Como os participantes deste estudo não terão contato com insetos, não existem riscos físicos.
Caso a pessoa não se sinta confortável com o procedimento ou com algum fator externo, terá total liberdade para interromper a entrevista, sem acarretar em prejuízos para a mesma.
Será garantida a privacidade, o sigilo e a confidencialidade dos dados de cada participante, utilizando um sistema de código nas fichas que identificam o participante.

Benefícios:
A longo prazo, este estudo trará benefícios a sociedade e indústria brasileira, pois visa compreender a representação social de insetos comestíveis em alimentos nos consumidores brasileiros, dando o suporte para o desenvolvimento de um produto alimentício à base de insetos que atenda as expectativas dos consumidores.

Comentários e Considerações sobre a Pesquisa:
Título do projeto na folha de rosto - Adequado
Nome do pesquisador responsável na folha de rosto - Adequado
Nome da representante da unidade proponente (nome, função, carimbo) - Adequado

Proposta de tese de doutoramento.

Equipe de Pesquisa:
- Dr. Heber Rodrigues Silva
- Prof. Dra. Lilian Regina Barros Mariutti
- Prof. Dr. Jorge Herman Behrens
- Prof. Dra. Flavia Maria Netto

No campo 'cronograma' do documento gerado pela Plataforma Brasil, as entrevistas e coletas de dados estão previstas para os meses de março a maio de 2018 - Adequado

No campo 'orçamento' do documento gerado pela Plataforma Brasil, o pesquisador relata um orçamento de 'R$200,00'. Esse orçamento é compatível com o orçamento de um projeto de pesquisa financiado pelo próprio pesquisador.

Considerações sobre os Termos de apresentação obrigatória:

Linguagem acessível ao sujeito da pesquisa - Adequado
Justificativa, objetivos e descrição de procedimentos - Adequado
Desconfortos, riscos e benefícios - Adequado
Garantia de esclarecimentos - Adequado
Liberdade na recusa ou retirada do consentimento - Adequado
Garantia de Sigilo - Adequado
Menção sobre ressarcimento - ou não - de despesas - Adequado
Menção sobre garantia de indenização diante de eventuais danos decorrentes da pesquisa - Adequado
Menção ao TCLE assinado em duas vias - Adequado
Menção ao CEP em caso de abusos ou reclamações de cunho ético - Adequado
Nome e contato com o pesquisador da pesquisa - Adequado
Rubrica do pesquisador e do voluntário em TCLEs com mais de uma página - Adequado

Conclusões ou Pendências e Lista de Inadequações:

Após readequação do TCLE, todos os items previstos pela Resolução 466/2012 foram contemplados.

Considerações Finais a critério do CEP:

- O participante da pesquisa deve receber uma via do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (quando aplicável).

- O participante da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (quando aplicável).

- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Se o pesquisador considerar a descontinuação do estudo, esta deve ser justificada e somente ser realizada após análise das razões da descontinuidade pelo CEP que o aprovou. O pesquisador deve aguardar o parecer do CEP quanto à descontinuação, exceto quando perceber risco ou dano não previsto ao participante ou quando constatar a superioridade de uma estratégia diagnóstica ou terapêutica oferecida a um dos grupos da pesquisa, isto é, somente em caso de necessidade de ação imediata com intuito de proteger os participantes.

Endereço: Rua Tessália Vieira de Camargo, 126
Bairro: Barão Geraldo **CEP:** 13.083-887
UF: SP **Município:** CAMPINAS
Telefone: (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@fcm.unicamp.br

- O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo. É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

- Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas e aguardando a aprovação do CEP para continuidade da pesquisa. Em caso de projetos do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma, junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial.

- Relatórios parciais e final devem ser apresentados ao CEP, inicialmente seis meses após a data deste parecer de aprovação e ao término do estudo.

-Lembramos que segundo a Resolução 466/2012 , item XI.2 letra e, "cabe ao pesquisador apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento".

-O pesquisador deve manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_992692.pdf	10/01/2018 21:25:26		Aceito
Projeto Detalhado / Brochura Investigador	Projeto_CEP_Consumidor_Insetos0901.pdf	10/01/2018 21:23:43	Antonio Bisconsin Junior	Aceito
Outros	Carta_Resposta_CEP0901.pdf	10/01/2018 21:22:21	Antonio Bisconsin Junior	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Insetos0901.pdf	10/01/2018 21:19:02	Antonio Bisconsin Junior	Aceito
Folha de Rosto	FolhaRostoAssinadaV2.pdf	23/11/2017 21:46:50	Antonio Bisconsin Junior	Aceito

Outros	AtestadoMatricula.pdf	20/11/2017 11:50:37	Antonio Bisconsin Junior	Aceit

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CAMPINAS, 02 de Fevereiro de 2018

Assinado por:
Renata Maria dos Santos Celeghini
(Coordenador)

Anexo II. Comprovante de cadastro da matéria-prima no SISGEN

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº AB16570

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**AB16570**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Grillus assimilis
Zophbas morio
Tenebrio molitor

Título da Atividade: **Insetos Comestíveis como Fontes Alternativas de Alimentos**

Equipe

Lilian Regina Barros Mariutti	**UNICAMP**
Antonio Bisconsin Junior	**Unicamp**
Heber Rodrigues Silva	**Unicamp**
Jorge Herman Behrens	**Unicamp**
Maria Aparecida Azevedo Pereira da Silva	**Universidade Federal de Sergipe**
Verena Silva Lima	**Unicamp**
Mari Silvia Rodrigues de Oliveira	**UFSM**
Lorena Aurora Januário	**Unicamp**
Rosires Deliza	**Embrapa**
Flávia Maria Netto	**Unicamp**
Juliana Azevedo Lima Pallone	**Unicamp**

Resultados Obtidos

Divulgação de resultados em meios científicos ou de comunicação

Identificação do meio onde foi divulgado: **COMPOSIÇÃO DE INSETOS COMESTÍVEIS. In:**

Data do Cadastro:	**01/11/2018 14:36:55**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **20:01** de **11/05/2023**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

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The use of alternative food sources to improve health and guarantee access and food intake

Author:
Lilian Regina Barros Mariutti,Kemilla Sarmento Rebelo,Antonio Bisconsin-Junior,Janne Santos de Morais,Marciane Magnani,Iriani Rodrigues Maldonade,Nuno Rodrigo Madeira,Andrea Tiengo,Mário Roberto Maróstica,Cinthia Baú Betim Cazarin

Publication: Food Research International

Publisher: Elsevier

Date: November 2021

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Examining the role of regional culture and geographical distances on the representation of unfamiliar foods in a continental-size country

Author:
Antônio Bisconsin-Júnior,Heber Rodrigues,Jorge H. Behrens,Verena S. Lima,Maria Aparecida Azevedo P. da Silva,Mari Silvia R. de Oliveira,Lorena A. Januário,Rosires Deliza,Flávia Maria Netto,Lilian Regina B. Mariutti

Publication: Food Quality and Preference

Publisher: Elsevier

Date: January 2020

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"Food made with edible insects": Exploring the social representation of entomophagy where it is unfamiliar

Author: Antônio Bisconsin-Júnior,Heber Rodrigues,Jorge H. Behrens,Maria Aparecida Azevedo P. da Silva,Lilian Regina B. Mariutti

Publication: Appetite

Publisher: Elsevier

Date: 1 June 2022

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