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POTENTIAL USES OF BRAZIL NUT FOR NUT-BASED BEVERAGES: DEVELOPMENT AND PERCEPTIONS BEYOND ITS COMPOSITION

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Victor Jonas da Rocha Esperança

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Tese de Doutorado em cumprimento às exigências do Programa de Pós-Gradução em Alimentos e Nutrição da Universidade Federal do Estado do Rio de Janeiro.

Orientador: Prof. Dr. Otniel Freitas Silva (Embrapa Agroindústria de Alimentos)

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SUMÁRIO

ABSTRACT

The Brazil nut (*Bertholletia excelsa*) among the native nuts is the one with the highest source of selenium, and its use in the development of fortified beverages based on fruits and vegetables can be an important and positive strategy from a nutritional point of view for the elderly public. However, lack of knowledge about the benefits, fear of new foods, the so-called neophobia, and concern about contamination factors and allergies associated with nuts, especially Brazil nuts, can impede the consumption of these plant-based products. In this sense, this Thesis aimed to develop a beverage enriched with Brazil nut selenium for the population over 60 years old and to study the potential and perception of these individuals about this beverage. The first chapter of this Thesis presents the introduction and state of the art on the elderly population in Brazil, the consumption of nuts, and the development of food products for this public. In the second chapter, a bibliographic review was carried out on vegetable beverages produced from tree nuts regarding sanitary, technological, microbiological, sensorial, and consumption aspects. The bibliographic search covered articles published between 2000 and 2022. In total, 54 selected articles for this review were categorized according to their thematic area, such as Food Technology (n = 37); Nutrition and Health (n = 19); Microbiological aspects (n = 10); and Sensory and consumption studies (n = 12). It was observed that the most cited nuts were almond, cashew, hazelnut, and walnut, and the least cited were Brazil nut, macadamia, pine nut, mallow, and sapucaia. The third chapter addressed a study on the raw material intended for the processing of nuts from two different harvests for microbiological analysis (from DNA extraction and amplification of 16S genes analysis of bacteria and ITS for fungi) and mycotoxins (aflatoxins AFB_1, AFB_2, AFG_1, and AFG_2) by HPLC-FD / KobraCell®. The samples showed a very different microbiome and aflatoxin profile. Genres such as *Rothia* (HA) and *Cronobacter* (HB) were found in abundance during the analysis of bacteria; as for fungi, the genera *Aspergillus, Fusarium, Penicillium,* and *Alternaria* were also considered prevalent in these samples. Soil pathogenic microorganisms are related to inadequate hygienic-sanitary production practices. Aflatoxins were found in the samples but within the limits established by Brazilian legislation. In the fourth chapter, a study evaluated Brazilian consumers' perception and consumption of nut-based beverages by evaluating an online questionnaire with socioeconomic and consumption questions. The almond beverage was the beverage

most consumed by the interviewees, and the Brazil nut beverage was the one that most aroused interest among the interviewees. Nut beverages were voted the healthiest, most sustainable, and nutritionally best than soy beverages and cow's milk. Consumers have considered possibly fully and partially replacing cow's milk and soy beverages with dried fruit beverages. In the last chapter, the consumption and perception of the elderly public about four beverages made from Brazil nuts and the application of a neophobia questionnaire were studied, where there was no difference for macronutrients, and all samples were microbiologically safe. Global acceptance showed that cashew and strawberry-flavored beverages were the most accepted. The online study showed that there were neophilic (1.8%), neutral (61.4%), and neophobic (36.8%) participants and that the presence of the selenium claim was one of the most important factors in the choice, followed by the low price and the presence of the agrobioversity claim.

Keywords: beverages, aging, elderly, aflatoxins, selenium, neophobia.

USO POTENCIAL DA CASTANHA-DO-BRASIL PARA ELABORAÇÃO DE BEBIDAS À BASE DE CASTANHAS: DESENVOLVIMENTO E PERCEPÇÕES PARA ALÉM DE SUA COMPOSIÇÃO

RESUMO

A castanha do Brasil (*Bertholletia excelsa*) dentre as *nuts* nativas é a que apresenta a maior fonte de selênio, e seu uso no desenvolvimento de bebidas fortificadas à base de frutas e vegetais, pode ser uma estratégia importante e positiva do ponto de vista nutricional para o público idoso. Contudo, o desconhecimento dos benefícios, o receio com novos alimentos, a chamada neofobia, e também a preocupação com fatores de contaminação e alergias associadas às *nuts* e principalmente à castanha-do-brasil podem ser impeditivos para o consumo desses produtos *plant-based*. Neste sentido, o objetivo desta Tese foi desenvolver uma bebida enriquecida com selênio de castanha-do-brasil para a população acima de 60 anos e estudar o potencial e a percepção desses indivíduos sobre essa bebida. No primeiro capítulo desta tese é apresentado a introdução e estado da arte sobre a população idosa no brasil, consumo de castanhas e desenvolvimento de produtos alimentícios para esse público. No segundo capítulo foi realizada uma revisão bibliográfica sobre bebidas vegetais produzidas a partir de nozes de árvores quanto à aspectos sanitários, tecnológicos, microbiológicos, sensoriais e de consumo. A busca bibliográfica abrangeu os artigos publicados entre os anos de 2000 e 2022. No total, foram selecionados 54 artigos para esta revisão, os quais foram categorizados de acordo com sua área temática como Tecnologia de Alimentos (n = 37); Nutrição e Saúde (n = 19); Aspectos microbiológicos (n = 10); e Estudos sensoriais e de consumo (n = 12). Observou-se que as nozes mais citadas foram amêndoa, caju, avelã e noz, e as menos citadas foram castanha-do-brasil, macadâmia, pinhão, malva e sapucaia. O terceiro capítulo abordou um estudo sobre a matéria prima destinada ao processamento de castanhas oriundas de duas safras diferentes para análises microbiológicas (a partir da extração de DNA e amplificação dos genes 16S análise de bactérias e ITS para fungos) e de micotoxinas (aflatoxinas AFB_1, AFB_2, AFG_1 and AFG_2) por CLAE-DF/ KobraCell®. As amostras apresentaram perfil de microbioma e de aflatoxinas bastante distintos. Gêneros como *Rothia* (HA) e *Cronobacter* (HB) foram encontrados em abundância durante a análise de bactérias, já para os fungos, os gêneros *Aspergillus, Fusarium, Penicillium* e *Alternaria* também foram considerados prevalentes nestas amostras.

Microrganismos de solo, patogênicos e relacionados às práticas inadequadas de produção higiênico-sanitárias. Já as aflatoxinas foram encontrados nas amostras, mas dentro dos limites estabelecidos pela legislação brasileira. O quarto capitulo foi realizado um estudo para avaliar a percepção e o consumo de bebidas à base de nozes pelos consumidores brasileiros, através de avaliação de um questionário *online* com questões socioeconômicas e de consumo. A bebida de amêndoa foi a bebida mais consumida pelos entrevistados e a bebida de castanha-do-brasil foi a que mais despertou interesse entre os entrevistados. As bebidas de nozes foram eleitas como as bebidas mais saudáveis, sustentáveis e nutricionalmente melhores em comparação com as bebidas de soja e o leite de vaca. Os consumidores consideraram a possibilidade de substituir total e parcialmente o leite de vaca e as bebidas de soja por bebidas de frutos secos. No ultimo capítulo foi estudado o consumo e a percepção do público idoso acerca de 4 bebidas elaboradas à base de castanha-do-brasil e a aplicação de um questionário de neofobia, onde não houve diferença para os macronutrientes e todas as amostras se encontravam microbiologicamente seguras. A aceitação global demonstrou que as bebidas sabor caju e sabor morango foram as mais aceitas. O estudo online demonstrou que haviam participantes neofílicos (1,8%), neutros (61,4%) e neofóbicos (36,8%); e que a presença da alegação de selênio era um dos fatores de maior importância na escolha, seguido do preço baixo e da presença da alegação da agrobioversidade.

Palavras-chave: bebidas, envelhecimento, idosos, aflatoxinas, selênio, neofobia

CHAPTER 1

1.INTRODUCTION

According to the World Health Organization, an individual who has 60 years of life or more is considered an elderly person (UN, 2023). In Brazil, the Brazilian Institute of Geography and Statistics (IBGE) projections in the year 2021 indicated that the group of elderly people aged 60 years or more represented 14.7% of the Brazilian population. It is estimated that this proportion will reach 25.49% in 2060, making public policies and actions more intensively necessary for this public.

Aging is a natural biological process that leads to the alteration and decline of some physiological functions in the human body (CHOCKALINGAM; SINGH; KATHIRVEL, 2024). Over the years, elderly adults will develop special nutritional needs because the senescence process can generate specific demands due to the natural impairment of absorption, use, and excretion of nutrients. In addition to the physiological impairment, the low intake of these nutrients can also affect the metabolism and health of the elderly because the elderly population, in general, tends to have lower consumption of healthy calories and food variability (ESTELLA ODOH et al., 2023; FISBERG et al., 2013).

Among the nutrients that can be compromised by poor-quality food is selenium (SHREENATH; AMEER; DOOLEY, 2021). Selenium is an essential mineral that exerts its biological effects as a component of several selenoproteins. These proteins involve several metabolic processes, including reproductive, thyroid, immune, and antioxidant functions. The antioxidant activity attributed to this mineral is explained by the essential component of the enzyme glutathione peroxidase (GPx), which prevents the accumulation of peroxide (HOOH) in cells, a source of free radicals that can damage cell membranes and other cellular components (FAIRWEATHER-TAIT et al., 2011; KIELISZEK, 2019). With advancing age, the accumulation of oxidized proteins, lipids, carbohydrates, and DNA is greater than in younger organisms, according to the free radical theory (AVERY; HOFFMANN, 2018; SILVA; FERRARI, 2011). In this sense, selenium intake by the elderly can be considered a strategy to delay and/or prevent these oxidative processes.

When ingested in insufficient amounts, selenium can make humans and animals infertile, and in the most severe deficiencies, it can lead to anomalies and health disorders (LI et al., 2020; MEHDI et al., 2013; SHREENATH; AMEER; DOOLEY, 2021). This mineral also influences the metabolism of neurotransmitters, and its adequate intake is important, as it causes an increase in systemic concentration and maintenance of brain functions. Regarding cognition, for example, selenium levels decrease as cognitive functions become impaired; these low selenium concentration values are also found in patients with Alzheimer's disease (BISHT; SINGH; KALDATE, 2021; KIELISZEK, 2019; LEIGH GIBSON; GREEN, 2002).

Foods considered a source of selenium are very diverse and available in Brazil. These main sources are oilseeds, beans, whole grain flours, viscera, fish (sardines), eggs, and milk (AVERY; HOFFMANN, 2018; KIELISZEK, 2019). Among the main sources, oilseeds can be highlighted as the most popular, with Brazil nuts (Bertholletia excelsa) being the largest source, reaching about 3 g of selenium in 100 grams of nuts (PHILIPPI, 2013).

In this sense, to offer an adequate and balanced intake of nutrients, thus avoiding nutritional deficiencies, fortified beverages are seen as a practical alternative and well accepted by the general public (DINI, 2019). The fortification of fruit and vegetable-based beverages has been an important and positive strategy from a nutritional point of view (ALÁN, 2019). The technologies used to preserve these products should improve the bioavailability of nutrients while preserving their physical-chemical properties, sensory characteristics, and acceptance by consumers (AHMAD; AHMED, 2019).

Beverages are indispensable in the human diet (ACAROZ; ARSLAN-ACAROZ; INCE, 2019). From this perspective, the increase in nutrients and bioactive compounds is seen as an improvement to the product and a benefit to the consumer (ISLAM; KABIR, 2019; VEIGA et al., 2019). Concerning the elderly, concern for sensory acceptance, mainly ease of swallowing, and simplicity of consumption are essential for preparing beverages (DAMULEVICIENE et al., 2018; SULMONT-ROSSÉ et al., 2018). Thus, the “oral comfort” of the elderly is a very relevant characteristic to be observed in the development of foods for this public (AGUILERA; COVACEVICH, 2023; GALLEGO et al., 2022; LORIEAU et al., 2018).

2.LITERATURE REVIEW

2.1 Elderly in Brazil

The aging process and death are events inherent to human life (COHEN; MARSISKE; SMITH, 2019). In this sense, healthy aging and quality of life are relevant factors for individuals. A study by Hambleton et al. (2015) pointed out that between the years 1965 and 2010, life expectancy in Latin America and the Caribbean exceeded international "minimum standard" targets and is improving relative to the region of the world with the increased human longevity.

In Brazil, life expectancy is increasing in ten years (2012-2021), the share of people aged 60 years or over increased from 11.3% to 14.7% of the population, while the younger population (0-29 years) has been shrinking over the past decade (Figure 1). These seniors are more concentrated in the Southeast (16.6%) and South (16.2%). Only 9.9% of residents in the North are elderly, with the states of Rio de Janeiro (19.1%) and Rio Grande do Sul (18.6%) having the highest concentration of elderly people, and Roraima has the lowest participation (7.7%) in the Brazilian territory (IBGE, 2022). When correlated with the year 1940, there is an excellent contrast since, at that time, he did not exceed 48.3 years of age (IBGE, 2021).

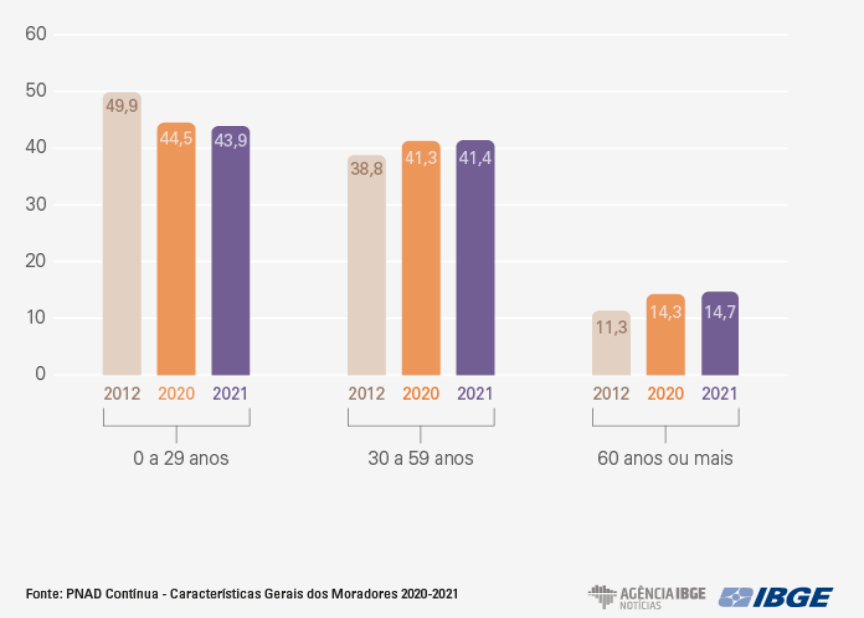

Figure 1: Resident population (%) in the last 10 years in Brazil in age groups. Source: IBGE (2022).

The longevity factor is an essential aspect of the development of a country, as it is through it that the quality of survival and expectation of its population is measured (SANTOS et al., 2020). Aging with quality of life is a challenge for individuals and society. Countries with elderly populations in high proportions are considered long-lived countries where there is quality of life for its citizens (LEINDECKER; BENNEMANN; MACUCH, 2020). The demand for academic and technical studies on longevity is necessary to help public policies in the economic, social, and psychological areas and, above all, in the health field, mainly faced with the growth of elderly proportions (PATRÍCIO et al., 2008).

In this sense, it is necessary to emphasize that the aging process of the population is an important factor to be analyzed and can be graphically observed by the changes in the format of the age pyramid in Brazil over the years (Figure 2), which follows the world trend of narrowing of the base (fewer children and young people) and enlargement of the body (adults) and top (elderly), thus representing the increase in elderly population density and having an expectation that in 2060, they will be 73 million, representing a growth of 152% in 40 years according to IBGE projections (IBGE 2021, b).

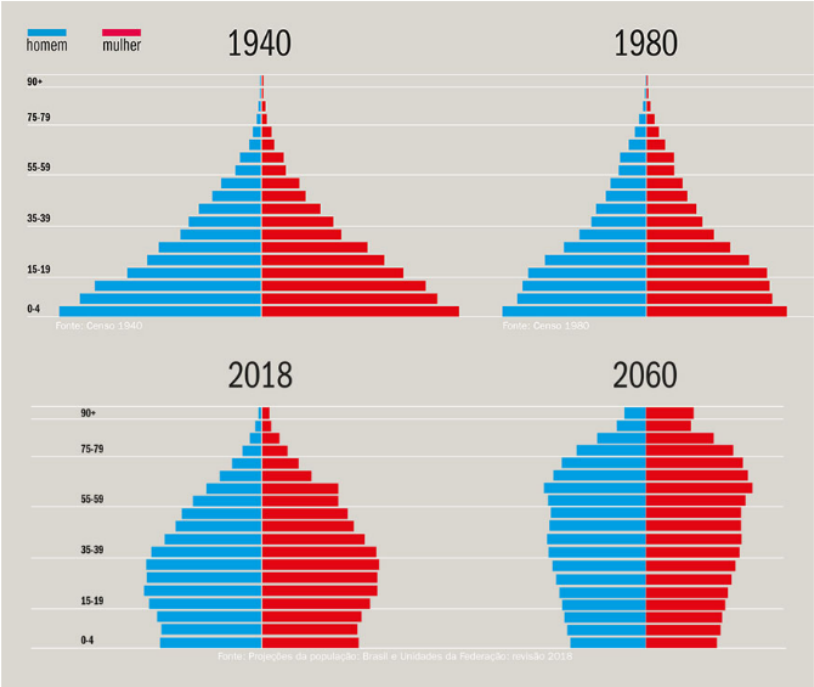

Figure 2: Age pyramid of the Brazilian population. Source: IBGE (2021).

2.2 Physiology of aging and health of the elderly.

The biological changes that occur with age in the human body affect mood, attitude towards the environment, physical condition, and social activity, and designate the place of the elderly in the family and society (CHOCKALINGAM; SINGH; KATHIRVEL, 2024; KOLCU et al., 2023). Senescence is one of the most complex biological processes to define and understand, and this complexity of the aging process leads to the realization that an integrative approach is needed to understand better aging mechanisms (ESTELLA ODOH et al., 2023).

There are several types and theories about senescence, several of which seek to explain a complex biological phenomenon that affects human beings. The main ones emphasize the changes that occur in the nervous system (BRUSENTSEV et al., 2017). Some theories are based on programmed development (TOWER, 2015), molecular crosslinking (BJORKSTEN, 1968), damage induced by free radicals (HARMAN, 1993), changes in immune functions (EFFROS, 2005), telomere shortening (KRUK; RAMPINO; BOHR, 1995) and the presence of senescence genes in the DNA (WARNER et al., 1987). Although there are several theories about aging, there is still no consensus on the subject. Many of the suggested mechanisms seem, in one way or another, to interact with each other and explain how this physiological process takes place (CHOCKALINGAM; SINGH; KATHIRVEL, 2024; ESTELLA ODOH et al., 2023)

Aging is an important risk factor for most chronic diseases and functional deficiencies (WAGNER et al., 2016). During the aging process, several changes occur in the human organism at the molecular, psychological, pathological, and physiological levels (PATEL; MCKINNON, 2018). Age-related anatomical and functional changes in sensory, neuromuscular, and cognitive systems are aggravating factors that impair balance and gait control during senescence (LORD; DELBAERE; STURNIEKS, 2018). Cardiovascular diseases (NOALE; LIMONGI; MAGGI, 2020), diabetes (VALENCIA et al., 2018), neurological diseases (SERAJI-BZORGZAD; PAULSON; HEIDEBRINK, 2019), anemia (HALAWI; MOUKHADDER; TAHER, 2017) and changes in the immune system (FUENTES et al., 2017) are prevalent at this stage due to changes in the organism. In this perspective of prevention and protection, weight balance, physical activity, and

careful eating are factors that positively affect the physical and mental health of the elderly (BALES; PORTER STARR, 2018; CUNNINGHAM et al., 2020; PAYNE et al., 2018).

2.3 Food for elderly

Aging can coincide with the decline of taste and smell functions, affect food intake, and negatively affect health (DEVERE, 2017). The loss of taste and perception of salty, sweet, and umami flavors also seems to decrease with age, caused by physiological changes and aggravated by aging events, such as polypharmacy and chronic diseases (SERGI et al., 2017). Furthermore, the loss of salivation capacity with age also seems to interfere with food consumption and hinder the process of digestion and absorption of nutrients (XU; LAGUNA; SARKAR, 2019).

In general, elderly Brazilians have a high inadequacy of nutrient intake (vitamins and minerals) recognized as protective against chronic diseases (BRASIL, 2014). This diet features a low weekly frequency of fresh or minimally processed foods, such as fruits and vegetables, and the replacement of large meals with snacks or ultra-processed foods (FISBERG et al., 2013). Despite their importance, epidemiological studies that present representative population data for Brazil on the dietary patterns of the elderly are still scarce in the literature, usually being developed in specific regions or cities (PEREIRA et al., 2020).

A study carried out in Rio Grande do Sul pointed out that the long-lived had, statistically, a better level of diet quality than the long-lived (IANISKI; GHENO; BÓS, 2019). On the other hand, another study at the national level showed that obesity is more prevalent in women than in elderly men, and this prevalence is inversely proportional with age from 75 years old; that is, the older the obesity rate is (COSTA et al., 2019).

Ferreira et al. (2017) identified three dietary patterns in the elderly people in the city of São Caetano do Sul (São Paulo); I the Traditional pattern (consisting of foods commonly consumed by the Brazilian population in their main meals: rice, beans, chicken, greens, vegetables, and olive oil); II - associated with male gender and practitioners of physical activity; the “pasta, pork and sweet” pattern, which was associated with males and retired elderly); III - the “coffee with milk and bread and

butter" pattern, which was associated with the age group of 80 years or older and elderly people who had difficulty chewing (FERREIRA ET AL., 2017).

The quality of the diet is influenced by physiological changes in terms of sensory, visual, auditory, and olfactory changes, leading to a decrease in food consumption in the elderly, leading to changes in body composition and also leading to deficiencies (CHOCKALINGAM; SINGH; KATHIRVEL, 2024; ESTELLA ODOH et al., 2023). Deficiencies of vitamins (Complex B, D, and C) and minerals (selenium, zinc, and magnesium) in the elderly are related to an increased risk of chronic non-communicable diseases (NCDs), including fatigue, cardiovascular diseases, and impairments in cognitive and neuromuscular function. In this sense, through public policies, authorities must implement ways to optimize the intake of essential nutrients as an integral part of their strategies to deal with CNCDs (BRUINS et al., 2019).

2.4 Development of enriched food products for the elderly

In the context of an aging population, developing products that meet the specific needs of the elderly while satisfying their sensory and hedonic expectations becomes a major challenge for the food industry and society (SULMONT-ROSSÉ et al. ., 2018). In this sense, food fortification is a common management approach for elderly people with reduced appetite and others who need to ingest nutrients adequately. With this perspective, the food industries have made efforts in recent years to improve the offer of foods dedicated to the elderly, in particular foods with modified texture and fortified foods (METHVEN et al., 2010; METHVEN; JIMÉNEZ-PRANTEDA; LAWLOR , 2016; ZARIM; ZAINUL ABIDIN; ARIFFIN, 2021).

In Brazil, ANVISA ORDINANCE No. 31 of 1998 (BRASIL, 1998) considers that fortified/enriched food or added nutrients are any food to which one or more essential nutrients naturally contained or not in the food are added to reinforce its nutritional value and/or prevent or correct demonstrated deficiency(s) in one or more nutrients, in the diet of the population or specific groups thereof. A "source" food claim must contain a minimum of 15% of the RDV of the respective vitamin or mineral. In comparison, a "high content" claim must contain a minimum of 30% of the RDV, and a claim of "increased "

a minimum increase of 25%, following ANVISA NORMATIVE INSTRUCTION No. 75, OF OCTOBER 8, 2020 (BRASIL, 2020).

Concerning the development of these products, the concern to value food-processing by-products capable of providing functional macro and micronutrients economically, as well as the use of emerging ingredients from various sources, represents an outstanding contribution to the development of functional and fortified foods (GULDIKEN et al., 2021). Furthermore, the concept of "oral comfort" when eating food should be considered by those who wish to design tailored food products for the elderly population (VANDENBERGHE-DESCAMPS et al., 2018).

Several studies point to developing enriched products for the elderly as a disease prevention and health maintenance tool. Fortified products such as yogurts (KERŠIENĖ et al., 2020; SIVIERI et al., 2017), beverages (CILLA et al., 2019; ICHINOSE et al., 2020; KASAPOĞLU et al., 2019) and meats (BAUGREET et al., 2016; FAROUK et al., 2018) are commonly found in the literature. Generally, food fortification for the elderly is related to increased levels of proteins, probiotics, and micronutrients (GARCÍA et al., 2019; SANTOS et al., 2016; SONG et al., 2019).

2.5. Selenium

2.5.1. Selenium mineral (Se)

Selenium is a mineral considered an essential trace element for human health, playing important roles, including in disease prevention (SHREENATH; AMEER; DOOLEY, 2021). For many years, research on selenium has provided controversial information about its benefits. However, it is currently understood that although this mineral can be toxic, it is also an essential micronutrient – and this depends on the concentration found in the diet (PRAUCHNER, 2020).The use of selenium in the human body began to be explored from the 1970s onwards, with the discovery of its role as part of the active site of the enzyme glutathione peroxidase, which protects the body against free radicals by manipulating hydrogen peroxide. (KABEL, 2014; KIELISZEK, 2019). Later, other enzymes were described as having selenium in their active sites, such as iodothyronine deiodinase – an essential mediator of the action of thyroid hormones (AVERY; HOFFMANN, 2018).

The participation of selenium in the composition of some proteins, called selenoproteins, has been widely studied. Selenium is incorporated into enzymes, mainly in the form of selenocysteine, but can also be found as selenomethionine (PRAUCHNER, 2020). Among the functions these selenoproteins perform, we can highlight their immunomodulatory activity. In this case, selenium acts as the redox center of the enzymes, essential for their biochemical activity and anticancer potential through different mechanisms through selenomethionine, for example (KHURANA et al., 2019).

Among the food sources of selenium, one of the most cited is the Brazil nut (*Bertholletia excelsa*), which contains up to 3 g of selenium / 100 grams of nut (PHILIPPI, 2013). A recent review demonstrated that studies with an average intervention of 11 weeks and average consumption of 9.42 g of Brazil nuts per day showed a significant effect on the increase in plasma selenium levels and an increasing effect on glutathione peroxidase (LI et al., 2020).

2.5.2. Selenium deficiency and excess

The recommended daily intake (RDI) of selenium by the Ministry of Health, through IN nº 75 of October 8, 2020, is 60 µg for adult men and women (BRASIL, 2005), while the recommendation adopted by the United States through the Recommended Dietary Allowance (RDA) is 55 µg per day for adults and elderly individuals of both genders, with the Tolerable Upper Intake Level (UL) being 400 µg per day (INSTITUTE OF MEDICINE (US), 2000).

Most of the time, selenium deficiency occurs due to inadequate intake of this mineral. Currently, it is estimated that this condition affects 500 million to 1 billion people worldwide and that it is related to the development of cardiovascular and myodegenerative diseases, infertility, cognitive decline, and immunological incompetence, leading to increased susceptibility to infections and, possibly to cancers (AVERY; HOFFMANN, 2018; SHREENATH; AMEER; DOOLEY, 2021).

Two diseases directly related to selenium deficiency are Keshan disease and Kaschin-Beck disease. Keshan disease is a cardiomyopathy described in China, where

heart muscle degeneration is observed, which can cause congestive cardiomyopathy, heart failure, cardiomegaly, and changes in the echocardiogram (KIELISZEK, 2019).

A study that evaluated the relationship between selenium supplementation and Keshan disease demonstrated that supplementation effectively prevented people from contracting the disease and that selenium deficiency is one of the causes of it (ZHOU et al., 2018). Kaschin-Beck disease is an endemic degenerative osteoarthritis, where chondrocyte necrosis occurs in various areas of hyaline cartilage – and although its cause is multifactorial, selenium deficiency has been associated with its development (KIELISZEK, 2019; ZHAO et al., 2013).

On the other hand, excess selenium can be toxic to the human organism, but intoxication by this mineral has rarely been observed. Acute selenium toxicity can occur in rare cases and triggers hypotension and tachycardia as the first symptoms. Toxic symptoms have usually occurred in individuals consuming 5 mg of selenium per day (AVERY; HOFFMANN, 2018; KHURANA et al., 2019; KIELISZEK, 2019).

Chronic selenium toxicity, called selenosis, has the signs and symptoms of brittleness and loss of hair and nails, gastrointestinal disturbances, skin rashes, garlic breath, fatigue, irritability, and nervous system abnormalities – and must be evaluated biochemically by measuring the concentration of selenium in tissues. Therefore, respecting the value established by the UL is extremely important, in addition to being aware of the form of selenium ingested, since inorganic selenium causes toxicity at much lower concentrations than organic selenomethionine (GAD, 2023; PRABHU; LEI, 2016).

2.5.3. Selenium and health benefits

Consumption of selenium is beneficial to human health in several ways. Recently, it was observed that consuming this and some other minerals can help with mood disorders by regulating neurotransmitters due to a modulation in the neuronal structure (SAMPAIO et al., 2019), helping maintain mental health. The study by Nascimento et al. (2019) also observed that elderly people with Alzheimer's had lower concentrations of selenium in plasma and erythrocytes than elderly people without the disease, establishing an important association between selenium and this pathology.

The effect of selenium supplementation and consumption by the elderly population has been reported in several studies worldwide. The study by Alehagen et al. (2020) evaluated for four years the daily supplementation of 200 µg of selenium associated with 200 milligrams of coenzyme Q10 in elderly people aged 70 to 88 years compared to a placebo. This study demonstrated that low selenium intake increased cardiovascular mortality and that supplementation could affect cardiovascular risk (ALEHAGEN et al., 2020).

The elderly population is considered for selenium supplementation because they are more likely to be deficient in this mineral. The adequacy of selenium intake in these cases may be necessary to, in addition to preventing both the development and progression of cardiovascular diseases, minimize the inflammatory process since the deficiency was also associated with increased inflammatory circulation and increased expression of cytokines and chemokines (GIACCONI et al., 2021). Furthermore, selenium and coenzyme Q10 supplementation has also been shown to improve kidney function in elderly patients significantly (ALEHAGEN et al., 2020b).

2.6 Brazil nut

The Brazil nut tree (Betholletia excelsa), native to the Amazon region, belongs to the Lecithidaceas family of the *Bertholletia* genus. Due to its structure, it is considered necessary for the local ecosystem. It produces fruits once a year, known as urchins, that measure from 8 to 15 cm in diameter (Figure 1. A) and takes more than a year to be formed – within these are found the seeds that contain the almonds (Figure 1. B) that we consume, which is called Brazil nut (BALDONI et al., 2020; MORI; PRANCE, 1990).

Figure 3. (A) *Pixidium* (woody capsule) with theirBrazil nuts seeds (Source: https://www.embrapa.br/busca-de-imagens/-/midia/1652001/castanha-do-brasil) and **(B)** Brazil nuts seeds and kernel (Source: https://www.embrapa.br/busca-de-imagens/-/midia/3715001/castanha-do-brasil).

The Brazil nut is considered one of the most important products of the Amazon rainforest, and its commercialization is one of the sources of income for many indigenous and riverside communities. The composition of this nut contains minerals, especially selenium, whose concentration is considered extraordinarily high (TAKEUCHI; EGEA, 2020). The selenium content found in 100 g of Brazil nuts is, on average, 3382 µg; in addition, the nut is recognized for its lipid content, which reaches an average of 63.5%, while for carbohydrates, it is 15.1% and proteins 14.5% (TBCA, 2020).

A recent study demonstrated that selenium in Brazil nuts is mainly found in the organic form, the most effective and least toxic source, and is located in a layer of tissue 1 to 2 mm below the surface of the seed, along its peripheral. When analyzing the selenium concentration of nuts, with the variation of levels in different batches, it was considered that one nut (5 g) would be enough to meet or even exceed the RDA for selenium, depending on the region where it was grown, for example (LIMA et al., 2019; PRABHU; LEI, 2016).

Brazil nuts also have a bioactive composition considered valuable, including phenolic compounds and flavonoids, such as gallic acid, 3,4-dihydroxybenzoic acid, and catechins; tocopherol and phytosterols, as well as proteins, amino acids, and dietary fiber. Nut lipids are considered beneficial for health due to the high content of monounsaturated fatty acids, such as oleic acid, and polyunsaturated fatty acids, such as linoleic acid, and the low concentration of saturated fatty acids, such as palmitic and stearic (ÖZCAN; JUHAIMI; USLU, 2018; TAKEUCHI; EGEA, 2020).

The Household Budget Survey of the Brazilian Institute of Geography and Statistics (POF/IBGE, 2019) evaluated, in the period 2017-2018, the consumption of nuts and nuts in kilos per capita per year in the regions of the country, and despite this consumption reflecting the consumption of Brazil nuts associated with other seeds, it is important to mention it. In the northern region, where Brazil nuts are widely cultivated, consumption was 52 g. In contrast, it was 21 g in the northeast region, the lowest consumption for all Brazil regions. The Southeast and Midwest regions consumed 59 g and 56 g, respectively, and the South region had the highest consumption, with a value of 268 g.

New evidence demonstrates the importance of consuming Brazil nuts, mainly as a source of selenium in the diet. Data indicate that the daily consumption of selenium through Brazil nuts in healthy individuals did not change the composition of the intestinal microbiota but increased important genera for human health and that there was a significant correlation of selenium and some other minerals with producing bacteria of short-chain fatty acids, demonstrating the benefit of consuming Brazil nuts as a source of selenium in the diet (HASHIMOTO, 2019).

On the other hand, one of the concerns for the consumption of nuts in general is associated with the risk of contamination by fungi and mycotoxins, especially the aflatoxin produced by *Aspergillus flavus* (PATERSON; LIMA, 2010). Studies point to the existence of four different types of aflatoxin common in Brazil nuts, which are AFB_1, AFB_2, AFG_1, and AFG_2 (FREITAS-SILVA; VENÂNCIO, 2011; TANIWAKI et al., 2017). This contamination occurs naturally, depending on these nuts' nutrient profile and water content (BHARDWAJ et al., 2023). It is a very important contamination because these toxins are associated with various health damages, such as immunosuppressive, carcinogenic, mutagenic, and teratogenic factors, even when present in small amounts in food (NAVALE et al., 2021). In this way, guaranteeing a safe product, free of contamination by aflatoxins, is essential for a productive and industrial chain (ALTOMARE; LOGRIECO; GALLO, 2021).

2.7 Plant-based beverages

Plant-based products are foods developed based on vegetables such as cereals (rice, oats, corn, sorghum), legumes (beans, soy, peas), oilseeds (peanuts, hazelnuts, walnuts, cashews, and Brazil nuts) (SILVA & SMETANA, 2022). These products are strongly emerging in the national and international markets and have been gaining prominence for being products often placed as sustainable vegan products and alternatives to milk (DRIGON et al., 2023).

Despite the sustainable and vegetarian perspective, these products are considered nutritionally inferior to milk and considered by several authors not to be an adequate substitute (PENHA et al., 2021; VAIKMA et al., 2021; VALLATH; SHANMUGAM; RAWSON, 2022). However, as these products are considered sustainable and practical, they can and should be included in the diet, not as a substitute, but as a food that tends to add nutritional value and be an option for people with allergies, intolerances or who wish to reduce their intake of animal products.

Until now, Brazil has no legislation to define these products about their denomination in the nutrition label. According to RISPOA (BRASIL, 2020), these products cannot be called milk, as they are not products derived from the secretions of female mammals. In this sense, the Bill (10556/18) of the Federal Chamber of Brazil is being discussed on these foods' denomination, specification, and labeling. This discussion is paramount since these products are often presented similarly to traditional dairy products on the market, either by packaging, appearance, or arrangement in the supermarket, sometimes leading the consumer to error.

Although these products are constantly on the rise, they are still unknown by many, especially in their composition, manufacturing, nutritional and health benefits. A study by Mintel (2023) on “global consumption trends” points out as predictions that consumers are increasingly concerned about sustainable products that bring nutrient benefits, are economically viable, and are locally produced. In this sense, developing and expanding knowledge of plant-based foods and beverages with these aspects are necessary, especially for an audience that has been growing in the country, as is the case of the elderly.

3. OBJECTIVE

3.1 General purpose

Development a beverage enriched with Brazil nut selenium for the population over 60 years old and to study the potential and perception of these individuals about this beverage.

3.2 Specific objectives

- Bring information reported in the literature on plant-based beverages made from tree nuts regarding health, technological, microbiological, sensory, and consumer aspects.
- Microbiological evaluation of Brazil nuts
- Evaluating Brazilian consumers' perception and consumption of nut-based beverages.
- Assess the understanding and acceptance of elderly people of a vegetable beverage made from Brazil nuts and fruit

4. ORGANIZATION OF THE THESIS

This thesis was organized into five chapters, as shown in Figure 4. The first chapter deals with an introduction, brief bibliographic review, and structure of the main theme of the thesis, addressing the subjects: aging, Brazil nuts, selenium, food, and food products for the elderly. The second chapter presents a bibliographic review of plant-based beverages produced from nuts concerning sanitary, technological, microbiological, sensorial, and consumption aspects. The third chapter of the thesis focuses on a study about Brazil nuts as a raw material in industry from two different harvests for microbiological analysis (from DNA extraction and amplification of 16S genes analysis of bacteria and ITS for fungi) and mycotoxins (aflatoxins AFB_1, AFB_2, AFG_1, and AFG_2) by HPLC-FD / KobraCell®. The fourth chapter o carried out a study to evaluate Brazilian consumers' perception and consumption of nut-based beverages. An online questionnaire was prepared with socioeconomic and consumer issues and published in digital media following established ethical standards. The fifth chapter of the Thesis is a study of the consumption and perception of the elderly public about four beverages produced based on Brazil nuts and the application of a neophobia questionnaire. After that, the general conclusion and future perspectives are presented to close this work.

5. THESIS FLOWCHART

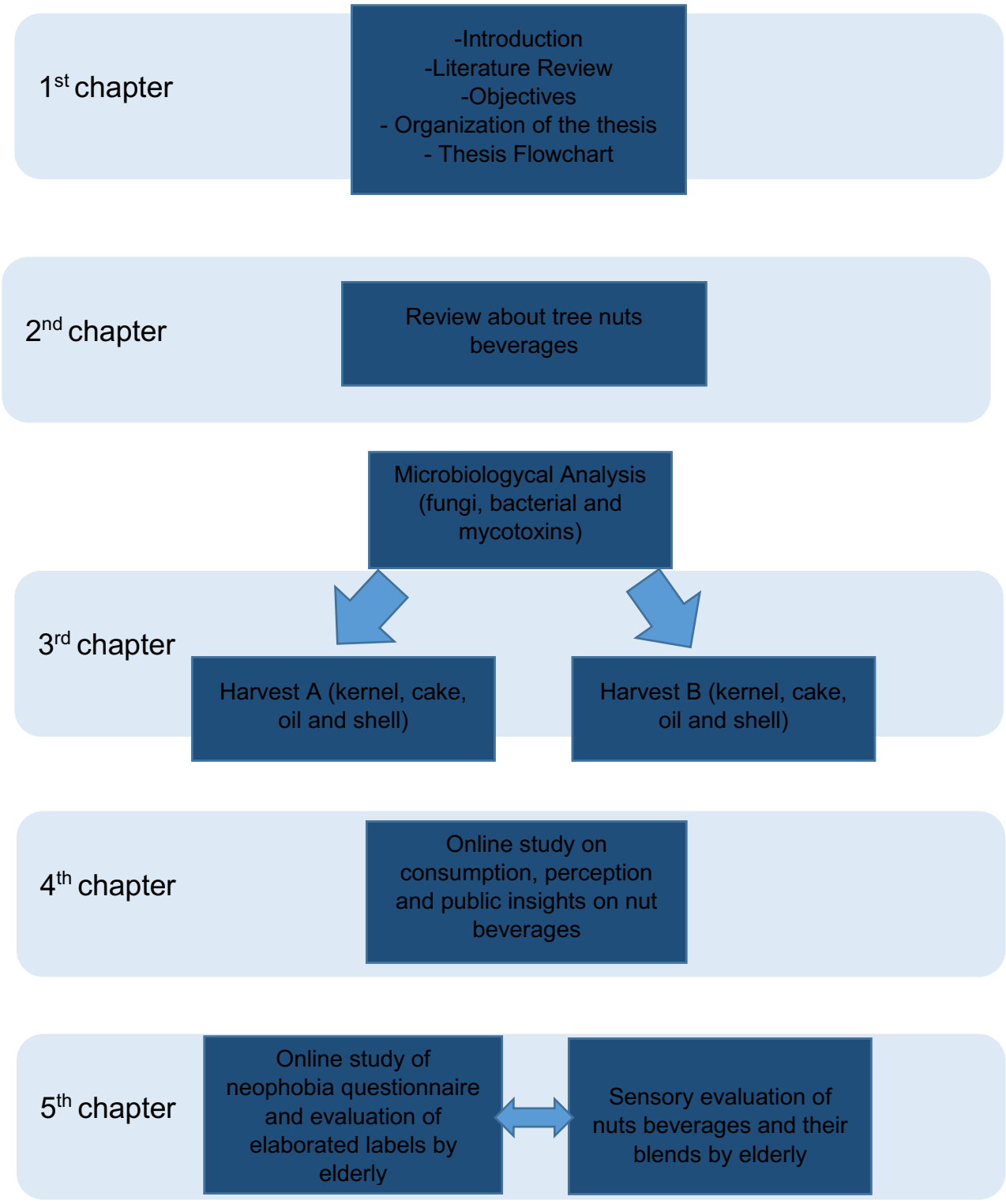

Figure 4: Thesis Structure

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CHAPTER 2 - A REVIEW ON PLANT-BASED TREE NUTS BEVERAGES: TECHNOLOGICAL, SENSORY, NUTRITIONAL, HEALTH AND MICROBIOLOGICAL ASPECTS

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ABSTRACT

The term tree nuts describes any nut that comes from a tree. The best-known and most consumed are almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut. This consumption has led to their use in the development of plant-based beverages. This review aims to bring information reported in the literature on plant-based beverages made from tree nuts regarding health, technological, microbiological, sensory, and consumer aspects. The bibliographic search covered the articles published between the years 2000 and 2022. In total, 54 articles were selected for this review, which were categorized according to their thematic area as Food technology (n=37); Nutrition and Health (n=19); Microbiological aspects (n=10); and Sensory and consumer studies (n=12). It was observed that the most mentioned tree nuts were almond, cashew, hazelnut, and walnut, and the lowest mentioned were Brazil nut, macadamia nuts, pine nut , mallow nut , and sapucaia. Finally, this review observed that beverages represent beneficial nutritional and functional profiles but cannot be considered as a substitute, in their entirety, for dairy milk, except in the case of allergies to this food. Technological aspects proved positive for obtaining these beverages, with high pressure being the most advantageous, considered safe, and very promising from nutritional, industrial, environmental, and functional aspects.

KEYWORDS: nuts, not- milk, dairy, legumes, vegetables, beverages.

1. INTRODUCTION

From a culinary point of view, nuts can be defined as any grain used in food with a high lipid content and protected by a thick husk. From a botanical point of view, they are defined as dry fruits composed of a hard inedible husk and a seed. The term “tree nuts” is associated to describe any nut from a tree, and this can typically include foods that do not meet the traditional botanical definition [1,2]. The best-known and most consumed tree nuts in the world are almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut [1,3–5].

The consumption and production of tree nuts have increased over the years [6–8]. In the last two years, the USA and Turkey were considered the largest producers of tree nuts in the world, with both productions exceeding 200 thousand tons in this period. On the other hand, the biggest consumers-importers of tree nuts were China and the countries of the European Union (USDA, 2021). Studies point to several health benefits in the consumption of fresh tree nuts and their products: cashew [9]; pecan [10]; almond [11]; Brazil nut [12]; pistachio [13]; hazelnut [14]. The positive effects have been mainly associated with these nuts’ lipid profile and the high mineral content [15].

Plant-based beverages are gaining great prominence in the food market [16], as they are considered healthy alternatives for consumption and because they present themselves as a substitute option for products traditionally made from dairy milk [17,18]. Beverages, in general are essential in the daily diet of human beings, as they are considered practical and accessible foods for consumption, in addition to being able to contain a large mixture of nutrients in a small volume of food [19]

However, some vegetable beverage may have limitations in the content of some nutrients essential to health, especially regarding the physiological needs of vulnerable groups such as children [20]. In this sense, to offer an adequate and balanced intake of nutrients, thus avoiding nutritional deficiencies, fortified beverages are seen as a practical alternative and well accepted by the public [21]. The fortification of fruit and vegetable drinks has been an important and positive strategy from a nutritional point of view [22]. The technologies used for the conservation of these products must enable an improvement in

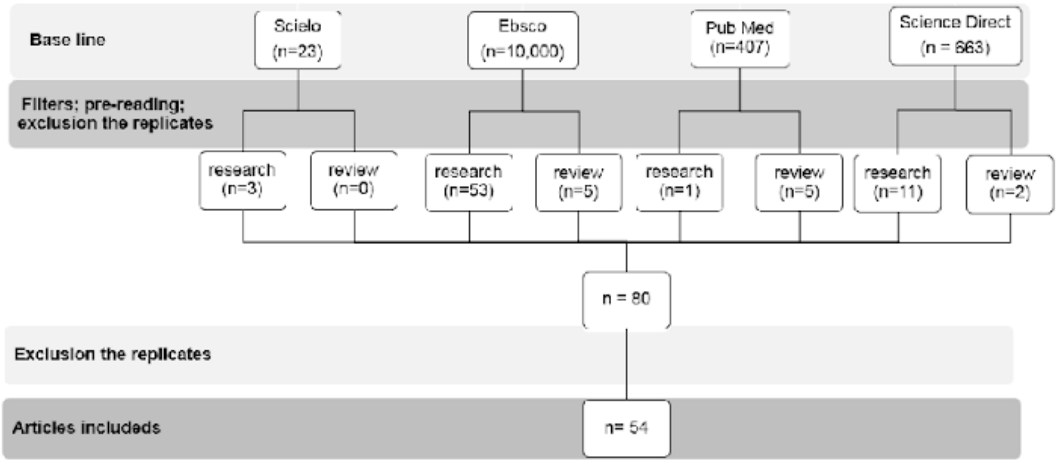
the bioavailability of nutrients with the preservation of physicochemical properties, stability, sensory characteristics, and acceptance by consumers [16,23–25]. From this perspective, the increase in nutrients and bioactive compounds is seen as an improvement to the product and a benefits to the consumer, and this has been perceived in the formulation of vegetable drinks [26–28].

Studies show that vegetable beverages are emerging in worldwide consumption; however, consumers still require more information on the composition and benefit of these beverages [26,29,30]. In this sense, this review aims to bring information reported in the literature on "plant-based" beverages made from tree nuts regarding health, technological, microbiological, sensory, and consumer aspects.

2. METHODOLOGY

The present work consisted of a bibliographic search carried out from June to September 2021 in the following databases: “Science Direct”, “Ebsco”, “Scielo” and “Pub Med”, where the descriptors used were: "nuts AND (beverages OR drink OR extract OR plant-based OR milk) AND NOT tiger". Articles between the years 2000-2022 were selected. For this study, only articles related to beverages/extracts made with tree nuts were included, thus excluding studies carried out exclusively on "coconut", "peanut", "tiger nut" and other fruits that did not fit the definition "tree nuts”. Works that used blends of “animal milk” with tree nuts were also excluded. After applying the filters and exclusion criteria, the articles were previously read (abstract and title), and the replicated ones were excluded, as shown in Figure 1.

Figure 1: Organizational chart of article selection during bibliographic search.



3. RESULTS

After reading and applying the exclusion and inclusion criteria, 54 articles were selected for this study, categorized according to their thematic area, as shown in Table 1. The defined thematic areas were Food technology (n=37); Nutrition and Health (n=19); Microbiological aspects (n=10); and Sensory and consumer study (n=12). An article may belong to more than one established category.

Most of the articles were related to the years 2020 (22.22%), 2019 (16.66%), 2021 (16.66%), and 2018 (11.11%), with the lowest prevalence in the years 2000, 2003, 2007, 2012 and 2013 with 1.8% each. After applying the criteria, there were no recovery articles from the years 2001-02, 2004-06, 2008, and 2010-11. It was observed that of the selected articles, those of the research type (n=43) were in greater quantity than those of the review type (n=11).

Regarding the prevalence of tree nuts, it was found that the most mentioned in the articles were almond (n=23), cashew (n=19), hazelnut (n=13), and walnut (n=11). The lesser mentioned were Brazil nut (n=4), macadamia (n=4), pine nut (n=3), mallow nut (n=2), sapucaia (n=2), Chilgoza pine (n=1), tucumã almond (n=1), Quercus acorn (n=1),

pistachios (n=1), cupuaçu almond (n=1) and pecan (n=1). Of the 54 articles selected, 13 presented beverages based on blends with fruits, vegetables, or other tree nuts. Regarding these blends, it was noticed that the most mentioned ingredients for the mixture were tree-nuts (n=4), (almond, macadamia, walnut, and hazelnut), soy (n=3), rice (n=3), mango (n=2), coconut (n=1), pea (n=1), oats (n=1), chocolate (n=1), prune (n=1), passion fruit (n=1), pineapple (n=1), mix vegetables (n=1), cocoa (n=1) and carob (n=1).

Table 1: Main tree nuts products beverages: Articles selected during the search categorized by thematic areas.

Tree nuts	Type of paper	Thematic field	Authors
Almond	Research	NH	[31]
Almond, hazelnut, walnut, cashew	Research	NH	[32]
Hazelnut	Research	FT	[33]
Hazelnut	Research	FT	[14]
Walnuts, hazelnuts	Research	NH	[34]
Blend soy and Brazil nut	Research	FT and MA	[35]
Walnut and blend soy-walnut	Research	FT and SC	[36]
Cashew nut	Research	MA	[37]
Blend Cashew nut and mango	Research	SC	[38]
Brazil nut	Research	FT	[39]
Almond, cashew nut	Review	NH	[17]
Almonds, cashews, hazelnuts, macadamia, almond and coconut, pea and almond, almond and cashew rice and hazelnut, oats and walnut, hazelnuts, walnut, and almond mix	Review	NH	[29]
Brazil nut	Research	MA	[40]
Sapucaia nut (*Lecythis pisonis Cambess*)	Research	FT	[41]
Walnut	Research	FT	[42]
Pine nut (*Siberian Pine*)	Review	FT, NH	[43]
Blend soy and Brazil nut	Research	FT and SC	[44]
Almond (*Prunus dulcis*), sapucaia nut (*Lecythis pisonis*), cashew nut (*Anacardium occidentale*), hazelnut (*Corylus avellana*) cupuaçu almond (*Theobroma grandiflorum*), tucumã almond (*Astrocaryum vulgare*), macadamia nut (*Macadamia spp.*)	Review	NH	[45]
Walnut	Research	FT	[46]
Hazelnut	Research	FT	[47]
Chilgoza (*Pinus gerardania*)	Research	FT	[48]
Almond, hazelnut	Research	FT and NH	[49]
Siberian Pine (*Pinus sibirica Du Tour*)	Research	FT and NH	[50]
Blend rice and Pine nut (*Pinus koraiensis*)	Research	FT	[51]
Almond	Research	FT, MA, and SC	[52]
Walnut	Research	FT	[53]
Walnut	Research	FT	[54]
Almond beverage, chocolate almond beverage, walnut beverage	Research	FT	[55]
Almond, Cashew, Hazelnut	Research	NH	[56]

Cashew	Research	FT and MA	[57]
Almonds, cashews	Review	FT	[58]
Almond	Review	FT, NH, and SC	[59]
Cashew nut	Research	FT	[60]
Almond, cashew nut (flavored with cacao or carob)	Research	FT and SC	[61]
Almond	Review	FT and MA	[24]
Cashew nut (blend prune, brown rice)	Research	FT and SC	[62]
Malva nut	Research	NH	[63]
Almond, walnut and hazelnut	Review	FT	[25]
Cashew nut (blend passion fruit)	Research	FT and SC	[64]
Cashew nut (blend with pineapple juice)	Research	FT and SC	[65]
Cashew nut (blend mango)	Research	FT and SC	[66]
Pistachios	Research	FT, MA, and SC	[67]
Quercus acorn	Research	FT and MA	[68]
Almond, hazelnut	Review	NH	[69]
Blend Brazil nut and baru, Brazil and Macadamia	Research	FT	[70]
Cashew, almond	Research	NH	[71]
Walnut (Juglans) and two types of hazelnuts (*Corylus avellana* almond; *Prunus dulcis*)	Research	MA	[72]
Malva nut (*Scaphium affine*)	Research	NH	[73]
Almonds	Review	FT	[74]
Almond	Review	NH	[75]
Walnut (vegetable blend)	Research	FT and NH	[76]
Cashew, almond, macadamia, pecan	Research	NH	[77]
Almond	Research	FT and MA	[78]
Almond, Cashew, Macadamia, Hazelnut	Research	NH	[79]

Thematic fields (FT = Food and Technology; NH = Nutrition and health; MA = Microbiology aspects; SC = Sensory and consumer studies)

4.DISCUSSION

Food Technology

Tree nuts beverages are foods that have complex colloidal structures in their matrix that directly impact the appearance, texture, mouthfeel, flavor, stability, and bioavailability of nutrients. Therefore, proper knowledge of these structures is essential for the preparation

of plant-based beverage substitutes for dairy milk, as this knowledge can be used to improve quality and functional attributes and contribute to the healthiness of the product [58]. Tree-nut matrices are not considered simple to manage. Their stability and rheology properties are poor when compared, for example, to animal milks and beverages or from other plants such as soybeans, which performed well concerning hydrocolloids [49]. In this sense, prior treatment and adequate processing, such as the use of high pressure, temperatures, pH modification, enzymes, fermentation, and homogenization, are necessary to improve the solubility, stability, and maintenance of the sensory characteristics of the product during storage [25,55]. The correct choice of extraction methods is also important to obtain a product with a better nutritional and bioactive profile, whether aqueous [48] or alcoholic [34,60].

The use of high-pressure was positive elaborating of tree nut beverages. A study by Valencia-Flores [78] points out that high-pressure is a potential alternative to conventional heat treatments, as it produces highly stable vegetable beverages from a microbiological, physical, and chemical point of view. Experiments by Gul et al. [47] pointed out that high pressure processing of hazelnut milk samples increased the solubility of proteins in water, and reduced the viscosity of the product. Therefore, this technology indicates a wide application for processing such products due to the improved preservation of the microstructure and rheological properties, lower energy consumption and greater efficiency due to changing viscosity.

The acceptance of the rheological properties of these beverages under technological treatment may vary according to the desired product. This is shown, for example, by the study by Atalar et al. [14], where the use of hazelnut milk under high pressure led to a high viscosity and consistency index and a high rigidity parameter in the product but provided the desired textural, melting rate, and rheological improvement, as they were related to ice cream formulations.

Regarding the use of temperatures, the physicochemical properties and oxidation stability of tree nut beverages can be affected by the intensity of the heat treatment and the storage period. Therefore, choosing the right time and temperature is important to ensure improvement in the parameters of the final product. According to Atalar et al. [33], heat treatments cause protein denaturation in these beverages, thus increasing aggregation.

Therefore, samples, when treated at high temperatures, have a higher level of aggregation than those treated at lower temperatures, thus decreasing their solubility, changing the content of soluble solids (ºBrix), and increasing the viscosity of the final product, in addition to impacting the increase in the hydroperoxide index during the storage period. On the other hand, the use of negative temperatures seems to have positive effects on the storage of tree-nut extracts. In the experiment by Demoliner et al. [41], this method proved to be a very interesting tool for obtaining and maintaining nutrients and concentrates rich in bioactive content from the extract obtained from sapucaia nut.

Heat treatment and high pressure have positive and negative effects regarding the maintenance of product quality, suggesting that these methodologies should be used as complementary in the preparation of tree nuts beverages. This concept is reinforced by Sardão et al. [68], who demonstrated that high pressure preserved the soluble solids content and color better than heat treatment. However, heat-treated samples showed superior antioxidant activities (ABTS/DPPH) compared to samples treated with high pressure. Regarding macronutrients, the results were similar between treatments.

The bibliographical survey also pointed out fermentation as a method to improve the technological properties of tree nut-based beverages [69,74]. However, this process can lead to nutritional losses, such as a decrease in carbohydrates, fibers, and minerals by up to 38.5% after fermentation [40]. Another resource used to improve the stability of tree nuts beverages is the use of additives. One of the arguments that reinforces this need is that sometimes the protein content of these beverages (depending on the tree nut) is not enough to stabilize an emulsion, requiring the use of emulsifiers such as lecithin [78], xanthan gum, and carboxymethylcellulose. However, with heat treatment, they can lose their technological effect, gum arabic [46], and the use of BHT with the objective of preventing the oxidation of these beverages [53].

Penha et al. [24] pointed out that for the preparation of vegetable beverages, it is necessary to apply the most efficient and viable method that will result in a final product with satisfactory physical stability and minimize the need for additives such as hydrocolloids and emulsifiers. However, Cardarelli and Oliveira [39] and Lee and Rhee [51] state that the use of physical processing added to aditives is an important factor in determining the adequacy of processing beverages prepared with tree nuts. A possible explanation for this

slight divergence in the literature is the year/time of publication, as over the years, the development of products with fewer additives and more ecologically sustainable means is gaining notoriety, and gaining demand, and interest in the current market.

With regard to sustainability (eco-friendly products), vegetable drinks have great potential and have been a major trend in the modern food industry because of their environmental, health, and ethical benefits [58]. An earlier study by McClements et al. [59] pointed out that water consumption in the cultivation of nuts to produce vegetable beverages was similar to that of animal milk. In this sense, two studies drew attention during the preparation of this work, as they developed beverages from tree nuts cultivated with reduced irrigation using almonds [52] and pistachios [67], showing that it was possible to prepare healthy, nutritious, and environmentally friendly beverages while also saving 66% and 74% of irrigation water by way of said cultivation modality. Another relevant study on this issue was one carried out by Rebouças et al. [64], who developed a drink that uses broken nuts with low commercial value, which contributes to greater profits in this sector and avoids food waste.

The technological processes and methodologies pointed out in this section seem to be promising within the tree nuts beverages industry. However, some important parameters at industrial levels need to be analyzed. Penha et al., [24] state that to incorporate these alternative and innovative techniques at an industrial level, the processes must still be evaluated according to each matrix, specifically to verify its cost-effectiveness and feasibility, which is still a challenge for the food industry.

Sensory aspects and consumer study

The market and demand for plant-based beverages are growing and expressive. However, there are great challenges regarding these products' sensory aspects and acceptance. Developing a healthy and sensory-pleasing product is not an easy task. Concerning tree nuts beverages, this situation is similar, and some resources have been used to improve the flavor to impact product acceptance.

Blending nuts, for example, has been one of the tools to improve the flavor and aroma of tree-nut drinks [64,65,76]. The study by Pinto et al. [62] corroborates this information

and appointed that blended tree nuts beverages were more accepted than beverages based on other cereals and vegetables (soy, oats, rice, quinoa) and demonstrated that it was technically possible to develop a vegetable-based beverage from brown rice, plums, and cashew nut, which has high overall acceptability. Blends have a positive impact on the taste and color of the final product. Felberg et al. [44] enhanced a beverage with a soy and Brazil nut blend. They pointed out that this mixture contributed to a change in the product's color (decreasing the yellow color and increasing the white color) and improved the acceptance of the product. This can be explained because most plant-based milks have a creamy appearance, and their color and lightness can be significantly different from those of bovine milk, which adds to the appearance of an enhanced beverage [59].

There is still no consensus on the adequate percentage of tree nuts and the ingredients for blends. The authors, in general, point out that this can vary according to the nut and beverage made. One hypothesis is related to the different percentages of sugars in fruits and vegetables used and the lipids of nuts that can influence the perception of product palatability. In addition, adding other beverage-formulating ingredients, such as sugars, can also interfere with the issue of flavor acceptance [65].

Fermentation was also mentioned as a tool with great potential to improve the sensory profile of these beverages. Bruno et al. [37] used fermentation to prepare a cashew nut beverage without significant changes in color and with good sensory acceptance. However, Cunha Júnior et al. [40] did not have the same results, as fermented Brazil nut beverages received low scores for “general acceptance” and “fermented taste”; nut flavor and “sweetness” were equal to the unfermented Brazil nut beverages tested. These differences in results regarding flavor can be explained by a lack of standardization and a combination of strains for fermentation for this purpose. Tangyu et al. [74] conclude that strain blending is still conducted with trial-and-error approaches and that possibilities for more rational selection and blending of strains with predictable synergistic interactions would be highly valuable for developing smarter fermentation processes and better products.

Regarding sensory acceptance, almond was considered a versatile tree nut applicable to beverages. Qamar et al. [25] pointed to the use of this nut in emulsions to improve flavor. One of the possible explanations for this improvement is that the perception of the general

taste of almond "milk" is quite similar to that of bovine milk [59], and consumers associate the taste with a known food since the experimenting with new foods and flavors can lead to difficulties in accepting a product.

Morais and Rodrigues [61] developed a beverage based on almond and cashew nut, to test the possible replacement of cocoa with locust bean poder, and pointed out that tree nuts were good vehicles to carry out this experiment. Sensory low scores were found by Lipan et al. [52] in the elaboration of almond milk powder, but which, according to them, can be improved with the application of adequate techniques.

One of the possible techniques indicated for this sensory improvement is the high-pressure process (HPP). The experiment by Sardão et al. [68] showed a preference for Quercus acorn drink when treated with HPP overheat treatment. Regarding color, although both treatments showed equivalent results, HPP showed better color retention, and consumers preferred this beverage. Plant-based beverages may have inferior flavors to the traditional bovine milk beverage. Another suggestion to overcome this problem is homogenization, which modifies insoluble emulsion particles to a size larger than that detected by the tongue (usually less than 50 μm), making the product more palatable [58].

Other factors that are also relevant within consumer studies are the aspects of labels and packaging that can influence the purchase of tree nut beverages. The use of labels and different nutritional claims has a positive impact on the perception of the drink in relation to quality, general impression, and purchase intention [66]. Cabral Rebouças et al. [38] point out in their study that the most relevant qualitative information in the purchase intention was the label illustration (especially the labels with the highlighted nut figure), nutritional information (0% lactose and 0% cholesterol), and functional claim term “prebiotic”. Also, according to these authors, the illustration itself was the biggest influence, even overlapping nutritional information and functional claims.

Nutrition and health

Composition and nutritional profile: Regarding the composition and nutritional richness of beverages based on tree nuts, it can be stated that there is a specific divergence in the literature regarding certain points, for example, the percentage and biological value of the product's protein and content of micronutrients such as calcium. The study by Lipan et al.

[52] states that a beverage made with almonds was a good source of Ca, K, and Zn and was rich in Mg, Cu, Mn, and monounsaturated fat. In this same perspective, the works by Sardão et al. [68] and Atalar et al. point out that beverages made from tree nuts were a good source of minerals in general. Bolarinwa et al. [36] used walnuts to enrich a soy-based beverage that resulted in a notable increase in protein and mineral content in the product. From this same angle, Dyshluk et al. [43] substituted raw milk for pine nut in the elaboration of "dairy" products and realized that they did not reduce the nutritional value of new dairy products, as they had a high proportion of protein, fat and minerals and were as rich in vitamins as their milk-based counterparts.

However, many studies indicate that tree-nuts and plant-based beverages, in general, are poor and inferior in nutritional content when compared to animal milk or even soy-based beverages (depending on the tree-nut) [17,18,32,62,69,79]. The wide variety of tree nuts can explain this divergence within the literature, each one with a unique nutritional profile and very peculiar characteristics [49]; as such, to generalize them would be a mistake. This nutritional variation can be observed both in the fresh nut and in the final drink [17,45], and even beverages, from the same nut can present variations in the countries where they are produced and marketed [29]. Another hypothesis that can be raised is that during the technological processing of these beverages there may be significant nutritional losses, thus reducing the nutrient profile of the final product [40,52]. In relation to plant-based beverages and tree nuts beverages, there is a certain consensus by the aforementioned authors that these beverages can be an alternative to animal milk only in case of allergies. However, the nutritional comparisons have not presented any concise indication for such replacement. What can be said so far is that tree-nut beverages can be nutritionally rich when compared to some fruit and vegetable beverages, but in relation to animal milk and soy are at their disadvantages. However, this nutritional profile can be improved upon and should be for fortifying tree nuts products.

Regarding the fortification and improvement of plant-based beverages, Jeske et al. [49] state that the choice of ingredients to improve the product must be a wiser and healthier choice than the addition of low-cost fortifiers and additives. In this sense, we can point out the studies by Pinto et al. [62] and Tiurikova and Peresichny [76], who used blends of tree nuts, and other vegetables that added nutritional, functional and biological value

to the final beverage, receiving good acceptance by consumers. Silva et al., [70] pointed out that cashew and almond beverages had less calcium than cow's milk; however, fortified samples had comparable values for total calcium and more calcium bioaccessibility than cow's milk. Craig et al., [29] state that specifc vitamins, such as D and B12, need to receive greater attention about fortification, as they are present and more bioavailable in foods of animal origin. Therefoe, tree nuts may have a greater appeal to the vegetarian public, as they lack vitamins in their diet.

Such information regarding the nutritional and functional profile generates a positive impact on consumers when they intend to purchase tree nuts beverages [17,38]. Therefore, improving these products regarding nutrient content, health benefits, and information should deserve attention and investment by industry and research [29,59,79]. In this aspect, tree nut beverages, depending on the nut that composes them, can present great bioactive potential regarding antioxidant activity due to their phenolic content [50,71]. In addition, considering the functional aspect, some studies have also pointed to the possibility of successfully enriching these drinks with prebiotics [38,64,65].

Health benefits and risks: Regarding the immunological safety of these drinks, the literature points out that, indeed, certain nuts can trigger an allergic response in some patients with hypersensitivity to the nutrients that make up these foods. Masiri et al. [56] pointed out in their study possible allergens in beverages prepared with almond, cashew, and hazelnut. It is possible that an individual is allergic to one or more of these nuts, as drinks may contain mixtures of nuts; it is essential that there is a specification in the labeling as to the composition so that allergic individuals can be informed about consumption [45]. However, it is common for other nuts to get mixed in intentionally or unintentionally (fraud and contamination), and identifying this contamination is of paramount importance. Ding et al. [42] established a strategy, based on DNA tracking, to detect the authenticity of tree nut beverages in local markets in China and found that some tree-nut beverages sold in the region had been contaminated with other nuts.

Tree nuts beverages are safe to drink, with the exception, of course, for the public allergic to nuts. Astolfi et al. [32] showed that these beverages are quite safe, with low contamination by toxic trace elements, including As, Cd, Hg, and Pb. These beverages can also have certain health benefits, such as improved blood glucose and cholesterol

levels [34,63] and dental health. A study by Townsend et al. [77] provided evidence that fluoride concentration varies among different plant-based beverages, but with few exceptions, and that these beverages contain significantly more fluorine than cow's milk; thus, they encourage such information to be placed on labels. This is enforced by Abd-elmonsif et al. [31], who pointed out that almond milk had good remineralization capacity and tooth enamel protection (in relation to calcium and phosphorus levels) when compared to soy, oat, bovine, and cocoa milks. However, it is necessary to emphasize that almond milk should not be sweetened with sucrose, this way, it can have a cariogenic potential [75].

Microbiological Aspects

A relevant aspect to consider in elaborating tree nut beverage is their relationship with the human body, especially regarding the intestinal microbiota. According to McClements et al. [59], there has been very little published research in this area, and at present, there is still a relatively poor understanding of how these plant-based beverages affect the human gut (microbiota) and what long-term health implications this has for humans. They state that more research is needed and that it seems to be a very promising study topic [58]

In this sense, studies were found in the literature on intestinal microbiota and functionality, which is the development of these beverages as a probiotic function. It is an enrichment of tree nuts beverages with microorganisms considered beneficial to intestinal health. Lipan et al. [52] showed that it is possible to enhance a powdered almond drink enriched with *Lactobacillus plantarum*.

A point raised is that tree nuts are a good substrate for developing these functional beverages. Barbosa et al. [35] stated that the drink made with Brazil nut was an excellent substrate for the fermentation of *Lactobacillus acidophilus*, *Bifidobacterium,* and *Streptococcus thermophilus*. This information also corroborates the results of Bruno et al. [37], who demonstrated that cashew nut milk was a good matrix for *Bifidobacterium animalis*, *Lactobacillus acidophilus*, and *L. plantarum.* However, another point to be evaluated is whether the beverages produced, besides being good substrates, present probiotic viability in the human intestine or if at least the numbers of colony-forming

units are in sufficient quantities after technological processing information that was slightly diverged in the findings. For example, Mattison et al [57] characterized a commercially available yogurt and found that the mean lactobacilli and Streptococcus thermophilus colony counts were greater than 10M colony forming units per milliliter, indicating they could provide a beneficial bacterial health benefit. Similar results were found by Sánchez-Bravo et al. [67] with lactic acid bacteria in the pistachio brew. However, Cunha Júnior et al. [40] pointed out that Brazil nut, despite being a good matrix for *Lactobacillus casei,* did not prove viable after processing. One explanation for this event is that the technological processes chosen for the beverage production can often affect the viability of microorganisms. The authors themselves argued that a suggestion to increase the viability of probiotics was their microencapsulation or even a pre-acidification of the nut to prepare of beverages.

Despite reports of nutritional loss and color change in beverages, which can often be accepted by the consumer [37,40], fermentation still stands as a useful strategy within tree nut beverage production. The use of mixed culture fermentation has excellent potential to improve the nutritional quality and sensory profile of tree nuts beverages, and the performance of crops is strongly dependent on species and strain [74]. The study by Mattison et al. [57] demonstrated that the fermentation of yogurt based on cashew nut was able to reduce some allergenic factors of this nut during production and that the final product had color, pH, and viscosity similar to standard cow's milk-based yogurt.

Finally, concerning to microbiological aspects, the issue of contamination and microbiological safety of these beverages were also found during the survey, and tree nut beverages, after proper processing, are considered safe. The study by Škrbić et al. [72] points out that 2 out of 17 investigated beverages were contaminated with mycotoxins, and the others had very low rates and were considered acceptable under local legislation. Sardão et al. [68] found that high-pressure treatment at 450 MPa for 5 min as well as heat treatment showed to be sufficient to inactivate *Enterobacteriaceae*, mesophiles, psychrophiles, and molds and yeasts over nine weeks of storage, such as heat treatment. Similar results were found by Valencia-Flores et al. [78] in the preparation of an almond drink during 20 days of storage. Therefore, it is possible to state that the combination of different preservation methods (pasteurization, fermentation, and refrigeration) is

sufficient to guarantee the microbiological stability of tree nut products without often resorting of chemical preservatives [40].

5.CONCLUSION

This review reinforces that although tree nut beverages can present a good nutritional and functional profile, they cannot be considered as a substitute, in their entirety, for cow's milk, except in the case of allergies to this food. Even so, these products can have their profile improved through fortification, which is necessary due to the great variability in the nutritional composition of these nuts. In addition to the lack of certain nutrients, the flavor is still a limiting factor in tree nut milks. In this sense, blends with other vegetables, fruits, and nuts for fortification and flavoring seem to be positive for improving these aspects.

Regarding processing, high pressure, and temperature were positive in the preparation of nut tree beverages, with high pressure being more advantageous in some points; however, such associated methodologies seem to be more positive, although more studies are needed to stipulate this. The use of additives, despite showing rheological improvements in beverages, can be replaced by adding other vegetables, nuts, or even high pressure to improve the properties in tree nut milks and beverages. Tree nut beverages are considered safe from a toxicological point of view and also from a microbiological point of view. Tree nuts beverages are excellent matrices for adding probiotics; however, their effect on the body still needs to be studied, and in vivo analyses need to be better clarified. Tree nuts beverages are very promising from a nutritional, industrial, environmental, and functional point of view for their growing consumer market when the correct techniques are used.

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Conflicts of Interest

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

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CHAPTER 3- EVALUATION OF THE SAFETY AND QUALITY OF BRAZIL NUTS *(Bertholletia excelsa)* USING THE TOOLS OF DNA SEQUENCING TECHNOLOGY (16 S AND ST) AND AFLATOXIN PROFILE

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ABSTRACT

Brazil nuts (BN) result from sustainable extraction and are widely exploited in the Amazon region. Due to the production characteristics in the forest and the nutritional characteristics of these nuts, the occurrence of fungal contamination and the presence of aflatoxins are extensively discussed in the literature as a great aspect of interest and concern. The BN samples, harvest A (HA) and harvest B (HB), from two different harvests were collected in an extractive cooperative in the Amazon region for microbiological analysis (from DNA extraction and amplification of 16S genes bacteria analysis and ITS for fungi) and mycotoxins (aflatoxins AFB1, AFB2, AFG1 and AFG2) by HPLC-FD / KobraCell®. The samples showed a very different microbiome and aflatoxin profile. Genres such as Rothia (HA) and Cronobacter (HB) were abundant during the analysis of bacteria; as for fungi, the genera *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* were also considered prevalent in these samples. Soil microorganisms, pathogenic and related to inadequate hygienic-sanitary production practices and aflatoxins, were found in the samples but within the established limits permitted by Brazilian legislation. However, attention to the quality and safety of this production process was discussed with the cooperative. Nuts have a diverse microbiota and are not restricted to fungi of the genus Aspergillus. The microbiological and

toxicological profile can be quite variable within the same nut in the same extraction region and be maximized by global climate changes. Therefore, it is necessary to advance sanitary educational actions in applying good production practices and inspection programs to ensure the sustainability and quality of the BN production chain.

KEY WORD: microbiome, agrobiodiversity, Amazon, extractivism.

CHAPTER 4 - PERCEPTION, KNOWLEDGE, AND INSIGHTS ON THE BRAZILIAN CONSUMERS ABOUT NUT BEVERAGES

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ABSTRACT

"Plant-based beverages", "plant-based milk" and "milk alternatives" are terms commonly used to refer to drinks made from plants, such as legumes, cereals, pseudocereals, and nuts. This study aimed to evaluate Brazilian consumers' perception and consumption of nut-based beverages. An online questionnaire was prepared with socioeconomic and consumption questions and disseminated in digital media following established ethical standards. The almond beverage was the most consumed by the respondents, and the Brazil nut beverage was the one that most aroused the most interest among the respondents. Nut beverages were elected as the healthiest, most sustainable, and nutritionally best than soy beverages and cow's milk. Fruits such as strawberries and bananas were cited as alternatives for flavoring these beverages. Consumers considered the possibility of fully and partially replacing cow's milk and soy drinks with nut beverages. Interest in these, along with plant-based milk was not restricted to the vegan segment, and these products were indicated as having strong potential for inclusion in the diets of omnivorous consumers. More studies on buying, purchase intention, acceptance, knowledge, and neophobia among Brazilian consumers are necessary to develop these beverages, especially with more representative samples of society.

Key-words: Consumption, Brazil, milk alternatives, plant-based beverages.

1. INTRODUCTION

"Plant-based beverages", "plant-based milk", and "non-milk beverages" or "milk alternatives" are terms commonly used to refer to beverages made from plants such as legumes (beans, soybeans, peanuts), cereals (oats, rice, sorghum), pseudocereals (quinoa, amaranth) and nuts (walnuts, Brazil nuts, almonds, hazelnuts, cashew nuts) [1]. Some of these nut-based products are relevant for their specific sensory characteristics and nutritional profiles, which differ from other plant-based groups. For example, they are rich in monounsaturated and polyunsaturated fats, proteins, and selenium[2].

The consumption of plant-based beverages in Brazil and the world is steadily rising.[3–7] Although milk consumption is still strong in relation to plant-based beverages, the market is promising. However, some information barriers still need to be overcome for better insertion of these products. In Brazil, milk and dairy products consumption is still higher than the consumption of nuts and other oilseeds. Although the southern region of Brazil has stronger demand for nuts than the country's other regions, the consumption there does not exceed 1.5% of total food consumption [8].

"Plant-based beverages" (PBBs) are sought, especially by vegan consumers, as milk substitutes with functional potential and health benefits. Among the potentials of these beverages are their pre and probiotic viability [9,10]. Concerns for the environment, animal welfare, and world hunger are also relevant factors that drive the expansion of demand for these beverages [11]. In this context, consumers often consider PBBs superior beverages in terms of health, wellness, sustainability, and nutritional value. However, these advantages are still controversial and inconsistent in the literature [12–14]. Another relevant question is whether these beverages are microbiologically safe[15].

The tendency to replace animals with plants as human food sources is global [16,52]. Despite being a subject of growing interest, by industry and the academic community, some points are still not well elucidated in the literature. In addition, the population's knowledge about these products and the factors that most impact their choices are matters that still need to be widely discussed. Thus, we aimed to evaluate Brazilian consumers' perception and consumption of beverages based on nuts.

2. METHODOLOGY

Study location and research method

A questionnaire was prepared with socioeconomic and consumption questions. This was made available online for eight months and disseminated via digital media under established ethical standards. The questionnaire was segmented as follows: Initial screening: Informed consent form; 1st section**:** questions related to socioeconomic status, subsequently classified according to the Brazilian criterion established by ABEP [51]; 2nd section**: "**Word Association*"* containing possibilities for four words or expressions related to the question: *"Write the expression, association, word, sensation, emotion or thought when saying: 'nut beverages'"*; 3rd section**:** questions about the consumption and knowledge of nut beverages; 4th section**:** buying intention and comparison among nut beverages, soy beverages, and cow's milk.

Study population and technique

The "Word Association", categorization and analysis were performed according to the proposal of Alcantara et al.[17] and Guerrero et al.[18], wherethe terms obtained in the word association test were grouped into categories and dimensions. The categorization process was carried out by two researchers with previous experience in the methodology, and the categorization was reviewed by an additional researcher. The final results were obtained by consensus. The frequency of mention of categories and dimensions was calculated by the number of consumers who mentioned the word in the test. Categories with under 5% mention were excluded. The Chi-square test was used to verify the difference between categories and dimensions according to consumer groups. After analyzing these results, we separated the respondents into two groups: "never tasted the drink" and "already tasted or consumed the drink".

Ethical considerations

Before being released to the public, the questionnaire was submitted to the Ethics and Research Committee of the Federal University of the State of Rio de Janeiro and was approved under CAAE protocol number 57364922.4.0000.5285.

An informed consent form (TCLE) was made available to the participants, where signatures were collected for permission to continue the study. All data considered sensitive were protected, and respondents were told-communicated that only general data would be used for academic purposes.

Statistical analysis

Descriptive statistics were calculated, followed by ANOVA with the Tukey post-test. Significant values of $p<0.05$ were considered in all analyses. To compare the frequency of socioeconomic data, the Chi-square test was performed.

3. RESULTS

Table 1: Main socioeconomic results of the respondents.

	Percentage (%)		
	Total participants (n=300)	**G1 (n=128)**	**G2 (n=172)**
Gender			
Female	71.7	78.1	66.9
Male	28.3	21.9	33.1
X^2 =4.586; p=0.0322			
Age			
18 - 25	18.0	19.5	16.9
26 - 36	30.7	32.8	29.1
37 - 47	18.3	22.7	15.1
48 - 58	12.0	12.5	12.2
59 or more	32.0	12.5	26.8
X^2=10.11; p=0.0386			
Income status*			
A	11.3	14.1	9.3
B1	22.0	25.8	19.2
B2	34.3	29.7	37.8
C1	21.7	7.8	9.3
C2	8.7	21.9	21.5

D/E	2.0	0.8	2.9
X^2=6.172; p=0.2898			
Education			
Elementary school**	4.3	3.2	5.2
High school	35.3	33.6	36.6
Undergraduate or higher degree	60.3	63.3	58.1
X^2=1.265 p=0.5313			
Eating Habit			
Omnivore	91.0	83.6	96.5
Vegetarian or vegan	9.0	16.4	3.5
X^2=14.95; p=0.0001			

Legend: G1= people who had tasted or regularly drink nut beverages; G2= people who had never tasted nut beverages. Values expressed in percentage (%) were evaluated by the Chi-square test. *Income categorization according to ABEP. ** Defined as through 9th grade.

Socioeconomic data, dietary style, and consumption.

The sample consisted of 300 individuals after excluding those who accessed the questionnaire but did not confirm their interest in participating. Most (57.3%) had never tasted a nut beverage (X^2 = 6.45; p=0.0111). However, most respondents (95%) expressed willingness to try these drinks. Women had tasted or regularly consumed nut beverages the most, differing significantly from men (X^2 = 4.586; p=0.0322) (Table 1).

Regarding of age, most respondents were 26-36 years old, followed by the 60 years or older group and, to a lesser extent, the 48-58-year-old group (X^2=27.83; p<0.0001). People aged 26-36 were the ones who consumed the most or had already consumed the beverages and were also the ones who had tasted them least proportionally, followed by the elderly (X^2=10.11; p=0.0386). However, that group was one of those with the slightest contact with the beverage. Most elderly respondents were willing to try the nut beverage;

only one elderly person reported not having any interest in tasting it, corresponding to 1.6% of the group in question.

For the economic level, the middle classes (B and C) were the most representative of the respondents, and the lowest classes (D and E) and highest (A) were the least prevalent (X^2=207.1; p< 0.0001). However, the economic level of the participants did not influence the opportunity to try the beverage (X^2=6,172; p=0.2898). Individuals with a college degree were the majority in the study (X^2=141.7; p<0.0001), but this factor also did not influence product experimentation (X^2=1.265; p=0.5313).

About the dietary style, respondents who declared themselves omnivores were in the great majority (91%) versus vegetarians/vegans (9%), differing significantly (X^2=201.7 p<0.0001). Of the declared omnivores (n=273), 38 respondents (13.9%) reported not consuming milk under any circumstance; of these individuals, only 3 (7.9%) were not willing to try the nut beverage (X^2= 26.95; p<0.0001). Of the omnivores who consumed milk (n=235), only four individuals (1.7%) were unwilling to try the nut beverage. Statistically equal proportions of vegetarians/vegans consumed (62.9%) and did not consume milk (37.1%) (X^2=1.815; p=0.1779), while both groups of vegetarians (100%) were willing to try the nut beverages. The eating habit was associated with whether respondents had already tasted the drink. Vegetarians already had greater contact with the drink compared to omnivores (X^2=14.95; p=0.0001).

Purchase intention and comparison between nut beverages.

Participants who had already tried a nut beverage were asked "Which nut beverages have you already tried?". Almond (71.9%) and cashew nut drinks (61.7%) received the highest mentions, while pecan (2.3%) and baru nut beverages (0.8%) were mentioned the least. When asked about "Which nut beverages do you dislike the most and which attributes of these beverages do you most dislike?", almond (27%) and cashew nut beverages (23.4%) were the most chosen, and the attributes flavor (57.8%) and texture (14.1%) were the most disliked. For all these questions, participants could choose more than one suggested option or add one that had not been presented.

The respondents in the group who had never tasted a nut beverage were asked "Which nut beverage would you be willing to taste?" Brazil nut (90.1%), cashew nut (85%), and

walnut (85%) received the most mentions, while baru nut (0.6%) and Portuguese chestnut (0.6%) were the least mentioned. Among the elderly, this trend continued with some minor changes; Brazil nut (95%) remained the most mentioned, followed by walnut (76%) and cashew nut (73.9%). For all these questions, participants could choose more than one suggested option or even add one that had not been presented.

The question “What health benefit do you believe is associated with this beverage?” was applied to all participants. The benefits most cited by both respondents (there was no difference between groups G1 and G2, p=0.879) were “antioxidant action” “prevention of inflammation" and "cholesterol reduction", and with fewer citations, "prevention of neurological diseases”. Fewer than 3% responded that they did not know/believe in any benefit linked to the product. For all respondents (consumers and non-consumers of nut beverages), more than 68% said they would be willing to consume or buy the beverage if it had a proven nutritional or health benefit, and less than 5% would not buy it. However, there was no significant difference between the groups (p=0.659). In a spontaneous question (no options offered) about which fruit flavor would be attractive for a nut beverage, the flavors strawberry (12.9%), banana (6.4%), grape (3%), açaí (3%), cashew fruit (2.6%) and apple (1.3%) were the most cited.

Table 2: Summary of pairwise comparisons for factor (Tukey, HSD) about relevant attributes for purchase intention of all respondents:

Categories	**Relevance**
Taste/flavor	$4.47^{a} \pm 0.98$
Price	$4.46^{a} \pm 0.96$
Health benefits	$4.40^{a} \pm 0.98$
Availability/access	$4.00^{b} \pm 1.10$
Functional claim	$3.95^{b} \pm 1.16$
Appearance	$3.84^{b} \pm 1.20$
Packaging	$3.49^{c} \pm 1.20$
Organic	$3.41^{c} \pm 1.26$
Brand	$2.99^{d} \pm 1.18$
Lactose-free	$2.94^{d} \pm 1.48$

Vegan	$2.73^{d} \pm 1.35$

Results are expressed as mean ± standard deviation on a 5-point scale where 1- not at all important; 3-indifferent; 5- very important. Subscript letters differ from each other in the same column with $p<0.05$ (ANOVA followed by Tukey post-test).

The most relevant factors for all respondents, when asked “What matters most when buying a nut beverage?” were “taste/flavor”, “price” and “health benefits”, and to a lesser extent "lactose-free", "vegan" and "brand" (Table 2).

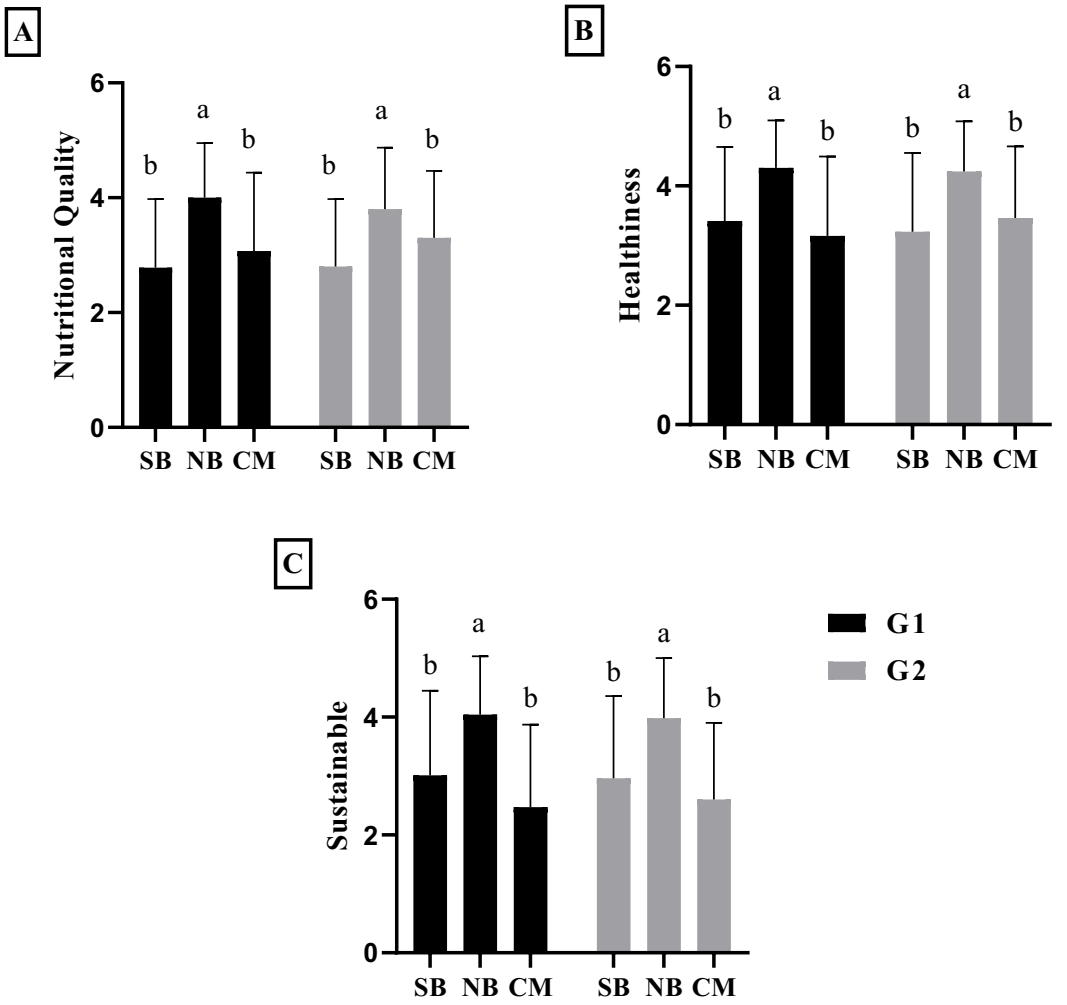


Figure 1: Results of comparisons between beverages when asked about nutritional superiority, the healthiest, and the most sustainable.

Legend: SB: soy beverage, NB: nut beverage, CM: cow’s milk. A: Results expressed as mean ± standard deviation on a 5-point scale where 1-much worse; 3-neither better nor worse; 5-much better. Letters differ significantly ($p<0.05$) among beveragess from the same group of respondents. B: Results expressed as mean ± standard deviation on a 5-point scale where 1-not

healthy; 3-neither healthy nor harmful to health; 5-very healthy. Letters differ significantly ($p<0.05$) among beverages from the same group of respondents. C: Results expressed as mean ± standard deviation on a 5-point scale where 1-not sustainable; 3-indifferent; 5-very sustainable. Letters differ significantly ($p<0.05$) among beverages from the same group of respondents.

Nut beverages were chosen by both groups as the healthiest, most sustainable, and nutritionally best beverage compared to soy beverage and cow's milk. However, these other beverages did not differ significantly, indicating that both cow's milk and soy beverages were less healthy, less nutritious, and not as sustainable (Figure 1), with scores averaging below 3 on the scale. When questioned about the possibility of substituting the soy beverage for the nut beverage, more than 50% of both groups (G1 and G2) said they were willing to substitute this beverage completely. Only 12.5% (G1) and 1.14% (G2) said they were not, showing that people who had never tasted it did not reject the idea of replacing their customary beverages. There was no significant difference between the groups regarding replacing soy beverages with a NB ($p=0.06$). The same question was asked regarding cow's milk, and G1 had the highest percentages distributed between the partial (36.7%) and total (35.2%) replacement options, demonstrating that people who had already tried NBs would be willing to replace them in somehow. In turn, in G2, there was a greater distribution of percentages between those who would replace partially (40.1%) and totally (20.3%) and those who were in doubt (maybe/I don't know) (28.5%), demonstrating that the people who had not tried a NB would be less willing to substitute their existing beverage preference (but not saying they wouldn't). In this question, there was a significant difference between the groups in the statements “would totally replace” ($p<0.01$) and “maybe/I don't know” ($p<0.05$), indicating that although the G2 respondents were willing to try NBs, partial replacement would be a greater possibility than the total in G1, because it was associated with insecurity/doubt regarding the beverage due to the fact they had never tried it, but it did not preclude the possibility of including it in the diet of this group.

In all, the word association (WA) terms were classified into 26 categories, and these into 10 dimensions (Table 3). G2 had the highest citation frequency compared to G1 for the categories consumption, health, negative characteristics, fatty/oily, positive characteristics, color/appearance, doubt, and lack of knowledge. In contrast, G1 had a

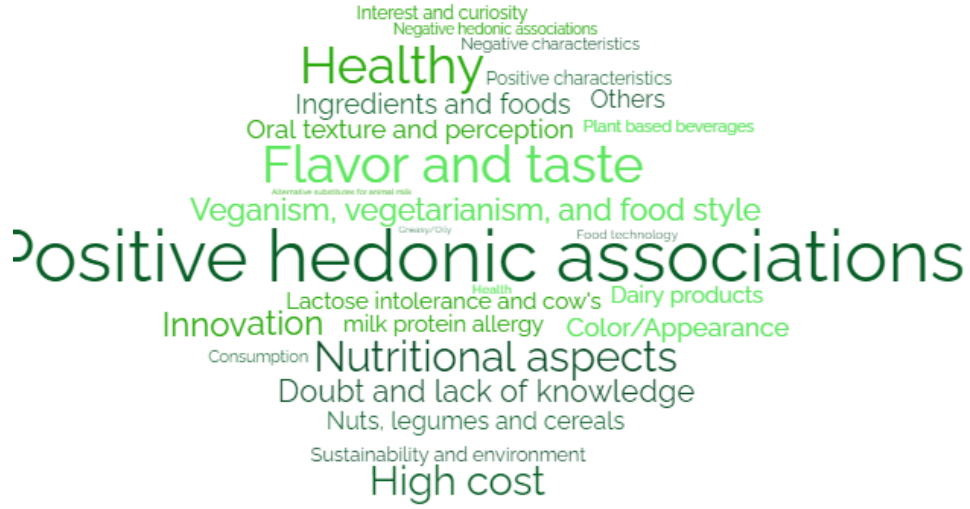
higher citation of sustainability and environment and positive hedonic associations, thus showing some divergences between these groups.

G2 had significant results in the description of WA for many different categories. While talking about consumption and positive health characteristics of nut beverages, the respondents often mentioned the negative sensory characteristics of the beverages; showing that people who have never tried the product did not have a convergent opinion about it and also did not have a more uniform opinion about it. This was confirmed since the category doubt/lack of knowledge was most cited by G2.

G1, composed of consumers who had had the experience of tasting one of these beverages, more often mentioned positive hedonic associations, demonstrating the acceptance of this product by this group; also citing sustainability and environmental factors as points that influence the purchase of this product. This same group demonstrated their opinion when they listed the nut beverage over the soy and milk beverages, regardless of which was nutritionally better.

Concerning the word cloud, which illustrates the results of the table with the most substantial categories cited in highlight, among the most cited by G2 was the category positive hedonic perception, despite being significantly smaller than G1. It is important to point out that nut beverages generated positive acceptance from these consumers who never had sensorial contact from experimentation. The G2 word cloud also corroborates what is reported in Table 3, with greater emphasis on more categories without as much uniformity of citations as in the G1 cloud. The respondents who had never tried a NB seemed not to have a general point of view about the product (Figure 2).

G1


Interest and curiosity
Negative hedonic associations
Negative characteristics
Healthy
Positive characteristics
Ingredients and foods
Others
Oral texture and perception
Plant based beverages
Flavor and taste
Veganism, vegetarianism, and food style
Food technology
Positive hedonic associations
Health
Lactose intolerance and cow's
Dairy products
Innovation
milk protein allergy
Color/Appearance
Consumption
Nutritional aspects
Doubt and lack of knowledge
Nuts, legumes and cereals
Sustainability and environment
High cost

G2

Figure 2. Word clouds from the most cited categories in the word association task of each consumer group.
Legend: G1= people who had tasted or were drinkers of nut beverages. G2= people who had never tasted a nut beverage.

Table 3. Percentage of respondents for each of the dimensions and categories identified in the word association test.

Dimension	Category	Examples	Total (n=300)	G1 (n=128)	G2 (n=172)
Consumption and sustainability			**39.33**	**42.18**	**37.20**
	Consumption	Consumption; breakfast; Christmas	13.33	10.15[b]	15.70[a]
	Sustainability and environment	Sustainable; natural; ecological	9.33	12.50[a]	6.98[b]
	Veganism, vegetarianism, and dietary style	Vegan; cruelty free; vegetarianism	16.66	19.53	14.53
Food and beverages			**32.33**	**31.25**	**33.14**
	Dairy products	Yogurt; milk; dairy beverage	12.00	14.06	10.46
	Ingredients and foods	Juice; liquor; sugar	20.33	17.18	22.67
Health and wellness			**49.33**	**52.34**	**47.10**
	Lactose intolerance and cow's milk protein allergy	Lactose intolerance; lactose-free; milk protein allergy Health; healthy product	11.33	11.72	11.04
	Healthy	Health, quality of life; neurological health	28.00	32.81	24.42
	Health		10.00	7.81[b]	11.62[a]
Innovation and food technology			**42.33**	**39.84**	**44.18**
	Innovation	Innovative; new; future; revolutionary	20.66	21.10	20.35
	Interest and curiosity	Interesting; I want to try; I am curious	15.33	10.94	18.60
	Food technology	Cold pressing; Tetra Pak use; manufacturing process	6.33	7.81	5.23
Negative characteristics			**46.00**	**43.75**	**47.67**
	High cost	Very expensive; high price; luxury product	24.33	25.00	23.84
	Negative hedonic associations	I don't like it; I wouldn't drink it; It is not good	9.33	8.60	9.88
	Negative characteristics	Strange; unnecessary; not available all year	12.33	10.15[b]	13.95[a]
Nutritional aspects			**36.33**	**32.81**	**38.95**

	Nutritional aspects	High calories; nutritious; vitamin; fiber; energy	29.33	26.56	31.40
	Greasy/oily	Fatty; oily; greasy	7.00	6.25[b]	7.55[a]
Nuts and other plant-based foods and beverages			**32.00**	**29.68**	**33.72**
	Plant-based beverages	Vegetable beverage; nut milk; vegetable milk	10.66	10.15	11.04
	Nuts, legumes, and cereals	Peanut; nut; oat; soy	14.66	14.84	14.53
	Alternative substitutes for animal milk	Alternative; milk substitute	6.66	4.70	8.14
Positive characteristics			**52.66**	**56.25**	**50.00**
	Positive characteristics	It's good for the body; strong; effective; medicinal	14.33	10.94[b]	16.86[a]
	Positive hedonic associations	Delicious, tasty, and great	38.33	45.31[a]	33.14[b]
Sensory aspects			**65.00**	**64.06**	**65.70**
	Color/appearance	Beige; milky; brown; cream	14.33	15.63[b]	13.37[a]
	Flavor and taste	Sweetened; watered down; sweet; flavorful	32.66	32.03	33.14
	Oral texture and perception	Creamy; greasy; light; consistent	18.00	16.40	19.20
Others			**36.00**	**34.37**	**37.20**
	Doubt and lack of knowledge	It is good; I never tried; I don’t know	22.66	19.53[b]	25.00[a]
	Others	No; nothing	13.33	14.84	12.20

Legend: G1= people who had tasted or were regularly drinking nut beverages; G2= people who had never tasted nut beverages. Superscript letters indicate significant differences between the groups for each category (Chi-square per cell test; $p<0.05$)

4. DISCUSSION

It is noteworthy that nut beverages, despite their increasing acceptance, are still very new and not widely consumed by the Brazilian public in general. This study revealed that most of the respondents had not tried NBs yet (Table 1) due to several factors, such as high price, rejection of new products, unavailability, and culture. However, a significant number of respondents (even omnivores) stated they would be willing to try this beverage, demonstrating that it is not limited to the vegan/vegetarian niche. Indeed, the vegetarian/vegan respondents reported having more contact with this type of beverage. In this regard, Cardello et al. [19] also observed the possibility of acceptance of plant-based beverages by both groups.

Neophobia can be considered an impediment accepting products such as PBBs. However, Brazilians generally have demonstrated a greater willingness to experiment and accept new plant-based products [20]. Although experimentation with the beverage was lower in our study, attributes such as "curiosity" and "innovation" were mentioned by this public, also reinforcing possible interest in this beverage (Figure 2). According to Mancini and Antonioli [21], institutional, technological, and cultural barriers still need to be overcome to include plant-based products in the Italian market.

Although some works have associated the difficulty of access to these beverages due to high price [3,22,23], our study indicated that the most diverse social classes present in Brazil have already had contact with this beverage (Table 1). However, according to Figure 2, high cost was mentioned mainly by those who had already consumed the beverage, as also indicated in Table 2, where price is identified as one of the most relevant factors for the purchase of these products, corroborating the results of other studies. Thus, we cannot deny that high prices can be a factor keeping these beverages from becoming more popular among consumers in general. Devising strategies that make these products cheaper and more accessible is necessary.

Another factor that can be considered influential in addition to price is the social and rational issues for the consumption of these beverages, whereby the lack of knowledge about the products and even the influence of the niche in which individuals are inserted can increase their chances of accepting PBBs in general [24]. Clegg et al. [3] noted that all

PBBs were more expensive than similar dairy products. In this same regard, Malek and Umberger [23] pointed out that more than half of the interviewed consumers had never bought plant-based products, mainly due to a lack of interest in new beverages in general, sensory characteristics, lack of familiarity, and price.

An interesting result of our study was that although the elderly were a small group in this study, most of them expressed willingness to try a NB. As in many other countries, the elderly are a large and increasing portion of the Brazilian population, so products aimed at them have good potential [8,25–27]. In that respect, Moss et al. [28] emphasized the need for better identification and characterization of the diverse segments of consumers in society so that in future studies, researchers can develop and improve PBBs to meet the needs and expectations of the various consumers and their peculiar preferences.

The NB made with almonds was the most consumed and tried, although considered both the most pleasant and least pleasant beverage in our study. This is because this nut has the greatest appeal and use for manufacturing of these beverages in several countries, including Brazil [3,11,29–31]. This result should be observed with more attention by the food industry since the use of regional nuts could gain greater prominence by making the product cheaper and promoting local agriculture. Reinforcing this information, Brazil nuts and cashews had the most mentions by respondents who had never tasted a NB. However, they said would be willing to try one, demonstrating that these are two nuts with strong potential developing of NBs in Brazil.

Taste was a fundamental attribute for purchase (Table 2) and acceptance of the beverage. Undoubtedly, this factor is highly relevant and is constantly attributed to the weaknesses of PBBs [24]. One of the possible explanations for flavor issues is that consumers often expect NBs to taste similar to milk, but this mimicry is not possible due to the unique characteristics of each nut compared to milk [19,30,32,33]. Another noteworthy point is that NBs differ sensorially from some PBBs, with a thicker texture, saltier flavor, and more umami notes [5].

Despite being an important factor, “taste/flavor” does not seem to be an impossible obstacle to overcome by the product. For example, in a study carried out in different regions of Europe, the individuals interviewed indicated they wanted smaller sensory

modifications for the alternative categories of PBBs, milk, and yogurt than for cheese and meat substitutes, where these modifications were greater and more significant[34]. In this sense, technologies can be applied to improve the taste and palatability of NBs [11,28,31,35]. Heat treatment, for example, can be used to improve the flavor profile of NBs. In this regard, Vaikma et al. [5] found that after treatment, hazelnut beverages had a higher content than almonds of pyrazines and benzaldehydes, important compounds for flavor nuances, thus improving their acceptance.

Another point suggested to improve this sensory aspect is the flavoring and aromatization of these NBs, as observed in studies by Acquah et al. [22] and Moss et al. [28], who reported that flavored PBBs were more accepted. In this sense, the respondents in the present study mentioned strawberry, banana, grape, açaí, cashew fruit, and apple as flavorings for NB blends, indicating good prospects for flavoring these beverages and consequently improving their public acceptance. Texture has also been mentioned in surveys as an unpleasant factor of NBs. This attribute is also a challenge to the marketing of PBBs [2,28].

Overwhelmingly, the respondents were likelier to buy NBs if they had proven health benefits. This was the opinion of both those who had already consumed them and those who had not. The concern and association between consumption and health are common in this sense, even if consumers do not know how to identify exactly which benefit is associated with this product. Our results also corroborate the findings of Acquah et al. [22], who observed that consumers were willing to purchase the concept beverage, citing innovation, taste, and health benefits as key drivers for purchasing a beverage made with tiger nuts.

Despite our initial expectation that the respondents in G1 would be more likely to have functional knowledge of the product than the members of G2, both mentioned the same associations of health benefits and nutritional quality (Figure 1 A), healthiness (Figure 1B) and sustainability (Figure 1C). The correct understanding by laypeople about these beverages is still somewhat debatable, and the absence of understandable didactic information can confuse the interpretation and knowledge of PBBs compared to milk [7]. Thus, the lack of specific regulations on plant-based products to inform consumers and strengthen the productive sector must also overcome [36].

The knowledge and perception of the composition of milk and its technological processes can be correlated and directly impact the consumers' opinions about its healthiness. When, consumers are unaware of simple processes such as pasteurization, filtration, and homogenization, this can lead them to believe that a product can be harmful to health [37]. Hence, these individuals often misunderstand their perception of the benefits of PBBs since they are unaware that these methods can also be applied to them.

Consumers are increasingly interested in functional products with greater added nutritional value and health benefits [22,38]. We also noted this in our study, as indicated in Table 2, which points to "health benefits" as one of the main factors for purchasing these products. As seen in Figure 2, this attribute was mentioned by both groups concerning NBs.

Regarding the nutritional aspect, the discussion about the superiority of PBBs to cow's milk is still controversial [2]. Silva and Smetana[13] reported the lack of micronutrient data for the comparison of PBBs with animal milk. Studies generally, studies point to PBBs as nutritionally inferior to milk [3,29,30]. Another question is related to the antinutritional factors found in these beverages, especially soy beverages[6] . Clegg et al. [3] reported that cow's milk has more energy, saturated fat, carbohydrates, protein, vitamins B2, B12, and iodine, and less fiber and free sugars than plant-based alternatives ($P < 0.05$). However, since nuts are excellent sources of fat, protein, and micronutrients like selenium [39–41], this statement is not valid for all PBBs, so that NBs can be a differentiator in this segment.

Although the respondents in G2 were willing to try NBs, partial replacement would be a greater possibility than total replacement in G1. The reason was associated with security/doubts regarding the beverages since respondents had never tried them, but it did not preclude the possibility of including NBs in the diets of the members of this group. These results can be explained because consumers who beverage milk often do so out of habit or because they like the taste, not just based on perceived health benefits or nutritional content [42].

Some further considerations on the nutritional issue are important since we observed that the general group would be willing to replace soy beverages and cow's milk with NBs. Replacing milk with PBBs is a good option because they are practical but not from a

nutritional point of view. Nevertheless, they are healthy products with good nutritional characteristics despite not meeting an individual's energy and nutritional needs [3,11,29]. Fortifying these beverages with minerals and vitamins, such as vitamin D, is a possible alternative to enhance competition with cow's milk[35,43] .

Sustainability also was a relevant factor in this study. Similar to the results found by Schiano et al. [44] in the comparison of PBBs with milk. The respondents in both groups stated that NBs are the most sustainable. This perception was greater in G1 (Table 3), indicating this is an important attribute for this group. This may be related to the lifestyle of people who already consume this beverage. McCarthy et al [42], in a study in New Zealand, suggested that for those drinking only non-dairy alternatives for sustainability reasons, milk from grass-fed cows can be attractive due to the lower carbon footprint, as long as the taste is appealing. However, replacing animal products with plant-based products is generally seen as a way to reduce the impact of eating habits on the environment[16]. Environmental impacts and animal welfare undoubtedly drive consumer demand for plant-based foods [4,45]

Measuring sustainability is not easy, so knowing whether consumers understand the points considered for this characterization is extremely important [42,44]. Moss et al.[28] reported the need to include this theme in sensory tests and examined what factors contribute to or characterize sustainability, similar to what we did in this study. How the public is informed about this topic directly influences people's perception of the product, and the type of packaging/labeling and organic status[44] . This demonstrates that didactic and clear labeling can improve the perception of the product [42].

The conclusion that NBs and PBBs are more sustainable than animal milk is still controversial. Most data on environmental impacts are related to soy and almond-based beverages, and the most common impact quantified has been greenhouse gas emissions[30]. In this sense, it is impossible to make a complete comparison between the beverages regarding environmental impact. Silva and Smetana[13] stated that overall, PBBs' environmental impact is lower than that of milk, with exceptions in some categories. However, the animal handling method (confined cattle produce less polluting gases) and the type of forage cultivation (with or without fertilizers) can variably impact sustainability and make this modality more or even less sustainable [46]. An argument also

in favor of the sustainability of NBs is that the byproducts of this category have good potential for other uses in the food industry, which can mitigate their environmental impact[47] . Another hypothesis raised is that harvesting of some nuts (Brazil nuts and cashew nuts, for example) is part of local sustainable extractivism[39,48] , leading to lower consumption of water and fertilizers (in comparison with soybeans) and less emission of greenhouse gases (in comparison with cattle), which is a positive point regarding the environmental impact of these products.

The greasy/oily category was more relevant for G2 (Table 3). This can be explained by the fact that they had never tasted NBs, and because nuts are oleaginous fruits, the expectation is that this beverage will be very fatty. Despite being rich in fat, tree nuts are rich in polyunsaturated fats and omega 3 (especially walnuts), making them beneficial for health [40,41,49]. Concerns about the allergenic potential and "lactose-free" were prevalent in both groups (Figure 2), although they were not important factors for purchasing the product (Table 2). Despite concerns about microbial contamination, NBs are safe for health [15,29], and "lactose-free" is a great advantage and attraction of these products, especially for vegans and lactose-intolerant individuals [10,50].

5. CONCLUSION

The consumption of beverages based on nuts and PBBs was not restricted to the vegan/vegetarian segment of this study. Therefore, these products had a strong potential for consumption by omnivorous consumers as a possible alternative to milk and soy-based beverages. Completely replacing milk with nut beverages and PBBs from a nutritional standpoint is still a hurdle, but fortifying these beverages can partially overcome this drawback hurdle by establishing nutritional equivalence. More studies emphasizing neophobia, product acceptability, and methods to improve the sensory acceptance of PBBs and NBs need to be performed and scaled up. It is also important to develop products aimed at age groups, such as the elderly. However, the most important factors are to reduce the cost of these beverages, publicize their health benefits, and make them more accessible, which is necessary and fundamental given their healthiness and adaptation in the market. It was also possible to observe the absence of exclusive and scarce studies concerning vegetable beverages by category (soy, oilseeds, cereals, and mainly nuts), to better understand the nutritional peculiarity of each product. Another

aspect is the lack of studies among Brazilian consumers. In this respect, in the sense of new research, more work is needed with larger samples of this population, as this was a challenge and a limitation encountered by this study. Despite the country's large population, economic potential, and global relevance, most studies were carried out in Europe, Asia, and North America and do not reflect the reality and characteristics of other, more diverse nations worldwide.

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CHAPTER 5 - ELDERLY PEOPLE'S EVALUATION AND UNDERSTANDING OF VEGETABLE BEVERAGES BASED ON BRAZIL NUTS (*Betholletia excelsa*)

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ABSTRACT

Aging is a natural process and, over time, can contribute to the development of special nutritional needs. In this sense, a growing demand for products that meet these specific needs, with sensory and microbiological quality. Plant-based beverages are an alternative for this public because of their practicality and sustainable appeal. The use of Brazil nuts for these beverages is relevant because of the value given to the country's agrobiodiversity and their nutritional quality, including their high selenium content. This study aimed to assess the understanding and acceptance of elderly people of a vegetable beverage made from Brazil nuts and fruit. This study was divided into two parts: (1) development and (2) sensory analysis of Brazil nut beverages and consumer perception of the purchase and its influencing factors for these beverages. In the first stage, four beverages were formulated with 5% Brazil nuts and 0.15% sucralose; three flavored with 10% different fruit pulp each (strawberry, cashew, and açaí) and pasteurized. Centesimal composition and microbiological analyses were carried out. For the sensory analysis, 100 elderly people took part in the overall acceptance and purchase intention tests. In the second stage of the study, an online questionnaire was administered nationally to 220 elderly people. This questionnaire consisted of a food neophobia scale, a choice test for eight pairs of nut beverage packages developed varying different label factors and questions about vegetable beverage consumption and socioeconomic data. For all the analyses, $p<0.05$ was considered. There were no differences in macronutrients, and all the samples were microbiologically safe. Overall acceptance showed that the cashew-flavored and

strawberry-flavored beverages were the most accepted. The online study showed that there were neophilic (1.8%), neutral (61.4%), and neophobic (36.8%) participants and that the presence of the selenium claim was one of the most important factors in the choice, followed by low price and the presence of the agrobioversity claim. Brazil nut vegetable beverages prove to be viable for the elderly, but further tests are still needed to improve the sweet taste and appearance of the product, as well as spread the word and raise awareness about the benefits and composition of these beverages more widely.

KEYWORD: plant-based, seniors, blend, neophobic, claim

GENERAL CONCLUSION

There was an attempt to address the thesis on the safety and quality aspects of Brazil nuts, from the raw material to the elaboration of a final product, a plant-based beverage intended for the vegan public.

The first challenge presented in this work was the study of the contamination and variability of the microbiome of Brazil nuts at different seasons. Good post-harvest practices were pointed out as an attention factor to be considered in processing for consumers' food safety. A relevant aspect observed was the predominant fluctuation of microorganisms between the first and second harvests and that global climate changes could affect this microbiological composition throughout the harvests in the environment of Brazil nut trees within the rainforest ecosystem.

Vegetable beverages, especially those made with "nuts," are gaining prominence in the production and consumption of the national and international markets. In this sense, it was possible to observe that beverages produced from Brazil nuts have great potential for inclusion in local consumption and also a good expectation for the adherence of different niches to veganism, such as for the elderly public and the omnivorous public. The elderly were an audience open to new food experiments and an interesting group for developing new nutritional products, given the growth and demand of this category. Despite the nutritional specifications, when often compared to cow's milk, vegetable beverages can be considered a healthy alternative for the composition of the diet, but not as a nutritional substitute for milk or an integral and complete food component. Another aspect observed was the addition of Brazil nuts to this beverage, as a differential from the point of view of selenium enrichment, a nutrient of great value for the healthy aging process, since several diseases, mainly neurological ones, may be associated with this deficiency. Flavoring nut beverages with fruits shown is an alternative for better accessibility and adherence to this beverage, as long as they are mixed with fruits that belong to everyday life and are of broad popular taste, as in the case of some mixtures used in this study. The appearance, texture, and price can still make it difficult for many people to access plant-based products. Neophobia can still be challenging for people to adhere to these beverages, so better disclosure of benefits, nutritional composition, health impacts, production, and processing should be expanded to the general population.

CONCLUSÃO GERAL

Um primeiro desafio apresentado neste trabalho foi o estudo sobre a contaminação da matéria prima oriundas de duas safras distintas a fim de verificar a variabilidade do microbioma presente na castanha-do-brasil ao longo das safras. As boas práticas no pós-colheita foram apontadas como um fator de atenção a ser considerado no processamento para a segurança alimentar dos consumidores, devido à contaminação por fungos e micotoxinas. Um aspecto relevante observado foi que houve uma flutuação de microrganismos predominantes entre a primeira e a segunda safra e que as mudanças climáticas globais podem impactar esta composição microbiológica ao longo das safras no ambiente dos castanhais dentro do ecossistema das florestas do tropico úmido.

As bebidas vegetais, em especial as feitas com "nuts", estão ganhando destaque na produção e consumo do mercado nacional e internacional. Neste sentido, foi possível observar que as bebidas desenvolvidas a partir de castanhas-do-brasil apresentam grande potencial de inserção no consumo local e uma boa expectativa para adesão de nichos diversos ao veganismo, como por exemplo, para o público idoso e o público onívoro. Os idosos demonstraram ser um público aberto a novas experimentações alimentares e um grupo interessante para o desenvolvimento de novos produtos nutricionais visto o crescimento e a demanda desta categoria. Apesar das limitações nutricionais, quando comparadas muitas das vezes ao leite de vaca, as bebidas vegetais podem ser consideradas uma alternativa saudável para composição da dieta, mas não como um substituto nutricional do leite ou um componente alimentar integral e completo. Outro aspecto observado, foi a adição da castanha-do-brasil nessa bebida, como um diferencial do ponto de vista de enriquecimento de selênio, um nutriente de grande valor para o processo de envelhecimento saudável, visto que diversas doenças, principalmente as neurológicas, podem estar associadas a deficiência deste. Saborizar bebidas à base de castanha com frutas mostrou ser uma alternativa para melhor aceitação e adesão desta bebida, desde que sejam misturadas com frutas que pertencem ao cotidiano e sejam de amplo gosto popular como no caso de alguns blends utilizados nesse estudo. O aspecto, a textura e o preço podem ainda dificultar o acesso de grande parte da população aos produtos *plant-based* como um todo. A neofobia ainda pode ser um desafio para adesão dessas bebidas,

por isso a melhor divulgação de benefícios, composição nutricional, impactos na saúde, produção e processamento devem ser ampliadas para a população em geral.

FUTURE DEVELOPMENTS

• Improve the sensory characterization of beverages through the development of new formulations to achieve texture, appearance, and strange sweetness;

• Carry out rheological tests to understand and improve the formation of phases presented on the beverages;

• Carry out campaigns, promote debates, and develop materials to value and encourage the use of Brazil nuts in plant-based products;

• Microbiological analysis of nuts ready for consumption in bulk sales;

• Develop new flavors with blends of other fruits and other plant-based products;

• Analyze the maximum limit of toxicity and safety of selenium in food products;

• Develop other Brazil nut products for the elderly.

• Analyze and improve the shelf life of beverages

DESENVOLVIMENTOS FUTUROS

- Melhorar a carcaterização sensorial das bebidas de castanhas através do desenvolvimento de novas formulações para alcançar textura, aparência e o dulçor desejável;
- Realizar testes reológicos para entender e melhorar a formação de fases apresentada pelas bebidas;
- Realizar campanhas, promover debates e desenvolver materiais para valorizar e incentivar o uso da castanha-do-brasil produtos plant-based;
- Analisar microbiologicamente castanhas prontas para o consumo em vendas à granel;
- Desenvolver novos sabores à base de castanhas com blends de outras frutas e demais produtos *plant-based*;
- Analisar o limite máximo para toxicidade e segurança de selênio em produtos alimentícios;
- Elaborar outros produtos de castanha-do-brasil para o público idoso;
- Analisar e aperfeiçoar a vida de prateleira das bebidas elaboradas a base de castanhas.