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Pâmela Gomes de Souza

SUCO BLEND DE BELDROEGA PRESSURIZADO: COMPOSTOS BIOATIVOS, ATIVIDADE ANTIOXIDANTE, BIOACESSIBILIDADE DE MINERAIS E ESTUDOS COM CONSUMIDORES

PRESSURIZED BELDROEGA BLEND JUICE: BIOACTIVE COMPOUNDS, ANTIOXIDANT ACTIVITY, MINERAL BIOACCESSIBILITY AND CONSUMER STUDIES

Rio de Janeiro
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Orientador: Dr. Anderson Junger Teodoro.
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(Eleanor Roosevelt)

RESUMO

O desenvolvimento de sucos blends com frutas e hortaliças não convencionais é uma alternativa para melhorar o potencial nutricional e bioativo dessas bebidas, assim como valorizar ingredientes subutilizados. No entanto, a presença de elevados teores de ácido oxálico em determinadas hortaliças utilizadas para a elaboração de sucos, pode reduzir a bioacessibilidade de minerais e contribuir para a formação de cristais de oxalato na urina. Nesse sentido, investigar pela primeira vez a eficiência do emprego de tecnologias emergentes, como a Alta Pressão Hidrostática (APH) e a Homogeneização à Alta Pressão (HAP) na redução desse antinutriente em sucos, traz informações inéditas à literatura. Esses métodos apresentam como vantagens a preservação dos nutrientes e bioativos, assim como das características sensoriais quando comparados à pasteurização. Além disso, essas tecnologias podem contribuir para uma produção sustentável dos produtos desenvolvidos, por apresentarem menores custos energéticos. Portanto, esse trabalho teve como principais objetivos desenvolver um suco misto contendo frutas e hortaliça não convencional, determinar as características físico-químicas e sensoriais, a atividade antioxidante, o teor de fenólicos totais e de ácido oxálico, assim como avaliar o impacto das tecnologias de alta pressão no conteúdo desses compostos. Além disso, a presente pesquisa objetivou testar métodos de extração e quantificação de ácido oxálico, através de adaptações aos protocolos existentes na literatura. Para atender tais objetivos, essa pesquisa foi dividida em quatro capítulos. No primeiro capítulo, foi elaborado um artigo científico que contemplou o levantamento bibliográfico sobre o potencial funcional e atividade anticâncer da beldroega (*Portula oleracea* L.), em base de dados, como Scopus, Periódicos Capes, Science Direct e Scielo, utilizando os descritores: *Portulaca oleracea*, nutrição, compostos bioativos, atividade antioxidante, desenvolvimento de produtos funcionais, e atividade anticâncer. Por meio das informações compiladas, foi visto que a beldroega (uma vegetal não convencional) apresenta teores de minerais como o potássio, cálcio, magnésio, fósforo e ferro, muito superiores aos encontrados no espinafre (um vegetalmente comumente presente no prato dos brasileiros), além de apresentar elevados teores de ácidos graxos ômega-3, evidenciando a importância de valorizar outras espécies de plantas na alimentação. Além disso, compostos oriundos do metabolismo secundário da beldroega foram associados à diversas propriedades farmacológicas, sendo algumas a atividade antimicrobiana, antidiabética, antimutagênica e anti-inflamatória. Um vasto estudo sobre a atividade anticâncer da beldroega, presente na literatura também foi apresentado no artigo científico, demonstrando os principais mecanismos moleculares envolvidos. No segundo capítulo foi elaborado um artigo científico sobre o desenvolvimento de um suco misto contendo suco de laranja (*Citrus sinensis)*, cupuaçu (*Theobroma grandiflorum)* e suco de beldroega (*Portulaca oleracea* L.), e foi avaliada as características físico-químicas, o potencial antioxidante e a análise sensorial envolvendo aceitação global e descrição das características sensoriais com o auxílio dos consumidores. Foram analisadas nove formulações diferentes (F1 a F9), variando as proporções dos ingredientes. A formulação F1 apresentou o melhor resultado para DPPH (82,9 ± 0,6 µmol TE/g). As médias de pontuação de aceitação global mostraram que não houve diferenças estatísticas entre F1, F5, F8 e F9. A formulação F8 foi selecionada como o protótipo promissor devido aos dados sensoriais, ORAC e fenólicos totais. As intensidades médias das características sensoriais, segundo o teste RATA, demonstraram que as amostras com as menores pontuações para a aceitação global estão associadas aos atributos "aparência consistente", "gosto ácido/azedo" e "consistência na boca". No terceiro capítulo, foi elaborado um artigo científico referente à investigação dos efeitos da APH (400 MPa/10 min), HAP (50 MPa e 100 MPa), assim como a comparação com pasteurização (90°C/1 min) e com os sucos controle (sem processamento), aplicando ou não uma etapa preliminar de filtração, no potencial bioativo, na redução de oxalato e na bioacessibilidade de cálcio e mangnésio. Os tratamentos de Alta Pressão (APH 400 MPa/10 min e HAP 100 MPa) mostraram o melhor potencial antioxidante. Todas as amostras apresentaram baixos teores de ácido oxálico (5,61 - 11,00 mg/100g em base fresca), podendo ser consideradas seguras para o consumo. As tecnologias de alta pressão provaram que são eficazes na redução do teor de ácido oxálico em sucos mistos. Esses foram os primeiros testes com essas tecnologias de processamento utilizadas na investigação da redução de oxalatos em sucos. A bioacessibilidade foi estimada usando o protocolo INFOGEST 2.0 recente e padronizado. Possíveis interações com proteínas reduziram a bioacessibilidade do cálcio nos tratamentos de pasteurização (40,5%) e HHP 400 MPa/10 min (47,9%). A bioacessibilidade do magnésio pode ter sido aumentada pelo efeito do tratamento HAP 100 MPa na redução do oxalato. As vantagens do uso de tecnologias emergentes no processamento de sucos devem ser vistas como motivos para incentivar o crescimento da aplicação desses métodos e contribuir para a produção sustentável desses produtos. No quarto capítulo foi elaborado um artigo científico referente à avaliação de três métodos de extração: M1(HCl 0.25N por 15 min à 100°C; sem precipitação), M2 (HCl 6M por 1 hora à 100°C; com precipitação) e M3 (HCl 0.25N por 15 minutos à 100°C; com precipitação) e dois de quantificação de oxalato (Espectrometria de Absorção Atômica com Chama - FAAS, e Espectrofotometria UV-vis), obtidos da literatura, e com adaptações testadas no trabalho para as amostras (beldroega-suco, beldroega-planta inteira, beldroega-folhas, espinafre-planta inteira). A ausência da etapa de precipitação no método M1 resultou em altos níveis de ácido oxálico nas matrizes investigadas, demonstrando que a ausência dessa etapa pode ter resultado na extração compostos interferentes. A quantificação de ácido oxálico por FAAS para M2 (6M HCl por 1 hora a 100°C) e M3 (0,25N HCl por 15 minutos a 100°C) nas amostras de folhas de beldroega e plantas inteiras de espinafre produziu resultados estatisticamente semelhantes. No entanto, a análise por espectrofotometria UV-vis para M2 e M3 mostrou discrepâncias significativas em todas as amostras avaliadas, sugerindo interferência de compostos coloridos na matriz alimentar.

Palavras-chave: Plantas Alimentícias Não-Convencionais; Frutos amazônicos; Antinutrientes; Oxalatos; Tecnologias emergentes; Digestão *in vitro*.

ABSTRACT

The development of blended juices with non-conventional fruits and vegetables is an alternative to enhance the nutritional and bioactive potential of these beverages, as well as to valorize underutilized ingredients. However, the presence of high levels of oxalic acid in certain vegetables used for juice preparation can reduce the bioaccessibility of minerals and contribute to the formation of oxalate crystals in urine. In this regard, investigating for the first time the efficiency of using emerging technologies such as High Pressure Processing (HPP) and High Pressure Homogenization (HPH) in reducing this antinutrient in juices provides novel information to the literature. These methods offer advantages such as preserving nutrients and bioactives, as well as sensory characteristics, compared to pasteurization. Furthermore, these technologies can contribute to the sustainable production of the developed products due to their lower energy costs. Therefore, the main objectives of this study were to develop a mixed juice containing unconventional fruit and vegetables, determine the physicochemical and sensory characteristics, antioxidant activity, total phenolic and oxalic acid content, and evaluate the impact of high-pressure technologies on the content of these compounds. In addition, the present research aimed to test methods of extraction and quantification of oxalic acid, through adaptations to existing protocols in the literature. To achieve these objectives, the research was divided into four chapters. The first chapter comprised a scientific paper that reviewed the functional potential and anticancer activity of purslane (*Portulaca oleracea* L.) using databases such as Scopus, Periódicos Capes, Science Direct, and Scielo, with descriptors: *Portulaca oleracea*, nutrition, bioactive compounds, antioxidant activity, functional product development, and anticancer activity. The compiled information showed that purslane (a non-conventional vegetable) contains mineral levels such as potassium, calcium, magnesium, phosphorus, and iron that are much higher than those found in spinach (a commonly present vegetable in Brazilian diets), in addition to high levels of omega-3 fatty acids, highlighting the importance of valuing other plant species in the diet. Moreover, compounds from the secondary metabolism of purslane were associated with various pharmacological properties, including antimicrobial, antidiabetic, antimutagenic, and anti-inflammatory activities. An extensive study on the anticancer activity of purslane present in the literature was also presented in the scientific paper, demonstrating the main molecular mechanisms involved. The second chapter presented a scientific paper on the development of a mixed juice containing orange juice (*Citrus sinensis*), cupuaçu (*Theobroma grandiflorum*), and purslane juice (*Portulaca oleracea* L.), evaluating the physicochemical characteristics, antioxidant potential, and sensory analysis involving overall acceptance and description of sensory characteristics with the help of consumers. Nine different formulations (F1 to F9) were analyzed, varying the proportions of the ingredients. Formulation F1 showed the best result for DPPH (82.9 ± 0.6 µmol TE/g). The average scores for overall acceptance showed no statistical differences between F1, F5, F8, and F9. Formulation F8 was selected as the promising prototype due to its sensory data, ORAC, and total phenolic content. The average intensities of sensory characteristics, according to the RATA test, demonstrated that samples with the lowest scores for overall acceptance were associated with attributes such as "consistent appearance," "sour/tangy taste," and "mouthfeel consistency." In the third chapter, a scientific paper was prepared regarding the investigation of the effects of High Pressure Processing (HPP) (400 MPa/10 min), High Pressure Homogenization (HPH) (50 MPa and 100 MPa), as well as a comparison with pasteurization (90°C/1 min) and control juices (no processing), with or without a preliminary filtration step, on the bioactive potential, oxalate reduction, and bioaccessibility of calcium and magnesium. The High Pressure treatments (HPP 400 MPa/10 min and HPH 100 MPa) showed the best antioxidant potential. All samples showed low levels of oxalic acid (5.61 - 11.00 mg/100g on a fresh weight basis), and can be considered safe for consumption. High-pressure technologies have proven effective in reducing oxalic acid content in mixed juices. These were the first tests with these processing technologies used in investigating oxalate reduction in juices. Bioaccessibility was estimated using the recent and standardized INFOGEST 2.0 protocol. Possible interactions with proteins reduced the bioaccessibility of calcium in pasteurization treatments (40.5%) and HPP 400 MPa/10 min (47.9%). Magnesium bioaccessibility may have been increased by the effect of the HPH 100 MPa treatment on oxalate reduction. The advantages of using emerging technologies in juice processing should be seen as reasons to encourage the growth of these methods and contribute to the sustainable production of these products. In the fourth chapter, a scientific paper was prepared regarding the evaluation of three extraction methods: M1 (0.25N HCl for 15 minutes at 100°C; without precipitation), M2 (6M HCl for 1 hour at 100°C; with precipitation), and M3 (0.25N HCl for 15 minutes at 100°C; with precipitation), as well as two oxalate quantification methods (Flame Atomic Absorption Spectrometry-FAAS, and UV-Vis Spectrophotometry), obtained from the literature and adapted for the samples (purslane-juice, purslane-whole plant, purslane-leaves, spinach-whole plant). The absence of the precipitation step in method M1 resulted in high oxalic acid levels in the investigated matrices, demonstrating that the lack of this step may have led to the extraction of interfering compounds. Quantification of oxalic acid by FAAS for M2 (6M HCl for 1 hour at 100°C) and M3 (0.25N HCl for 15 minutes at 100°C) in purslane-leaves and spinach-whole plant samples produced statistically similar results. However, UV-vis spectrophotometry analysis for M2 and M3 showed significant discrepancies in all evaluated samples, suggesting interference from colored compounds in the food matrix.

Keywords: Non-conventional Edible Plants; Amazonian Fruits; Antinutrients; Oxalate; Emerging Technologies; *In vitro* Digestion.

SUMÁRIO

O presente trabalho segue as normas da tese no formato de artigo definido pelo Programa de Pós-Graduação em Alimentos e Nutrição.

Dessa forma, a tese está dividida em 4 capítulos:

CAPÍTULO I - Potential Functional Food Products and Molecular Mechanisms of *Portulaca oleracea* L. on Anticancer Activity: A Review. **Artigo publicado na revista Oxidative Medicine and Cellular Longevity, em 20 de setembro de 2022**.

CAPÍTULO II - Mixed juice with purslane (*Portulaca oleracea* L.), a non-conventional edible plant: a strategy to provide consumers a healthy and sustainable product. **Artigo submetido na revista Journal of Food Science and Technology, em 18 de julho de 2024.**

CAPÍTULO III – Effects of Hig Pressure treatments on physicochemical characteristics, phenolic compounds, antioxidant potential and bioaccessibility of Calcium and Magnesium in mixed orange (*Citrus sinensis*), cupuaçu (*Theobroma grandiflorum*) and purslane (*Portulaca oleracea* L.) juice. **Artigo não publicado.**

CAPÍTULO IV - Evaluation of oxalic acid extraction and quantification methods in the different purslane (*Portulaca oleracea* L.) matrices and spinach (*Spinacea oleracea*). **Artigo aceito para publicação na revista MethodsX, em 12 de julho de 2024.**

Para fins de cumprimento de pré-requisito da disciplina Elaboração de Artigos, segue anexado à tese a seguinte publicação:

APÊNDICE - Food neophobia, risk perception and attitudes associations of Brazilian consumers towards non-conventional edible plants and research on sale promotional strategies. **Artigo publicado na revista Food Research International, em 28 de fevereiro de 2023.**

INTRODUÇÃO GERAL

O termo Plantas Alimentícias Não-Convencionais (PANC) refere-se a plantas ou partes de plantas que possuem uso restrito pela população e distribuição geográfica limitada devido a alimentação atual apresentar muita homogeneidade e monotonia, com consumo repetitivo de poucas espécies (KINUPP; LORENZI, 2014; TULER; PEIXOTO; SILVA, 2019).

O uso de PANC na alimentação proporciona versatilidade culinária, aumento na variedade e na qualidade da dieta alimentar, além de já ser comprovado cientificamente que algumas espécies apresentam propriedades funcionais e elevado teor de nutrientes, superiores às hortaliças convencionais (SCHMEDA-HIRSCHMANN et al., 2005; SANTOS et al., 2021).

A beldroega (*Portulaca oleracea* L.) está incluída no grupo das PANC (BRASIL, 2010). É nativa da África, mas atualmente encontra-se distribuída em várias regiões do Brasil, e suas folhas, talos, flores e sementes são consideradas comestíveis (KINUPP; LORENZI, 2014; SILVA; CARVALHO 2014).

Os compostos fenólicos estão relacionados com a maior parte dos efeitos benéficos atribuídos a saúde, e estão presentes na beldroega, sendo responsáveis pela atividade antioxidante que contribui para a prevenção de processos degenerativos (ZHOU et al., 2015). Além disso, a beldroega apresenta diversas propriedades farmacológicas, sendo algumas dessas, a atividade anti-inflamatória, antidiabética, antimutagênica e anticâncer (SOUZA et al., 2022).

As folhas da beldroega possuem elevados teores de minerais como o cálcio, magnésio, potássio, ferro e zinco (OLIVEIRA et al., 2013; VIANA et al., 2015). Para que o organismo humano possa exercer suas funções vitais é importante que os minerais que são ingeridos a partir dos alimentos estejam bioacessíveis (SILVA et al., 2020). Desta forma, investigar a bioacessibilidade de mineirais no suco blend de beldroega faz-se necessário.

O ácido oxálico é um antinutriente que está presente em grande quantidade (em torno de 234g-622,5 mg/100g) nas folhas da beldroega, e os teores podem variar de acordo com a composição do adubo e o estágio de maturação da planta (PALANISWAMY, 2004; POEYDOMENGE; SAVAGE, 2007). O ácido oxálico pode reduzir a disponibilidade de minerais para os seres humanos, podendo originar problemas de hipocalcemia e hipossideremia, ao se complexarem com o cálcio e ferro, respectivamente (PALANISWAMY et al., 2004). Assim, esse antinutriente deve estar presente em níveis seguros nas matrizes alimentícias que estejam prontas para o consumo.

A elaboração de blends com frutas é muitas vezes realizada para melhorar a qualidade nutricional, de bioativos e sensorial de bebidas (FARAONI et al., 2012). Aliada aos diversos benefícios à saúde que a beldroega possui, o uso dessa hortaliça para a produção de um suco misto proporciona uma opção nutritiva, sustentável e ainda pouco explorada comercialmente. O cupuaçu é um fruto nativo da Amazônia, com elevado teor de compostos fenólicos e potencial antioxidante (PUGLIESE, 2010). O uso de ingredientes para compor o suco misto de beldroega, como a polpa de cupuaçu, pode contribuir para a valorização da biodiversidade brasileira. Além disso, o suco de laranja é amplamente comercializado no Brasil, sendo popularmente conhecido pelo elevado teor de vitamina C, além da boa aceitação sensorial (MURAKAMI et al., 2020). Dessa forma, o suco de laranja pode contribuir com o sucesso comercial do produto.

Apesar das vantagens que a produção de sucos mistos contendo frutas e PANC pode fornecer, a aceitação sensorial dessas bebidas pelos consumidores e a compreensão das características sensoriais associadas com a aceitação e rejeição das formulações é essencial para o sucesso comercial desses produtos. Testes como a aceitação global e o Rate-All-That-Apply (RATA) são úteis essa finalidade (PERYAM.; PILGRIM, 1957; MEYNERS; JAEGER; ARES, 2016).

A pasteurização é o processamento térmico convencional utilizado para a conservação de sucos de frutas na indústria, garantindo a segurança e estendendo a vida útil do produto (KOUTCHMA et al., 2016). As tecnologias emergentes usadas para a conservação de alimentos compreendem os métodos térmicos ou não térmicos, e são capazes de preservar os nutrientes e parâmetros de qualidade (BUTZ; TAUSCHER, 2002; CULLEN; TIWARI; VALDRAMIDIS, 2012). O emprego de tecnologias que utilizam o tratamento não térmico permite inativar os microrganismos e algumas enzimas de interesse presentes nos alimentos, sem alterar as características sensoriais e nutricionais que comumente são afetadas pelos processamentos térmicos convencionais (BARBA; ESTEVE; FRÍGOLA, 2012). A Alta Pressão Hidrostática (APH) e a Homogeneização a Alta Pressão (HAP) são tecnologias não térmicas consideradas eficientes no processamento de sucos (AUGUSTO; TRIBSTI; CRISTIANINI, 2018).

Sucos mistos contendo frutas e hortaliças podem conter elevados teores de oxalatos. Diversos métodos têm demonstrado eficiência na redução de oxalatos em matrizes alimentícias, como a pasteurização, campo elétrico pulsado, ultrassonicação e tratamento enzimático (HUYNH et al, 2022). No entanto, os efeitos das tecnologias de alta pressão na redução de oxalatos em sucos ainda não foi relatado na literatura e essa será a primeira pesquisa a investigar os impactos de tais métodos nos teores desse antinutriente. Os principais métodos utilizados para quantificação de oxalato em matrizes alimentícias incluem titulação, espectrofotometria no UV-vis e Cromatografia Líquida de Alta Eficiência (CLAE) (SAVAGE et al., 2000; CHARRIER et al., 2002; ADENIYI; ORJIEKWE; EHIAGBONARE, 2009; NAIK et al., 2014). A quantificação de oxalato de cálcio por Espectrometria de Absorção Atômica foi descrita na AOAC (1990) para determinar indiretamente o teor desse antinutriente, por meio da concentração de cálcio encontrado na amostra. O emprego da Espectrometria de Absorção Atômica com Chama para análise de determinação de oxalato pode ter vantagens comparado ao método espectrofotométrico, quanto à seletividade e interferência de compostos da matriz na quantificação do analito de interesse. Dessa forma, faz-se necessário investigar e comparar as duas técnicas análises. Além disso, avaliar a eficiência de métodos de extração empregados na literatura, e o efeito da inclusão da etapa de precipitação no aumento da seletividade para a quantificação, pode trazer mais confiabilidade aos resultados.

Dessa forma, esse trabalho teve como objetivos: compilar os dados da literatura em formato de artigo de revisão bibliográfica sobre o potencial bioativo, funcional e anticâncer da beldroega; desenvolver formulações de suco misto contendo laranja, cupuaçu e beldroega em diferentes proporções desses ingredientes e avaliar o potencial antioxidante, teores de fenólicos totais e aspectos sensoriais associados a aceitação do produto pelos consumidores; avaliar os impactos das diferentes tecnologias de alta pressão (APH e HAP), assim como a pasteurização na preservação de nutrientes e bioativos, na redução de oxalato e na bioacessibilidade de cálcio e magnésio no suco misto desenvolvido; e por fim, investigar a eficiência de diferentes métodos de extração e quantificação de oxalato por espectrofotometria no UV-vis e Espectrometria de Absorção Atômica com Chama no espinafre e em matrizes contendo beldroega.

OBJETIVO GERAL

Esta pesquisa teve como objetivo desenvolver um suco misto contendo frutas e PANC, determinar as características físico-químicas, sensoriais, o potencial antioxidante e o teor de fenólicos totais, assim como investigar os efeitos das tecnologias Alta Pressão Hidrostática e Homogeneização a Alta Pressão no teor desses compostos, e pela primeira vez na literatura, no conteúdo de oxalato. Além disso, este trabalho teve como objetivo testar métodos de extração e de quantificação de oxalato por espectrofotometria no UV-vis e FAAS, por meio de modificações em protocolos descritos na literatura,

OBJETIVOS ESPECÍFICOS

- Realizar uma revisão bibliográfica sobre os benefícios nutricionais e farmacológicos da beldroega (*Portulaca Oleracea* L.);
- Desenvolver sucos mistos contendo laranja (*Citrus sinensis*), cupuaçu (*Theobroma grandiflorum*) e beldroega (*Portulaca Oleracea* L.), e identificar a melhor formulação, de acordo com as variações nas proporções dos ingredientes, por meio de análises de atividade antioxidante e sensoriais;
- Avaliar o impacto das tecnologias de alta pressão na redução do oxalato, na preservação de compostos bioativos e na bioacessibilidade de cálcio e magnésio.
- Comparar dois métodos de análise de oxalato (espectrofotometria no UV-vis e Espectrometria de Absorção Atômica com Chama) em matrizes da beldroega e espinafre, realizando adaptações aos protocolos descritos na literatura, assim, como avaliar diferentes métodos de extração e o uso da etapa de precipitação na melhora da seletividade para a quantificação do analito de interesse.

REVISÃO BIBLIOGRÁFICA

1. ALIMENTAÇÃO SUSTENTÁVEL E A RELAÇÃO COM PANC E FRUTAS

No atual contexto global, questões ambientais, sociais e econômicas estão cada vez mais interligadas com a produção e consumo de alimentos (PORTILHO; CASTAÑEDA; CASTRO, 2011). A evolução agrária, especialmente a partir da "Revolução Verde", provocou várias mudanças no panorama da agricultura, que passou a se tornar globalizada (MANIGLIA; NETO, 2020).

A Revolução Verde teve início no Brasil a partir da década de 1960 e constitui um modelo agrícola caracterizado pela combinação de insumos químicos (adubos e agrotóxicos), mecânicos (tratores) e biológicos (melhoramento genético). O objetivo era aumentar a produção de alimentos para atender à crescente demanda e melhorar a segurança alimentar no país. No entanto, há uma contradição entre os dados da fome e desnutrição com a elevada produção de alimentos, evidenciando que a prática de monocultura não é eficiente na garantia da segurança alimentar (NUNES et al., 2021). Além disso, a modernização do campo perpetuou o modelo latifundiário e monocultor, excluindo a agricultura familiar e dificultando o acesso dos pequenos produtores às novas tecnologias (FARIAS, 2015).

A intensificação dos sistemas de monocultura também tem gerado outros impactos negativos, como a degradação do solo e a redução da biodiversidade, que ameaçam a segurança alimentar (ECHER, 2020; FAO, 2018). Segundo Wilson et al (2012), cerca de 30 mil espécies no mundo possuem partes comestíveis e 7 mil foram cultivadas e colhidas ao longo dos anos. No entanto, apenas um número restrito de espécies vegetais entra na cadeia produtiva de alimentos, incluindo arroz, soja, milho, trigo, cana-de-açúcar, feijão, batata, mandioca, banana e amendoim (NUNES et al., 2021). O Brasil possui a maior biodiversidade do mundo e sua diminuição compromete a sustentabilidade ambiental, a disponibilidade de recursos naturais e, consequentemente, a vida no planeta. Em contrapartida, a conservação e utilização sustentável da biodiversidade brasileira trazem benefícios imensos para a humanidade (KINUPP; LORENZI, 2014; MAPA, 2010).

A busca por soluções alternativas que promovam a alimentação sustentável é crucial. Nesse cenário, as Plantas Alimentícias Não Convencionais (PANC) se destacam como opções que trazem diversas vantagens, como o fortalecimento da agricultura familiar e a redução dos impactos ambientais. As PANC são espécies de plantas ou partes comestíveis de plantas (frutos, frutas, folhas, flores, rizomas, sementes, casca, polpa) que não são convencionalmente consumidas, porém podem apresentar elevado potencial nutricional (KINUPP; LORENZI, 2014). Além disso, os sistemas de policultura em que as PANC são cultivadas minimizam as perdas decorrentes de insetos e doenças, assim como utilizam de maneira mais eficiente os recursos disponíveis, como água, luz e nutrientes (REZENDE, 2020). Algumas frutas nativas do Brasil também podem ser consideradas como PANC, dependendo do local em que estão sendo consumidas. Isso porque esse termo é considerado relativo, ou seja, algumas espécies podem ser classificadas como PANC em uma região, e em outras não, dependendo se o consumo não for convencional pela população local (KINUPP; LORENZI, 2014).

O Brasil é amplamente reconhecido como um dos países com a maior diversidade de espécies de frutas, as quais possuem um potencial ainda não explorado. As condições climáticas favoráveis do país e expansão territorial contribuem para a biodiversidade dos seis principais biomas, como a Amazônia, Mata Atlântica, Cerrado, Caatinga, Pantanal e Pampas (FARIAS et al., 2023; PEREIRA et al., 2013). Algumas espécies de frutas nativas brasileiras são consideradas fontes de vitaminas, fibras, minerais e compostos bioativos que apresentam elevada atividade antioxidante, anti-inflamatória e outras funções que oferecem diversos benefícios para a saúde dos consumidores. Esses efeitos benéficos podem ser aproveitados na produção de uma variedade de produtos, como néctares, sucos, geleias e polpas (FARIAS et al., 2023).

Do ponto de vista econômico, a valorização e a comercialização de PANC e frutas nativas têm o potencial de abrir novas oportunidades para a inovação e desenvolvimento de produtos alimentícios sustentáveis. Isso pode diminuir a dependência de produtos agrícolas não sustentáveis e promover práticas que preservam a biodiversidade. Além disso, esses produtos podem contribuir com a segurança alimentar da população. Portanto, incentivar o consumo de PANC e frutas nativas é crucial para enfrentar os desafios ambientais, sociais e econômicos.

1.1 Beldroega (*Portulaca oleracea* L.)

A beldroega (*Portulaca oleracea* L.), pertencente à família Portulacaceae, é uma planta nativa dos países do Oriente Médio, com ampla dispersão global (ZHOU et al., 2015). Essa espécie apresenta diversos cultivares com diferenças morfológicas, e adaptados aos diferentes climas e regiões (UDDIN et al., 2014; DANIN et al., 2016). A *Portulaca oleracea* silvestre (não cultivada) possui talos com colorações mistas (verde e vermelho), folhas ovaladas e carnosas, e flores amarelas de comprimento menor ao das folhas (ALAM et al., 2014). Pode ser encontrada nas ruas e calçadas, e em terrenos arenosos (RAHIMI et al, 2019). No Brasil, a beldroega (Figura 1) é comercializada em algumas feiras agroecológicas, e é categorizada como uma PANC.

A *Portulaca oleracea* contém quantidades elevadas de minerais como o potássio, cálcio, magnésio, fósforo e ferro (OLIVEIRA et al., 2013; NEPA/UNICAMP, 2011). No entanto, devido aos elevados teores de oxalato de cálcio encontrados na beldroega, o consumo desta planta por indivíduos com propensão ao desenvolvimento de cálculos renais deve ser moderado, e métodos de redução desses compostos devem ser utilizados (SAVAGE et al., 2000; PALANISWAMY et al., 2004; HUYNH et al., 2022).

Essa hortaliça possui altos teores de compostos fenólicos e elevadas quantidades de ácidos graxos ômega-3 (SIRIAMORNPUN; SUTTAJIT, 2010; SOUZA et al., 2022).

Figura 1. Beldroega obtida de uma feira agroecológica do Rio de Janeiro, Brasil.
Fonte: O próprio autor.

Os flavonoides são uma classe de compostos polifenólicos encontrados na beldroega. Essa espécie contém sete tipos distintos de flavonoides: kaempferol, myricetina, luteolina, apigenina, quercetina, genisteína e genistina. Essas substâncias estão associadas com as propriedades antioxidantes, anti-inflamatórias, antibacterianas, antiviral e anticâncer dessa planta (XU; YU; CHEN, 2006; LIM; QUAH, 2007; GALLO; CONTE; NAVIGLIO, 2017).

Diversas propriedades farmacológicas foram amplamente estudadas e relatadas na literatura para a beldroega, assim como a atuação contra diversos tipos de câncer (SOUZA et al., 2022).

1.2 Cupuaçu (*Theobroma grandiflorum*)

O cupuaçuzeiro (*Theobroma grandiflorum* (Willdenow ex. Spreng.) k. Schum.) é uma árvore nativa da região amazônica, e pertencente à família Sterculiaceae (NIU et al., 2019). Essa espécie pertence ao mesmo gênero do cacau (*Theobroma cacao*), e pode ser encontrada nos estados da Bahia, Pará, Acre, Maranhão, Tocantins e Mato Grosso. No entanto, o cupuaçuzeiro também é cultivado em outros países, como Venezuela, Colômbia, Equador, Peru, México, Costa Rica, Panamá e Suriname (MARTIM, 2013; PUGLIESE et al., 2013; COSTA et al., 2022).

O cupuaçu (Figura 2), fruto do cupuaçuzeiro, possui importante contribuição econômica para agricultura familiar da região amazônica, com destaque para a polpa, muito utilizada na produção de sucos, iogurtes, bolos, sorvetes, geleias, entre outros produtos (SILVA; PIERRE, 2021). O cupuaçuzeiro cresce em sinergia com outras espécies nativas da floresta tropical, o que contribui para um cultivo agroecológico e sustentável (PUGLIESE et al., 2013).

Figura 2. Foto do cupuaçu.

Fonte: Embrapa, 2014.

A polpa de cupuaçu possui uma coloração branca-amarelada e características sensoriais desejáveis, devido à presença de compostos voláteis que contribuem para o aroma do fruto e um sabor forte, muito apreciado pelas comunidades locais e no comércio internacional (BOULANGER; CROUZET, 2000; PEREIRA; RODRIGUES, 2018; DA SILVA et al., 2024).

A incorporação da polpa de cupuaçu em diversos produtos alimentícios pode contribuir para melhorar o potencial nutricional e bioativo das formulações elaboradas, por apresentar elevados teores de fibras e compostos associados às propriedades antioxidantes, como os flavonoides (YANG et al., 2006; CLÍMACO et al., 2019; VRIESMANN; PETKOWICZ, 2009).

A presença de alguns flavonoides como a (+)-catequina, (−)-epicatequina, isoscutelareína 8-O-β-D-glucuronídeo, hipolaetina 8-O-β-glucuronídeo, quercetina 3-O-β-D-glucuronídeo, quercetina 3-O-β-D-glucuronídeo 6″-metil éster, quercetina, kaempferol e isoscutelareína 8-O-β-D-glucuronídeo 6″-metil éster e teograndinas I e II foi relatada para o cupuaçu (PUGLIESE et al., 2013). Os bioativos fitoquímicos presentes no cupuaçu contribuem para a atividade antioxidante do fruto e no desenvolvimento de produtos alimentícios funcionais por meio do uso dessa espécie como ingrediente em diferentes formulações (FREITAS et al., 2017).

1. 3 Laranja (*Citrus sinensis*)

A família Rutaceae é a mais importante no gênero *Citrus,* do ponto de vista econômico, com destaque para o grupo das laranjeiras doces (*Citrus sinensis* (L.) Osbeck), cultivado mais expressivamente nos pomares dos países citrícolas (BASTOS et al., 2014). A laranjeira "Pera" (Figura 3) é a cultivar mais importante do Brasil, caracterizando-se como uma árvore de porte médio (atingindo de 5 a 10 metros de altura), copa de formato esférico, flores brancas, com laranjas que possuem elevado teor de suco e baixa acidez (LORENZI et al., 2006; BASTOS et al., 2014).

Figura 3. Foto da laranjeira, cultivar "Pera".

Fonte: Embrapa, 2021.

A laranja é originária do sul asiático, mais precisamente da China, tendo sido introduzida no Brasil logo no início da colonização (BRASIL, 2015). Apesar de não ser uma fruta originária do Brasil, pode agregar valor nutricional e sabor em formulações contendo frutas nativas e PANC. No Brasil, o estado de São Paulo é o maior produtor de laranja, destinada principalmente à exportação de suco concentrado e congelado (IBGE, 2022; SANTANA, 2022). O suco de laranja integral 100%, pasteurizado, passou a ter mais de destaque e consumo em meados do ano 2000, sendo considerado de qualidade superior em termos sensoriais, quando comparado ao suco concentrado (processo em que a água é extraída dentro dos evaporadores, e posteriormente o suco é reconstituído pelos engarrafadores). No entanto, como o volume ocupado pelo suco integral é alto, o custo para o armazenamento resfriado é elevado (CITRUS BR, 2024).

O suco de laranja contém elevados teores de vitamina C, um nutriente essencial para a manutenção da integridade epitelial, e a sua deficiência pode evoluir para o desenvolvimento da doença escorbuto (MILES; CALDER, 2021). A vitamina C, assim como os compostos fenólicos presentes no suco de laranja, estão associados com o elevado potencial antioxidante dessa bebida (STINCO et al., 2015).

2. SUCOS MISTOS DE FRUTAS E HORTALIÇAS NÃO-CONVENCIONAIS

As preferências dos consumidores em relação aos produtos alimentícios estão sempre evoluindo, e há um crescimento contínuo na busca por alimentos naturais e saudáveis, com alto valor nutricional (LAN et al., 2023). Frutas e hortaliças desempenham um papel crucial na alimentação, fornecendo elevadas quantidades de nutrientes, como vitaminas, açúcares, minerais e fibras, assim como de fitoquímicos bioativos associados ao potencial antioxidante e à prevenção de doenças crônicas não transmissíveis (BHARDWAJ; PANDEY, 2011; WALLACE et al., 2020; THEBA et al., 2024). O consumo de sucos mistos de frutas e hortaliças é uma alternativa para aumentar a ingestão de nutrientes e compostos bioativos presentes nestes alimentos (LAN ET AL., 2023).

O suco misto é definido pela legislação brasileira como a mistura de frutas, a combinação de frutas e vegetais, a mistura de seus sucos ou partes comestíveis (BRASIL, 2009). Apesar da vasta biodiversidade brasileira, a maioria das espécies vegetais com potencial alimentício são negligenciadas. No Brasil, as plantas ou partes de plantas (cascas, talos, sementes e flores) que são subutilizadas na alimentação são denominadas de Plantas Alimentícias Não-Convencionais (PANC) (KINUPP; LORENZI, 2014). O desenvolvimento de sucos mistos utilizando frutas e hortaliças não convencionais pode resultar em um melhor aproveitamento das espécies vegetais, além de contribuir para a valorização da biodiversidade (BHARDWAJ; PANDEY, 2011).

A redução do impacto ambiental também pode ser alcançada através do cultivo de PANC, por não necessitar da utilização de agrotóxicos, além do apoio aos pequenos produtores e da contribuição com o desenvolvimento local que podem ser atingidos por meio da produção e comercialização sustentável (FINK et al., 2018). Além disso, a incorporação de frutas e hortaliças não convencionais em um suco de uma única espécie, rico em nutrientes, porém rejeitados sensorialmente devido às características como a alta acidez e sabor ruim, podem melhorar a aceitabilidade dessas bebidas (BHARDWAJ; PANDEY, 2011).

Apesar do potencial nutricional e bioativo de sucos mistos de frutas e hortaliças, essas bebidas podem conter elevados teores de ácido oxálico (VANHANEN; SAVAGE, 2015). O conteúdo de oxalato endógeno também é proveniente do metabolismo do ácido ascórbico em humanos (NGUYEN; SAVAGE, 2020). A ingestão contínua de sucos de frutas e hortaliças com altos teores de ácido ascórbico pode aumentar a excreção de oxalato na urina (VANHANEN; SAVAGE, 2015). O emprego de processos apropriados para o tratamento de sucos é crucial para um melhor aproveitamento do potencial nutricional e bioativo dessas bebidas, assim como para a redução de riscos associados à ingestão de antinutrientes.

3. TECNOLOGIAS DE ALTA PRESSÃO NO PROCESSAMENTO DE SUCOS

Na indústria de sucos, enquanto a pasteurização é eficiente na conservação dos produtos desenvolvidos, as tecnologias de alta pressão não apenas reduzem os microrganismos, mas também preservam melhor os nutrientes e as características sensoriais das bebidas (ROOBAB et al., 2021). Além da conservação de alimentos, o tratamento por alta pressão pode resultar na obtenção de novas texturas e modificações nas características reológicas dos produtos alimentícios (AUGUSTO et al., 2012).

Os primeiros alimentos processados por alta pressão foram introduzidos no mercado japonês em 1990, e apesar da falta de equipamentos adequados nas primeiras aplicações, ao longo das décadas essa tecnologia passou a ser reconhecida mundialmente por seu potencial no processamento de uma ampla variedade de produtos alimentícios (THAKUR; NELSON, 1998; RASTOGI et al., 2007). Os processamentos por alta pressão hidrostática (APH) e a homogeneização à alta pressão (HAP) são tecnologias modernas e não convencionais que foram inicialmente exploradas como métodos de conservação de alimentos. Embora possuam nomenclaturas similares, essas abordagens diferem significativamente em termos de princípios e aplicação (AUGUSTO et al., 2018).

3.1 Alta Pressão Hidrostática (APH)

O processamento por APH baseia-se em dois princípios científicos. O princípio de *Le Chatelier* é aquele em que qualquer fenômeno, transição de fase e reação química, juntamente com redução de volume, é favorecido pelo aumento da pressão e vice-versa. Já o princípio de Pascal ou Isostático é aquele em que a pressão é transmitida de forma instantânea e uniforme por todo o alimento, independente da forma, tamanho e composição, através do contato direto com o meio sob pressão (BALASUBRAMANIAM; MARTINEZ-MONTEAGUDO; GUPTA, 2015; NASCIMENTO et al., 2013). A condição adiabática da APH faz com que independentemente do tamanho e do formato do alimento, a temperatura sofra pouca variação com o aumento da pressão, sendo o aumento de temperatura de 3°C a cada aumento de 100MPa, evitando assim, a deformação ou aquecimento do alimento (CHAWLA et al., 2011). O equipamento de APH pode ser observado na Figura 1.

Figura 1. Equipamento de Alta Pressão Hidrostática.

Fonte: Lima, 2020.

A técnica APH altera estruturas de moléculas de alto peso molecular como as proteínas e carboidratos, enquanto moléculas menores como pigmentos, vitaminas, compostos voláteis, e compostos associados a características sensoriais e nutricionais são preservados, havendo modificações químicas mínimas já que esta tecnologia não afeta as ligações covalentes (MORAIS, FERREIRA; ROSENTHAL, 2014). A aplicação de pressões pelo equipamento de APH, em que a água geralmente é usada como meio transmissor da pressão, varia de 100 a 800 MPa sobre alimentos sólidos ou líquidos, embalados ou não, podendo durar segundos ou minutos, além de poder ultrapassar 1000 MPa (FDA, 2011; ROMANO; ROSENTHAL; DELIZA, 2015; WANG et al., 2016). Para o processamento de sucos, geralmente são empregadas pressões entre 400 e 600Mpa por menos de 10 min (FARKAS, HOOVER, 2000).

Um estudo conduzido por Yildiz et al., (2020) demonstrou que o tratamento por APH (300 Mpa/1 min) preservou o conteúdo de compostos fenólicos totais em um suco de morango (143,53 ± 2,80 mg GAE/ 100 ml), com teor significativamente similar ao suco controle (137,81 ± 0,91 mg GAE/100 mL).

Varela - Santos et al., (2012) avaliaram o impacto da APH nos compostos bioativos de um suco de tomate. O conteúdo fenólico aumentou significativamente ($p<0,05$) entre 3,38% e 11,99% para amostras tratadas com 350 MPa (30, 90 e 150 s) 450 Mpa (30, 90 e 150 s) 550 MPa (30 e 90 s). De acordo com os autores, o aumento no teor total de fenólicos pode ser atribuído a uma maior extração de alguns componentes antioxidantes após o processamento sob alta pressão. Segundo a teoria de *Le Chatelier*, durante a aplicação de pressão, o volume do sistema tende a diminuir. Neste processo, o solvente de extração penetra nas células para se ligar aos componentes bioativos. Além disso, as células submetidas à pressurização apresentam maior permeabilidade (VARELA-SANTOS et al., 2012).

O aumento de compostos fenólicos paralelo ao da atividade antioxidante (pelo método FRAP) em um suco de jabuticaba submetido à APH (200, 350 e 500MPa por 5, 7,5 e 10 min) foi observado por Inada et al., (2017). Os autores também identificaram uma correlação positiva entre o conteúdo de compostos fenólicos e a atividade antioxidante.

Com relação aos teores de vitamina C, Salar et al., (2021) observaram um decréscimo no conteúdo desse composto em bebidas de maqui, submetidas a pressões de 450 e 600 MPa por 180 s, quando comparado à amostra controle. Resultados diferentes foram relatados por Patras et al., (2009), tendo sido observado que o conteúdo de vitamina C foi preservado em amostras de morango submetidas a pressões de 600 MPa/15 min. No entanto, em pressões de 400 e 500 Mpa/15 min, o conteúdo de vitamina C foi reduzido.

Além dos benefícios associados à preservação das características sensoriais e nutricionais, a APH é uma das principais tecnologias emergentes que pode substituir métodos térmicos convencionais de conservação de forma ecologicamente sustentável, tendo em vista que o emprego da tecnologia permite redução do custo de energia e dos custos de operação (MORAIS, FERREIRA; ROSENTHAL, 2014).

3.2 Homogeneização à Alta Pressão (HAP)

O processo de HAP ou alta pressão dinâmica (por se tratar de um processo contínuo) consiste em pressurizar um fluido para que este passe rapidamente por uma válvula estreita, com elevada velocidade, gerando cavitação e altas tensões de cisalhamento no produto durante o processo (AUGUSTO et al., 2018). Esse fenômeno resulta em um aumento instantâneo de temperatura cuja magnitude é determinada pela intensidade da pressão aplicada (CALLIGARIS et al., 2012). As pressões podem variar de 20 à 200 MPa (HAP) e de 300 à 450 MPa para Homogeneização de Ultra Alta Pressão (HUAP), com a tecnologia aplicada apenas para produtos líquidos ou pastosos (CALLIGARIS et al., 2012; ZAMORA; GUAMIS, 2015; SENEVICH; MATHIS, 2018). O equipamento de HAP pode ser observado na Figura 2.

Figura 2. Equipamento de Homogeneização à Alta Pressão.

Fonte: Lima, 2020.

O principal efeito do HAP é a redução do tamanho das partículas dispersas, afetando assim a estabilidade física, além de inativar microrganismos, preservar compostos bioativos e manter as características sensoriais dos produtos (LIMA; ROSENTHAL, 2022). A inativação dos microrganismos ocorre por meio da desestruturação mecânica das células, causada por gradientes de pressão e velocidade espaciais, turbulência e cavitação gerados (CALLIGARIS et al., 2012). O HAP é frequentemente combinado com outras tecnologias emergentes, como ultrassom, radiação ultravioleta e campos elétricos pulsados (LIMA; ROSENTHAL, 2022).

Nos sucos de frutas ocorrem interações complexas entre a fase insolúvel, composta pela polpa contendo células de tecido de frutas, seus fragmentos, e estruturas poliméricas insolúveis, e a fase viscosa, que consiste em uma solução aquosa de polissacarídeos solúveis, açúcares, sais e ácidos intracelulares. Assim, o processamento por HAP faz com que os tecidos e células da polpa sejam desestruturados, liberando o material intracelular para a fase viscosa, e, dessa forma, alterando as propriedades de ambas as fases, inclusive as características reológicas (AUGUSTO et al., 2018).

Segundo um estudo realizado por Marszalek et al., (2023), os compostos fenólicos totais apresentaram degradação de 15% em um suco de maçã submetido a Homogeneização a Ultra Alta Pressão (300 Mpa/1 min). Em contrapartida, Benjamin e Gamrasni (2020) realizaram um estudo comparativo entre sucos de romã submetidos a tratamento por HAP (100 e 150 MPa) e o processamento resultou em níveis mais elevados de atividade antioxidante e teor de compostos fenólicos totais em comparação com o suco fresco. Além disso, não foi evidenciado degradação significativa de vitamina C em nenhuma das condições testadas. Segundo os autores, os resultados sugeriram que as temperaturas relativamente baixas de aquecimento e as pressões de homogeneização utilizadas (inferiores a 200 MPa) não foram suficientes para afetar o teor de vitamina C.

De forma semelhante, Sentandreu et al., (2020) não observaram degradação para o conteúdo de vitamina C em amostras de suco de tangerina submetidas à HAP 150 MPa. No entanto, a perda de vitamina C foi observada em pressões de 75, 100 e 125 MPa (com 3 ciclos) para amostras de néctar de rosa mosqueta, em análises conduzidas por Saricaoglu et al., (2018). Diferenças nas condições de processamento, como o número de ciclos, pode resultar na redução desses compostos nas amostras pressurizadas.

As múltiplas passagens (quatro ciclos) de um suco de groselha preta pelo equipamento de HAP (50 e 150 Mpa) contribuíram para o aumento da atividade antioxidante (KRUSZEWSKI; ZAWADA; KARPINSKI (2021). Um aumento dos compostos relacionados à atividade antioxidante de um suco de morango submetido a pressões de 100 MPa (dois a cinco passagens) também foi observado por Karakam; Sahin; Oztop, 2015).

Portanto, a aplicação HAP em sucos resulta em melhorias significativas nas propriedades funcionais, na estabilidade dos componentes bioativos e comportamento reológico, assim como na redução do tamanho das partículas (WELLALA et al., 2020).

4. DESENVOLVIMENTO DE ALIMENTOS FUNCIONAIS

A crescente preocupação com a saúde e a qualidade de vida tem impulsionado pesquisas na área de alimentos, destacando a dieta como uma alternativa para uma vida mais longa e saudável e fonte de novos conhecimentos. (VIZZOTO; KROLOW; TEIXEIRA, 2010). Nesse contexto, as doenças crônicas não transmissíveis são vistas como um dos maiores desafios de saúde pública global, e a ingestão de alimentos funcionais tem mostrado potencial para melhorar a expectativa de vida da população (SILVA et al., 2016; ROCHA et al., 2021).

A expressão "alimentos funcionais" surgiu no Japão na década de 1980 e é também conhecida como alimentos para uso específico de saúde (FOSHU, do inglês *Foods for Specified Health Use*). Esse conceito foi resultado de um programa financiado pelas autoridades japonesas com o propósito de diminuir os gastos com saúde pública e controlar o avanço das doenças crônicas (SILVA et al., 2021). Entre 1995 e 1998, mais de 100 especialistas da área de alimentos chegaram a um consenso sobre a definição de alimentos funcionais. No entanto, definições alternativas variaram entre institutos de alimentos e nutrição (BAKER et al., 2022). No Brasil, a Portaria n° 398 de 1.999, da Secretaria de Vigilância Sanitária, define os alimentos funcionais como todo alimento ou ingrediente que, além de oferecer funções nutricionais básicas, também provoca efeitos metabólicos ou fisiológicos, além de trazer benefícios à saúde, desde que seja seguro para o consumo sem a necessidade de supervisão médica.

Diversos estudos mostram que a aceitação dos consumidores de alimentos funcionais não é incondicional, sendo o sabor um dos principais fatores determinantes, além da qualidade do produto, preço, conveniência e a credibilidade das alegações de saúde. Além disso, os consumidores tendem a avaliar esses alimentos principalmente como comuns, considerando que, embora os benefícios funcionais possam agregar valor, não superam as propriedades sensoriais (SIRÓ et al., 2008).

Os alimentos funcionais possuem matrizes complexas, com polifenóis, carotenoides, minerais e vitaminas, porém nem todo conteúdo dessas substâncias estará disponível para absorção no trato gastrointestinal. Assim, a análise de bioacessibilidade possibilita determinar a quantidade desses compostos que são libertados da matriz e estarão disponíveis para serem absorvidos (NICOLESCU et al., 2023).

4.1 Importância da análise sensorial

A análise sensorial é definida como a ciência utilizada para evocar, medir, analisar e interpretar reações das características dos alimentos, percebidas pelos sentidos visão, olfato, sabor, tato e audição (AMERINE; PANGBORN; ROESSLER, 1965). Os métodos afetivos usados na análise sensorial mensuram o quanto a população gostou de um produto com o objetivo de avaliar a preferência ou aceitabilidade (DUTCOSKY, 2019). Para que um produto alimentício tenha sucesso no mercado, os consumidores precisam gostar da aparência, aroma, sabor e textura, sendo o resultado da aceitação global, ponto crucial para o atendimento desse objetivo (SIRANGELO, 2019). A escala hedônica é amplamente utilizada em estudos de aceitabilidade de produtos alimentícios (DUTCOSKY, 2019).

Além dos métodos afetivos, existem os métodos descritivos que auxiliam na caracterização quali e/ou quantitativa dos produtos (MURRAY et al., 2001). Alguns métodos descritivos, como a Análise Descritiva Quantitativa (ADQ), requerem avaliadores treinados para a realização dos testes, tornando-se dispendiosos para a obtenção dos resultados (MURRAY et al., 2001; ARES, 2015). O uso de testes descritivos utilizando consumidores, como o Rate-All-That-Apply (RATA), podem ser mais rápidos e fáceis de serem executados (ARES et al., 2014; CADENA et al., 2014).

Portanto, a aplicação dos testes de aceitação global e RATA podem auxiliar no alcance da melhor formulação de suco misto a ser comercializada, assim como na compreensão das características sensoriais que contribuíram com os resultados de aceitação e rejeição.

4.1.1 Escala hedônica

A escala hedônica é utilizada na avaliação do grau de “gostar” ou “desgostar”, assim como na verificação da “indiferença” atribuída pelos avaliadores (consumidores) em relação aos produtos testados (PERYAM; PILGRIM, 1957). A graduação de 9 pontos é a escala mais utilizada em pesquisas de aceitabilidade para adultos, enquanto escalas hedônicas de 7 ou 5 pontos são mais empregadas em estudos com crianças, para facilitar a compreensão (KROLL, 1990; DUTCOSKY, 2019).

4.1.2 Rate-All-That-Apply (RATA)

O teste Check-All-That-Apply (CATA) é um questionário de múltipla escolha com uma lista de termos (atributos da amostra) apresentado aos consumidores para que possam marcar todas as opções que considerarem aplicáveis na descrição do produto (Ares, 2015). Esse método pode ser traduzido para o português como "Marque tudo que se aplique" (DUTCOSKY, 2019). O teste Rate-All-That-Apply (RATA) é uma variante do CATA, no qual os termos são apresentados aos avaliadores (consumidores) para que possam ser marcados se considerados aplicáveis, assim como a intensidade percebida em relação as características, podendo ser utilizada uma escala de três pontos (baixa, média ou alta) ou de 5 pontos (variando de 1 = "ligeiramente aplicável" e 5 = "muito aplicável") (ARES et al., 2014). Esse método possibilita discriminar melhor as amostras semelhantes, mas que se diferenciam na intensidade das características sensoriais (MEYNERS; JAEGER; ARES, 2016).

4.2. Importância da análise de bioacessibilidade de minerais

Os minerais são elementos inorgânicos que desempenham diversas funções importantes em uma variedade de processos metabólicos e fisiológicos, como a contração muscular, o ritmo cardíaco, a condução de impulsos nervosos, o transporte de oxigênio e as funções imunológicas (WILLIAMS, 2005; SANTOS; PALLONE, 2022). O cálcio e o magnésio são minerais encontrados em elevadas quantidades em vegetais folhosos (OLIVEIRA et al., 2013). O magnésio está envolvido em mais de 600 reações enzimáticas, incluindo metabolismo energético e síntese de proteínas (BAAIJ; HOENDEROP; BINDELS, 2015). Por outro lado, o cálcio desempenha um papel importante nas funções esqueléticas e regulatórias, sendo fundamental para manter as concentrações plasmáticas dentro de faixas estreitas e prevenir doenças como osteoporose, câncer e doenças cardiovasculares (VICTORIA, 2016).

A bioacessibilidade pode ser definida como a fração de um composto liberado da matriz alimentar durante o processo de digestão gastrointestinal e que está acessível para a absorção no intestino (JENZER; BÜSSER; SADEGHI, 2016). Já o conceito de biodisponibilidade se refere à fração de um composto que está disponível para suprir as demandas fisiológicas nos tecidos alvos (MOURÃO et al., 2005).

Os ensaios de bioacessibilidade *in vitro* simulam as condições fisiológicas do organismo e as etapas que ocorrem a digestão no trato gastrointestinal, levando em consideração as três áreas (boca, estômago e intestino) (TOGNON, 2012).

Diferentes métodos de digestão *in vitro* têm sido desenvolvidos ao longo dos anos, sendo esses, os métodos estáticos e dinâmicos. Os modelos dinâmicos são mais complexos e tendem a simular de forma mais realista os processos de digestão, considerando a cinética, movimentos peristálticos e tamanho de partículas. Já os modelos estáticos são realizados por meio de uma sequência de simulações no trato gastrointestinal (NAKATSUBO, 2017). Modelos de digestão estática são caracterizados por sua simplicidade e eficácia, e oferecem a vantagem de serem facilmente validados devido à elevada reprodutibilidade dos ensaios (COLOMBO et al., 2021). Uma representação esquemática da simulação de uma das etapas do modelo estático INFOGEST pode ser observada na Figura 1.

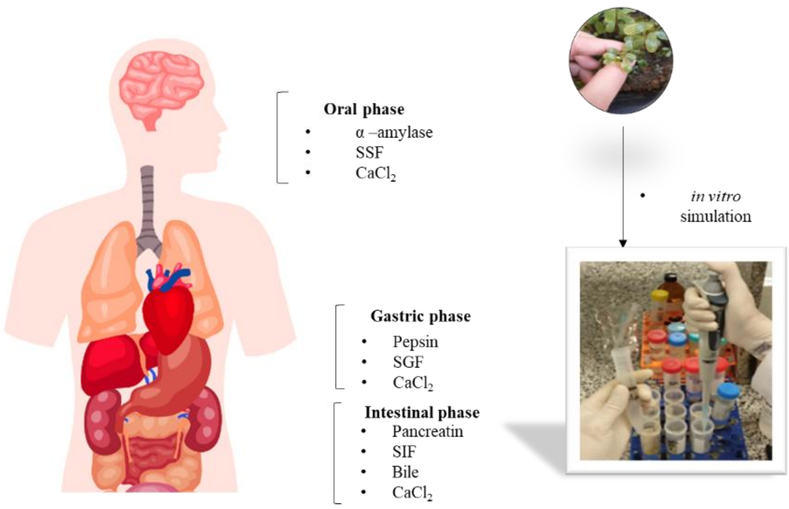

Figura 1. Representação de etapa de simulação de digestão *in vitro* utilizando o protocolo INFOGEST. Fonte: Do próprio autor.

O protocolo harmonizado INFOGEST, atualizado em 2019, é um modelo estático de digestão in vitro que padroniza parâmetros como concentrações de enzimas e sais, volumes de amostra, temperatura, pH e tempo de digestão, visando melhorar a reprodutibilidade e otimizar os ensaios de bioacessibilidade de componentes nutricionais (EGGER et al., 2017; COSTA-SANTOS; PALLONE, 2022; COSTA-SANTOS et al., 2024).

5. MÉTODOS DE EXTRAÇÃO E QUANTIFICAÇÃO DE ÁCIDO OXÁLICO

O ácido oxálico é um antinutriente que pode ser encontrado em elevadas quantidades em hortaliças como a beldroega e o espinafre (NAIK et al., 2014; VANHANEN; SAVAGE, 2015). Esse composto forma oxalato solúvel em água ao se complexar com o Na^{+}, K^{+} e NH_4^{+}, e oxalato insolúvel quando se complexa com o Ca^{2+}, $Fe2^{+}$ e $Mg2^{+}$ (SAVAGE et al., 2000). A formação de oxalatos pode reduzir a bioacessibilidade de minerais, ou seja, a fração do elemento essencial disponível para a absorção, além de contribuir para a formação cristais na urina em indivíduos com pré-disposição (CHARRIER et al., 2002; SAVAGE; CHARRIER; VANHANEN, 2003; VANHANEN; SAVAGE, 2015).

Diferentes métodos de extração e quantificação de oxalatos são encontrados na literatura. Na Tabela 1 é mostrados alguns trabalhos com dados de extração e quantificação de oxalatos em diferentes matrizes vegetais.

Os métodos titulométricos incluem o processo de precipitação para isolar o oxalato, eliminando assim, possíveis compostos interferentes da matriz (ADENIYI et al., 2009; OLIVEIRA; KAMINSEKI; ROSTELATO-FERREIRA, 2017). No entanto, a titulação requer maiores quantidades de amostra e para analitos presentes em pequenas concentrações, apresenta menor sensibilidade quando comparada a métodos instrumentais, como a análise por CLAE. Diversos trabalhos foram publicados na literatura pelo mesmo grupo de pesquisa em que o oxalato foi analisado por CLAE (SAVAGE et al., 2000; CHARRIER et al., 2002; SAVAGE; CHARRIER; VANHANEN, 2003; SAVAGE; DUBOIS, 2006; VANHANEN; SAVAGE, 2015; NGUYEN; SAVAGE, 2020). Esse método de análise é considerado eficiente na quantificação de oxalatos em alimentos. A espectrofotometria no UV-vis e a Espectrometria de Absorção Atômica com Chama são métodos instrumentais que podem ser empregados na quantificação desse antinutriente (NAIK et al., 2014; AOAC, 1990). Esses métodos são menos empregados e podem ser alternativos à análise por CLAE. No entanto, a Espectrofotometria no UV-vis pode ter algumas desvantagens como a interferência de compostos cromóforos da matriz que podem absorver a radiação na mesma faixa de comprimento de onda do analito de interesse, ocasionando alterações nos resultados. Assim, a inclusão da etapa de precipitação se faz necessária, assim como a comparação da espectrofotometria no UV-vis com a Espectrometria de Absorção Atômica com Chama, pois essa última pode ter um grande potencial para quantificar com maior seletividade o oxalato.

Tabela 1. Métodos de extração e quantificação de oxalatos em matrizes vegetais

Amostras	Métodos de extração	Métodos de análise	Referências
Acelga; espinafre; talos de ruibarbo; beterraba, brócolis, cenoura e pastinaca. As amostras foram analisadas cruas e cozidas.	Oxalato solúvel (50 mL de água nanopura à 80°C, por 15 min). Oxalato total (50 mL HCl 2M à 80°C, por 15 min).	CLAE	Savage et al., (2000)
Chá verde; chá preto; chá oolong.	Infusão em 245 mL de água nanopura `a 90°C por 5 minutos.	CLAE	Charrier et al., (2002)
Batata inglesa; batata-doce inhame branco; inhame amarelo; taro; grão de trigo; soja; espinafre.	Oxalato insolúvel (10 mL de HCl 6M, por 1hora). Inclui etapa de precipitação com 5% de $CaCl_2$.	Titulação	Adeniyi et al., (2009)
Folhas de beldroega	30 mL de HCl 0,25 N à 100°C, por 15 min. Reação de oxi-redução com 5 mL de H_2SO_4 2 N e 2 ml de KMnO4 0.003 M.	Espectrofotometria	Naik et al., (2014)
Suco blend (espinafre, maçã, aipo, pepino, pimentão verde e vermelho, limão, salsa) feito no liquidificado e na prensa a frio.	Oxalato solúvel (40 mL de água nanopura à 80°C, por 20 min). Oxalato total (40 mL HCl 0,2M à 80°C, por 20 min).	CLAE	Vanhanen; Savage (2015)
Tomate (*in natura*), extrato de tomate	250 Ml de HCl 0,25 N à 70 °C, por 1 hora. Inclui etapa de precipitação.	Titulação	Oliveira; Kaminseki; Rostelato-Ferreira (2017).

*CLAE (Cromatografia Líquida de Alta Eficiência).

CAPÍTULO I

Potential Functional Food Products and Molecular Mechanisms of Portulaca oleracea L. on Anticancer Activity: A Review

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Oxidative Medicine and Cellular Longevity

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Abstract

Portulaca oleracea Linn. (*P. oleracea* L.) has recently gained attention as a functional food due to the chemical composition of this plant regarding bioactive compounds. The special attention to the use of *P. oleracea* as an ingredient in functional food products is also due to the promotion of sustainable food. It is an unconventional food plant, and its consumption may contribute to preserving biodiversity due to its cultivation in a polyculture system. Food sovereignty may be achieved, among other strategies, with the consumption of unconventional food plants that are more resistant in nature and easily cultivated in small places. *P. oleracea* grows spontaneously and may be found in streets and sidewalks, or it may be cultivated with seeds and cuttings propagation. The culinary versatility of *P. oleracea* opens up opportunities to explore the development of sustainable, functional food products. This mini-review shows that functional food products developed from *P. oleracea* are already available at the research level, but it is expected that more scientific literature focusing on the development of *P. oleracea* functional products with proven anticancer activities may be released in the near future. Polysaccharides, some phenolic compounds, alkaloids, and cerebrosides are associated with the inhibition and prevention of carcinogenesis through *in vitro* and *in vivo* investigations. The anticancer activities of *P. oleracea*, its bioactive compounds, and the involved molecular mechanisms have been reported in the literature. The importance of further elucidating the cancer inhibition mechanisms is in the interest of forthcoming applications in the development of food products with anticancer properties for implementation in the human diet.

1. Introduction

The common purslane (*P. oleracea* L) is a herbaceous succulent annual plant from the Portulacaceae family, native to the Middle East and India [1, 2]. It may be found on roadsides, gardens, and cultivated areas in the tropical and subtropical regions [3, 4]. There are various cultivars of *P. oleracea* distributed worldwide, mainly with morphological differences, with the common purslane having green-red stems, obovate leaves, yellow flowers, and single-layered petals, while the ornamental purslane produces flowers of different colors [1]. The stems and leaves have a slightly acid and salty taste and are usually consumed in salads, soups, and stews [5, 6]. It is an edible plant in regions of European, Mediterranean, African, and Asia countries and Australia [6]. In Brazil, *P. oleracea* is known as an "unconventional food plant", a term referring to plants that are not part of the usual consumption of most of the population in a particular region, country, or even the planet because basic food is very homogeneous, with the use of few food species [7].

P. oleracea has a high nutritional value and many antioxidant properties due to its phenolic compound and omega-3 fatty acid abundance, particularly α-linolenic acid. It is well-known in traditional Chinese medicine [2]. for its use in diuretic, febrifuge, antiseptic, antispasmodic, and vermifuge treatments [8]. Among its various pharmacological properties are its anti-inflammatory [9], antioxidative [10], renoprotective [11], neuroprotective [12], hepatoprotective [13], and muscle-relaxing effects [14].

Anticarcinogenic activities have been reported for *P. oleracea*. Investigations were carried out to screen the activities for antihepatocellular carcinoma [15, 16], colon cancer [17], glioblastoma multiforme [18], ovarian cancer [19] sarcoma [20], lung cancer [16], anti-cervical [21], gastric cancer [22], and pancreatic cancer [23]. *P. oleracea* contains bioactive compounds with antioxidant properties, act on metastasis and invasion, modulate the immune system, and inhibit tumor formation [19, 24 25, 4].

Thus, this mini-review aimed to assemble the anti-cancer effects of bioactive compounds of P. oleracea, demonstrating the molecular mechanisms and the potential for the development of functional food products with anticancer properties.

2. The nutritional value and bioactive compounds of *P. oleracea*

Proximate analyses of *P. oleracea* components including leaves, seeds, stems, buds, and flowers, have been performed. Ash, fiber, protein, and fat approximate contents of P. oleracea leaves as 20.56%, 36.27%, 12.82%, and 3.75%, respectively, are found on a dry matter basis [26]. *P. oleracea* also contains minerals in its leaves with concentration values approximate such as potassium (3710 mg/100 g of dry matter), calcium (2390 mg/100 g), nitrogen (2170 mg/100 g), magnesium (580 mg/100 g), phosphorus (350 mg/100 g), sulfur (200 mg/100 g), iron (32.4 mg/100 g), manganese (5.8 mg/100 g), boron (2.8 mg/100 g), zinc (2 mg/100 g), and copper (1.1 mg/100 g) [26]. This study showed higher levels of potassium, calcium, magnesium, phosphorus, and iron when compared to those of spinach (336 mg/100 g of dry matter, 98 mg/100 g, 82 mg/100 g, 25 mg/100 g, and 0.4 mg/100 g, respectively) [27].

P. oleracea contains high amounts of Omega-3 fatty acids, as discussed by Siriamornpun and Suttajit [28] that found higher levels of Omega-3 fatty acids in fresh leaves, with 523.146±2.29 mg/100 g, while, for stems and flowers, the authors reported 148.87±3.30 mg/100 g and 216.17±1.16 mg/100 g, respectively. Other plants (analysis of leaves in dry matter) contain lower levels of Omega-3 fatty acids than *P. oleracea*, such as mint (194.9 mg/100 g), watercress (179.6 mg/100 g), spinach (129.2 mg/100 g), parsley (124.8 mg/100 g), and broccoli (110.3 mg/100 g) (analysis of leaves in dry matter) [29]. Omega-3 fatty acids may have pharmacological effects such as anti-hyperlipidemic, antimicrobial, anti-inflamatory, neuroprotective and nephroprotective activities [3, 30,31 32; 11]. *P. oleracea* also contains high levels of tocopherols, vitamin A, β-carotene and ascorbic acid [3, 32, 33, 34]. Antimicrobial and antioxidant activities were related to these compounds [3, 33].

High concentrations of oxalic acid have also been detected in *P. oleracea.* The intake of oxalic acid provided by the diet with *P. oleracea* may form complexes with minerals such as calcium and iron (insoluble salts) or sodium, magnesium, and potassium (soluble salts), reducing their bioavailability and possibly leading to the development of kidney stones through the formation of calcium oxalate crystals [35]. Thus, consumption of *P. oleracea* should be moderated by individuals with a propensity to develop kidney stones. Amounts of 23.45±0.45 g, 5.58±0.18 g, and 9.09±0.12 g of total oxalates per kilogram of fresh weight oxalates were obtained in fresh leaves, stems, and buds, respectively, with 75.0% being soluble oxalates in the stems and buds, and only 27.5% in the leaves [36]. The authors reported a 66.7% reduction ($p < 0.001$) of soluble oxalates after cooking the leaves for a short time, discarding the water, and pickling them with white vinegar [36]. Some other bioactive compounds from secondary metabolism of *P. oleracea* such as flavonoids, alkaloids, terpenoids and their pharmacological activity can be seen in Table 1.

Flavonoids (a class of phenolic compounds) in *P. oleracea* were associated with anti-fertility, antimicrobial, antioxidant and antidiabetic effects [37, 38, 39, 40].

Combined effects of polyunsaturated fatty acids, flavonoids and polysaccharides on hypoglycaemic, hypolipidaemic and insulin resistance reducer effects through ingestion of P. oleracea seeds in clinical test with humans were observed [40]. Other phenolic compounds (Polyphenols and phenolic acids) in *P. oleracea* have antioxidant and antimutagenic effects [41, 42, 43].

Other bioactive compounds with pharmacological importance in *P. oleracea* are alkaloids and terpenes. Anticancer, anti-inflamatory and antioxidant effects were described for alkaloids found in this plant while hepatoprotective, antibacterial, antifungal and anti-hypoxia effects were described for terpenes of *P. oleracea* [44, 45, 46, 47, 48].

Table 1. Some classes of bioactive compounds from secondary metabolism of P. oleracea and their pharmacological activit

Compounds	Plant Structure	Form (fresh or dry)	Pharmacological Activity	References
Flavonoids	Aerial part	Dry	Anti-fertility	[37]
	Aerial part	Dry	Antimicrobial	[38]
	Leaves	Fresh	Antioxidant	[39]
	Seeds	Dry	Antidiabetic	[40]
Polyphenols	Leaf, steam and flower	Dry	Antioxidant	[41]
	Whole plant	Fresh	Antimutagenic	[42]
Phenolic acids	Aerial parts	Dry	Antioxidant	[43]
Alkaloids	Aerial part	Dry	Anticancer	[44]
	Whole plant	Fresh	Anti-inflamatory	[45]
	Whole plant	Dry	Antioxidant	[46]
Terpenes	Whole plant	Dry	Hepatoprotective, antibacterial and antifungal	(47]
	Aerial part	Dry	Anti-hypoxia	(48]

3. Potential antioxidant of the *P. oleracea*

This plant is rich in antioxidants such as vitamin A, tocopherols, ascorbic acid, beta-carotene, and phenolic compounds [49, 33]. Beta-carotene was found in *P. oleracea* with content ranging from 21 µg/g to 30 µg/g of fresh mass in leaves and 3.6 µg/g to 6.5 µg/g of fresh mass in stems [50]. The antioxidant potential was measured at different growth stages (15, 30, 45, and 60 days) of aerial parts of *P. oleracea* [49]. The total phenolic content (TPC) for the young shoots at 15 days was significantly lower than at 30, 45, and 60 days, while the ascorbic acid content (AAC) did not show a significant decrease from the developing to the mature stage. According to the study, the IC50 value of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity ranged from 1.30±0.04 mg/ml (60 days) to 1.71±0.04 mg/ml (15 days), while the ascorbic acid equivalent antioxidant content (AEAC) values ranged from 229.5±7.9 mg AA/100 g (15 days) to 319.3±8.7 mg AA/100 g (60 days), the TPC varied from 174.5±8.5 mg GAE/100 g (15 days) to 348.5±7.9 mg GAE/100 g (60 days), the AAC varied from 60.5±2.1 mg/100 g (60 days) to 86.5±3.9 mg/100 g (15 days), and the ferric reducing antioxidant power (FRAP) ranged from 1.8±0.1 mg GAE/g (15 days) to 4.3±0.1 mg GAE/g (60 days). Thus, mature plants (60 days) of *P. oleracea* had higher TPC and antioxidant activities than immature plants.

The dry weights of the samples (leaves, flowers, and stems) from two different locations were investigated for potential antioxidant activity by Silva and Carvalho [41], who found that stems had a higher total phenolic content and total antioxidant activity than the flowers and leaves. The oil from seeds, leaves, and stems of *P. oleracea* were analyzed and found that the peroxide value was significantly higher for seed oil and the lowest for stem oil [51]. Furthermore, the highest ascorbic acid content was found for *P. oleracea* seed oil (41.67%), followed by leaf oil (32.29%), and the highest DPPH was obtained for leaf oil (12.55%), followed by seed oil (2.05%). Values for lettuce (IC50 = 17.07 mg/ml), artichoke (IC50 = 18.14 mg/ml), turmeric (IC50 = 21.14 mg/ml), spinach (IC50 = 22.87 mg/ml), and escarole (IC50 = 32.2 mg/ml) were reported by Tiveron et al. [52], showing that *P. oleracea* presents the lowest IC50 necessary to reduce 50% of DPPH free radicals.

4. Functional food products and *P. oleracea*

The *P. oleracea* plant may be used as an ingredient in functional food products due to its nutritional value and bioactive compounds that will be incorporated into the formulations.

The use of the *P. oleracea* plant as food may not only enhance the nutrients and bioactive composition of functional products but also influence their sensory and technological characteristics. Although it is well-known that sensory acceptance by consumers is essential for a product's commercial success on the market, few studies in the literature have reported the application of *P. oleracea* in products and its performance or the sensory profile of such products.

Regarding the technological aspect, the incorporation of the durum wheat flour with 5% of *P. oleracea* to bread resulted in the improvement of the rheological characteristics, an increase in antioxidant properties, and a decrease in the Omega-6-to-Omega-3 ratio, which is beneficial for human health, in addition to improving the sensorial quality [53].

The durum wheat spaghetti fortified with 10% of *P. oleracea,* a potential functional food, was appreciated by consumers. It showed a high concentration of α-linolenic acids (Omega-3), total phenolic compounds, and antioxidant properties, so that, considering 100 g of pasta per day, it is possible to obtain 75 mg of essential linoleic acid and 9 mg of linolenic acid, along with a four-fold increase in total phenolic compounds [54]. The Omega-3 fatty acids can also inhibit carcinogenesis and slow tumor growth, as demonstrated by *in vitro*, *in vivo*, and clinical investigations [55].

The analysis of bread incorporated with four different concentrations of *P. oleracea* powder (0%, 5%, 10%, and 15%) showed increasing water absorption capacity, stability under the mixer, and softening levels as the *P. oleracea* powder concentration in the samples increased. The protein, fat, total ash, moisture, and fiber contents also increased along with the *P. oleracea* concentrations [56]. However, the bread with 15% of *P. oleracea* powder showed a decreased farinograph quality number and presented the lowest scores for sensory properties and color, taste, texture, and overall liking. The optimized formulation containing 10% of *P. oleracea* powder had the highest acceptance.

P. oleracea has also been used to produce powder mixtures with two other plant species, *Amaranthus hybridus* L. and *Chenopodium berlandieri* L. The powder mixtures containing *P. oleracea* showed more significant contents of phenolic compounds, with an increase in the antioxidant activity [57].

Another innovative functional product assessed was a fermented *P. oleracea* juice added with a selected lactic acid bacteria. Results demonstrated an increase in total antioxidants, preserved vitamin C, A, and E levels, and increased contents of vitamin B2 and phenolic compounds. In addition, decreased levels of pro-inflammatory mediators and reactive oxygen species were observed, with a consequent increase in the restorative characteristics of the use of *P. oleracea* juice for intestinal inflammation and epithelial injury [58].

The combination of yogurt or coconut plant extract or coconut cream with fresh leaves of *P. oleracea* reduced the overall oxalate content by simple dilution. The soluble oxalate content decreased from 53.0% to 10.7% when *P. oleracea* leaves were added to yogurt. However, the coconut plant extract and coconut cream had no effect on the percentage of soluble oxalate content but provided the mixture with an acceptable flavor [59].

The addition of fresh purslane leaves (ranging from 1% to 10%, w/w) to tomato sauces resulted in a decrease of total soluble solids from 9.57 °Bx to 9.20 °Bx, beneficially impacting sugar reduction. On the other hand, the amount of protein significantly increased from 0.12% to 1.83% from the lowest to the highest concentrations, respectively [60].

5. Bioactive compounds of *P. oleracea* on anticancer activity

P. oleracea presents phytochemicals and nutrients associated with anticarcinogenic properties. The 12% reduction in the activity of the mutagenic nitrosation mixture may be attributed to the ascorbic acid (vitamin C), α and β-carotene, chlorophyll, and polyphenols of the *P. oleracea* extract obtained through a standard juice extractor [42].

Phenolic compounds such as kaempferol and apigenin from a hydroethanolic extract of *P. oleracea* have effects *in vitro* against human glioma cells, and homoisoflavonoids showed *in vitro* selective cytotoxic activity for SF-268, NCI-H460, and SGC-7901 cell lines, as shown Table 2 [61, 18].

Polysaccharides from *P. oleracea* act on free radicals through the antioxidant mechanism, modulating the immune system, which may be preventive and therapeutic in rat ovarian and gastric cancer and mouse cervical cancer and sarcomas, as shown Table 2 [19, 20, 22, 21, 62].

Another bioactivity from *P. oleracea* is portulacacerebrosie A, a cerebroside compound that suppresses the invasion and metastasis of liver cancer HCCLM3 cells and acts in leucocythemia treatment are show in Table 2 (24, 63].

Polysaccharides showed activity against ovarian cancer by inhibiting the red blood cell (RBC) hemolysis in the spleen, thymocyte, and T and B lymphocyte proliferation [19]. These compounds also act against cervical cancer through Sub-G1 phase cell cycle arrest triggering DNA damage, inhibit the growth of transplantable sarcoma 180, increase the number of white blood cells (WBC), CD4+ T-, the $CD4^+/CD8^+$ ratio, IL-12, and TLR-4, decrease IL-10 and HeLa cell proliferation, reduce the production of cytokine/chemokine and the expression levels of CD80, CD86, CD83, Bax, and downregulate the Bcl-2 level in a concentration-dependent manner. In addition, polysaccharides inhibit the protein expression levels of TLR4, myeloid differentiation primary response 88 (MyD88), TNF receptor associated Factor 6 (TRAF6), activator protein-1 (AP-1), and factor nuclear kappa B (NF-κB) subunit P65 [20, 21, 63, 64].

In gastric cancer, interleukins (IL-2 and IL-4) and TNF-α were enhanced by polysaccharides that also provide dose-dependent protection against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induced oxidative injury by enhancing Superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH-Px) [22]. In addition to acting against ovarian, gastric, and cervical cancer, polysaccharides also work against intestinal cancer by stimulating the TLR4-PI3K/AKT-NF-κB signaling pathway and Anti-NF-κB activity along with two upstream ROS and NO mechanisms [18, 62], showing the importance of studying these molecules in *P. oleracea* matrices.

The cerebroside compound, Portulacerebroside A, affects leukemia and cervical, liver, esophageal, breast, and colon cancer and cancer stem cells [16, 24, 63, 65, 66]. Some mechanisms involved with Portulacerebroside A have increased RNA expressions and protein levels of Bax/Bcl-2, caspase-3, and caspase-9, protein expression levels of TIMP-2 and nm23-H1, inhibition of the mRNA expression of MTA1, MMP-2, and MMP-9, RhoA, Rac1/Cdc42, MMP-2, and downregulation of the expression of the

Notch1 and β-catenin genes. Alkaloids inhibited lung and breast cancer through moderate cytotoxic activities against A549, weak cytotoxic activities against K562, and low cytotoxic activity against MCF-7 and MDA-MB-435 cells.

Some possible mechanisms of P. oleracea for anticancer activity are represented in Figure 1. The bioactivity of P. oleracea and the potential to develop new products from this underused plant in some regions deserve attention regarding its valorization as a functional food and its pharmacological properties. Different anticancer mechanisms of P. oleracea were explored and reported in this review. Aqueous extracts, seed oil, and hydroethanolic extracts present cytotoxicity to cancer cell lines while chloroform extract does not have cytotoxic activity [67]. Further studies will be needed to determine anticancer activity in particular food matrices and beverages.

Table 2. Bioactive compounds of *P. oleracea*, types of extracts, and molecular mechanisms for cancer inhibition

Experimental model		Compounds	Types of extract	Types of cancer inhibited	Mechanisms and Results	References
In vitro	*In vivo*					
	Rats	Polysaccharides	Aqueous extract	Ovarian	Scavenge superoxide anion, (DPPH-), nitric oxide, and hydroxyl radicals Inhibit RBC hemolysis spleen, thymocyte, T and B lymphocyte proliferation	[19]
Human cancer cell lines SF-268, NCI-H460, K-562, SGC-7901, and SMMC-7721		Homoisoflavonoids	Hydroalcoholic extract		Homoisoflavonoids showed in vitro cytotoxic activities towards four human cancer cell lines	[61]
Treatment of HeLa cell	Mice	Polysaccharides	Aqueous extract	Cervical	Sub-G1 phase cell cycle arrest, triggering DNA damage Inducing apoptosis	[21]
	Mice	Polysaccharides	Aqueous extract		Inhibit the growth of transplantable sarcoma 180 Increase in the number of white blood cells (WBC) and CD4+ T-lymphocytes Increase in the $CD4^+/CD8^+$ ratio	[20]
	Rats	Polysaccharides	Aqueous extract	Gastric	Interleukin-2 (IL-2), interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF-α) was enhanced Provide dose-dependent protection against MNNG-induced oxidative injury by enhancing SOD, CAT, GSH-Px	[22]

Experimental model		Compounds	Types of extract	Types of cancer inhibited	Mechanisms and Results	References
In vitro	*In vivo*					
Human lung (K562 and A549) and breast (MCF-7 and MDA-MB-435) cancer cell lines		Alkaloids	Hydroalcoholic extract	Lung Breast	moderate cytotoxic activities against A549 and weak cytotoxic activities against K562. The compounds showed low cytotoxic activity against MCF-7 and MDA-MB-435 cells.	[54]
Human hepatocellular carcinoma cells			Seed alcoholic extract	Hepatocellular	Significantly reduced the cell viability of HepG2.	[15]
The uterine cervical carcinoma (U14) cell line		Polysaccharides	Aqueous extract	Cervical	Upregulated the expression of CD80, CD86, CD83 Increase in IL-12, TLR-4, Decrease in IL-10	[29]
Human HL60 cell line		Portulacerebroside A	Aqueous extract	Leukemia	Mitochondrial membrane potential ROS accumulated Increase in RNA expressions and protein levels of Bax/ Bcl-2, caspase-3, and caspase-9 ERK1 / 2, JNK1 / 2 and p38 MAPK pathway were blocked	[49]

Experimental model		Compounds	Types of extract	Types of cancer inhibited	Mechanisms and Results	References
In vitro	*In vivo*					
HepG2 and A-549 cell lines			Seed oil	Liver Lung	Significant cytotoxicity and inhibition of growth of the liver cancer (HepG2) and lung cancer (A-549) cell lines	[20]
Human liver cancer HCCLM3 cells		Portulacerebroside A	Aqueous extract	Liver	Increase in RNA and protein expression levels of TIMP-2 and nm23-H1 Inhibition of the mRNA expression of MTA1, MMP-2, and MMP-9 Suppression of the protein expression of MTA1, RhoA, Rac1/Cdc42, MMP-2, but not RhoC and MMP-9	[23]
Cervical cancer HeLa cells, esophageal cancer Eca-109 cells and breast cancer MCF-7 cells			Seed oil	Cervical Esophageal Breast	Stronger inhibitory effect on the proliferation of MCF-7 cells and significantly inhibited the proliferation of HeLa cells and Eca-109 cells	[51]
PANC-1 cancer cell line			Aqueous extract	Pancreatic	Significant effect on apoptosis in pancreatic cell line and high expression of P53 and reduction of CDK gene expression	……
Human colon adenocarcinoma (HCT-15) and normal (Vero) cell line			Chloroform extract	Colon adenocarcinoma	Chloroform extract does not have cytotoxic activity and was not safe to normal Vero cell line.	…….

Experimental model		Compounds	Types of extract	Types of cancer inhibited	Mechanisms and Results	References
In vitro	*In vivo*					
Colon cancer cells (HT-29) and HT-29 cancer stem cells			Ethyl alcohol extract	Colon Stem cells	Inhibited the proliferation of both HT-29 cancer cells and HT-29 cancer stem cells Significantly decreased the expression of the Notch1 and β-catenin genes in both cell types	[52]
The human cervical cancer HeLa cells.		Polysaccharides	Aqueous extract	Cervical	Decrease HeLa cell proliferation Upregulate Bax level and downregulate Bcl-2 level in a concentration-dependent manner Inhibit the protein expression levels of TLR4, MyD88, TRAF6, AP-1 and NF-κB subunit P65 Reduce the production of cytokine/chemokine	[50]
The mouse cervical carcinoma U14 cells		Polysaccharides	Aqueous extract	Intestinal	Dendritic cell (DC) apoptosis in U14-bearing mice Increase intestinal DC survival Stimulate the TLR4-PI3K/AKT-NF-κB signaling pathway	[48]
Human glioblastoma cancer cell line (U-87)			Hydroethanolic extract		Cytotoxicity and apoptogenic effects Anti-NF-κB activity along with two upstream ROS and NO mechanisms	[17]

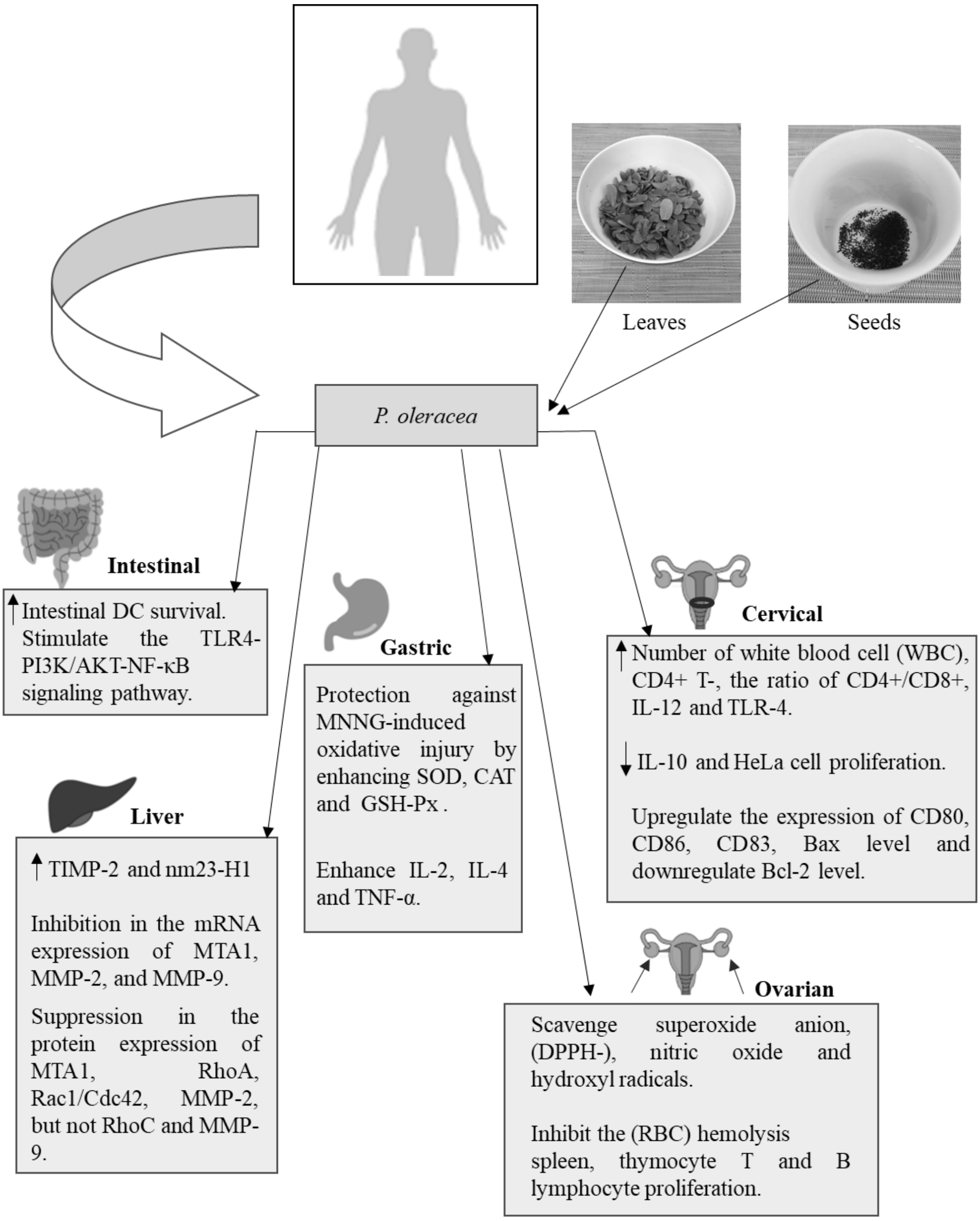

Figure 1. Some possible mechanisms of *P. oleracea* for anticancer activity.

6. Conclusion

The *P. oleracea* plant may be promising for developing and innovating potential functional food products. The high levels of antioxidants such as phenolic compounds, carotenoids, and other nutrients such as minerals and Omega-3 fatty acids are supported by functional food studies. Research has indicated the anticancer activity of *P. oleracea* extracts. Polysaccharides, some phenolic compounds, alkaloids, and cerebrosides detected in *P. oleracea* and contained in aqueous extracts, seed oil, and hydroethanolic extracts are associated with inhibition and prevention of carcinogenesis. However, more studies are needed to prove the anticancer activity of food products containing *P. oleracea* as an ingredient to promote health benefits to the consumers.

Data Availability

All data are included within the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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CAPÍTULO II

Mixed juice with purslane (*Portulaca oleracea* L.), a non-conventional edible plant: a strategy to provide consumers a healthy and sustainable product.

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CAPÍTULO III

Effects of High Pressure treatments on physicochemical characteristics, phenolic compounds, antioxidant potential and bioaccessibility of Calcium and Magnesium in mixed orange (*Citrus sinensis*), cupuaçu (*Theobroma grandiflorum*) and purslane (*Portulaca oleracea* L.) juice

Não publicado

CAPÍTULO IV

Evaluation of oxalic acid extraction and quantification methods in the different purslane (*Portulaca oleracea* L.) matrices and spinach (*Spinacea oleracea*)

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MethodsX

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Abstract

Purslane (*Portulaca oleracea*) and spinach (*Spinacea oleracea*) are species with elevated levels of oxalic acid, an antinutrient that interferes in the bioaccessibility of minerals such as calcium and iron. Evaluating methods to determine oxalic acid content with reduced matrix interference, such as employing Flame Atomic Absorption Spectrometry (FAAS), can enhance the specificity of determinations. The different matrices of purslane (whole plant, leaves, and juice) and spinach (whole plant) were tested using three extraction methods (M1, M2, and M3). The oxalic acid content was evaluated by UV-vis spectrophotometry and FAAS (Flame Atomic Absorption Spectrometry). The absence of the precipitation step in M1 resulted in high levels of oxalic acid in the investigated matrices. The quantification of oxalic acid by FAAS for M2 (6M HCl for 1 hour at 100°C) and M3 (0.25N HCl for 15 minutes at 100°C) in the samples of purslane leaves and spinach whole plants yielded statistically similar results. However, the analysis by UV-vis spectrophotometry for M2 and M3 showed significant discrepancies in all evaluated samples, suggesting interference from colored compounds in the food matrix.

- Comparison of methods of extraction
- Comparison of UV-vis spectrophotometer and FAAS in the quantification of oxalic acid
- Analysis of antinutrients in plant matrices

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Method name: Determination of oxalic acid by Flame Atomic Absorption Spectrometry (FAAS) and UV-vis spectrophotometry.
Keywords: Oxalic acid, Antinutrients; Extraction; FAAS; UV-vis spectrophotometry

Specifications Table

Subject area	Chemistry
More specific subject area	Quantitative analyses
Method name	Oxalic acid quantitative analysis
Name and reference of original method	Naik et al., (2014) [12]; AOAC (1990) [8]; Adeniyi et al., (2009) [13].
Resource availability	NA

Background

Oxalic acid is found in large quantities in vegetables such as spinach and purslane. This antinutrient can form soluble oxalates (complexes with sodium, potassium, and ammonium) and insoluble oxalates (complexes with calcium, magnesium, and iron) [1]. When complexed with calcium, oxalic acid can form calcium oxalate crystals in the kidneys, reducing the bioavailability of this mineral [2]. The recommended daily intake of oxalic acid is 50-200 mg/day, with the minimum lethal dose for adults being 5 grams. [3; 4; 5]. Some techniques for reducing oxalic acid in food are already known and employed, such as cooking and bleaching [5]. However, juices are commonly produced without prior heat treatment of the vegetables, resulting in potentially high oxalic acid content in the final product [6]. The most commonly methods of analysis to quantify oxalic acid in food matrices are titration, High Performance Liquid Chromatography (HPLC) and UV-vis spectrophotometry [2; 7; 8; 9; 10]. UV-vis spectrophotometry has numerous advantages, such as being an inexpensive method for quantifying a variety of organic compounds. This method is also widely used coupled with HPLC. [11]. For UV-vis spectrophotometry, chromophore compounds can absorb within the same range as the analyte of interest when the method is used without coupling with complementary techniques for characterization. The indirect determination of oxalic acid in canned vegetables through calcium quantification using the Atomic Absorption Spectrometry (AAS) is described in AOAC (1990). FAAS is an Atomic Absorption Spectrometry that utilizes a flame as an atomizer. This technique can be an alternative to other established methods for determining oxalic acid amounts, with fewer interferences from chromophore compounds. This study aimed to compare three extraction methods, two quantification techniques (FAAS and UV-Vis spectrophotometric) and also investigate the inclusion of the precipitation step to form and separate calcium oxalate.

Method details

Sample preparation

Method of Extraction 1 (M1)

This method was performed according to the reference [12]. Triplicate samples of 0.5g of sample (freeze-dried) or 2g (liquid) were weighed and digested with 30mL of 0.25N HCl for 15 min at 100°C. After cooling to room temperature, the volume was adjusted to 50 mL with 0.25 N HCl. The precipitation step was not performed in M1.

Method of Extraction 2 (M2)

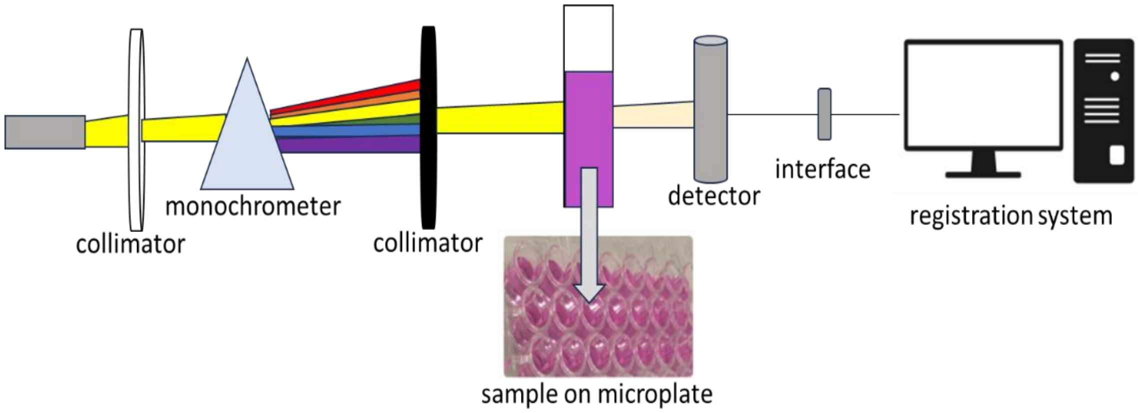
The M2 was performed according to reference [13], and with adaptations. For extraction, triplicate samples of 0.5g (freeze-dried) or 2g (liquid) were weighed and digested with 10mL of 6M HCl for 1 hour at 100°C. The digested solution was completed to 100 ml with ultrapure water, filtered and cooled. For precipitate calcium oxalate, concentrated NH_4OH was dripped before the color changed from salmon pink to faint yellow, and the pH was adjusted to 4-4.5, monitored using methyl red indicator for visualization of the color change. The precipitation step consisted of adding 15 mL of 5% $CaCl_2$, followed by centrifugation of the suspension (2500 rpm/5 min) and decantation of the supernatant. The precipitate was dissolved in 10 ml of 20% H_2SO_4 and completed to 50 ml with ultrapure H_2O.

Method of Extraction 3 (M3)

For executing M3, the extraction followed the methodology described in reference [12], with adaptations. Triplicate samples of 0.5g (freeze-dried) or 2g (liquid) were weighed and digested with 30mL of 0.25N HCl for 15 min at 100°C. The precipitation step was executed as described by Adeniyi et al., (2009), detailed in M2. The precipitate was dissolved in 10 ml of 20% H_2SO_4. The resulting precipitate (calcium oxalate) was adjusted to 50 ml with ultrapure 0.25N HCl.

Quantification of oxalic acid by UV-vis spectrophotometry

The UV-vis spectrophotometer (Fluo Star Omega, BMG Labtech, Ortenberg, Germany) was used to measure light absorption at a wavelength of 528 nm. The method was based on the oxidation reaction of oxalic acid by $KMnO_4$, adapted of Naik et al. (2014). For each sample (M1 and M2) or the reagent blank (containing only water), 25 µL of the extracts were mixed with 125 µl of 2N H_2SO4 and 50 µl of 0.003M $KMnO_4$. The mixtures were incubated at 27°C, for 10 min.

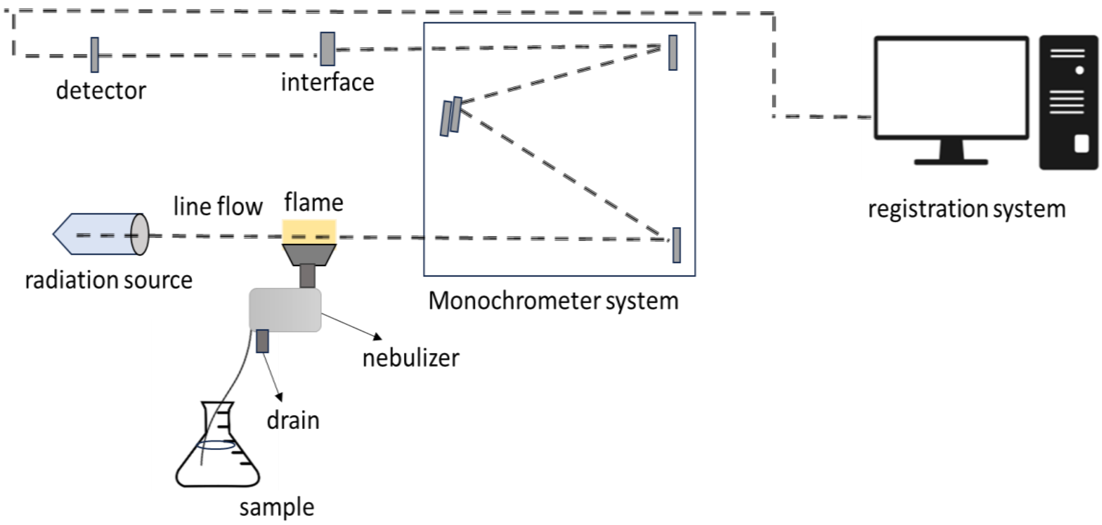


Standard oxalic acid solution for analysis in UV-vis spectrophotometry

The potassium permanganate solution was prepared, and as the concentration of oxalic acid increased, the absorbance decreased. Different concentrations of the oxalic acid standard curve were prepared from the 1mg/mL stock solution, ranging from 0.1 to 0.9 mg/mL.

Quantification of oxalic acid by Flame Atomic Absorption Spectrophotometry (FAAS)

The indirect measurement of oxalic acid was evaluated by quantifying the total calcium content of the samples using a flame atomic absorption spectrometer (Analyst 200, Perkin-Elmer, Norwalk, USA). The determination of Ca was conducted through the specific wavelength of a hollow cathode lamp set at 423 nm, in a flow rate of 2.7/0.6 L/min of air/acetylene gas input. A 0.5% lanthanum oxide solution was added to the final sample solution to prevent interferences of phosphate ions. The same procedure was executed for the analytical blanks, used for quantifying extracts M2 and M3. A standard solution of 1000 mg/L Ca was used to prepare the analytical curve, distributed in five equidistant concentrations ranging from 0.5 to 5.0 mg/L.



Standard calcium solution for analysis in FAAS

Calcium standards were prepared with lanthanum oxide 10%, HNO_3, and ultrapure water.

Results and discussion

Formula for determination of oxalic acid in the UV-vis spectrophotometry

The final absorbance values for the standard curve were obtained using the formula (B-A) as presented in Table 1. The linear regression was obtained through the equation (y=0.9958x+0.054) and the determination coefficient (R2 = 0.9806) is shown in Fig. 1.

Table 1
Calculation of the final absorbance for the development of the standard

	Standard curve	
Concentration of oxalic acid (mg/mL)	Absorbance (A)	Final absorbance = (B-A)
0.1	1.6335	-0.1311
0.2	1.5675	-0.1971
0.3	1.3955	-0.3691
0.4	1.2785	-0.4861
0.5	1.17	-0.5946
0.6	1.0685	-0.6961
0.7	1.004	-0.7606
0.8	0.9335	-0.8311
0.9	0.8635	-0.9011

*B(reagente blank). The absorbance value obtained for B was 1.7646.

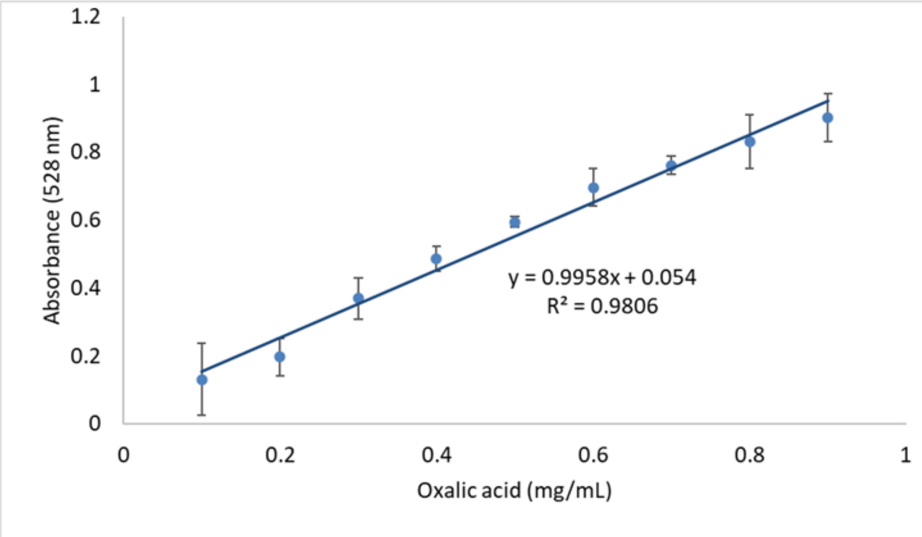


Fig. 1. Regression line for quantification of oxalic acid in triplicate.

To calculate the final absorbance of the sample (FA), the absorbance of the sample (S) was subtracted from the absorbance of the reagent blank (B). Thus, FA = B-S. For determining the linear regression equation, the analyte concentration was obtained by the following formula:

$y=ax+b$

FA = 0.9958x + 0.0054, where x represents the final concentration of the analyte in mg/mL. To express the result in grams (g) of weighed sample, the concentration must be multiplied by the final volume of the extract (50 mL). This calculation yields the amount of analyte present in the sample. Afterward, simply express the result as a percentage for 100g of the sample (%).

Formula for indirect calculation of oxalic acid in the FAAS

The linear regression equation for calcium quantification through the standard curve is shown in Fig.2. The final absorbance of the sample (FA) can be calculated by absorbance readings of the reagent blank (B) subtracted by the absorbance of the sample (S).

Therefore, FA = S-B.

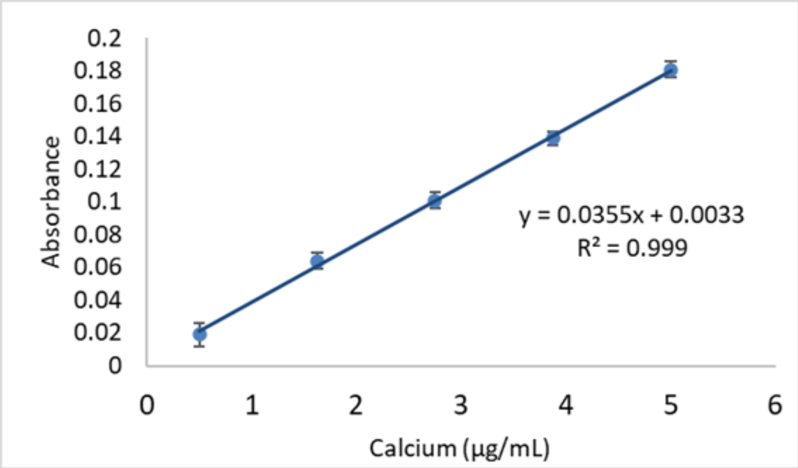


Fig. 2. Regression line for quantification of calcium in triplicate.

The formula used to calculate the oxalic acid content was determined indirectly through the quantification of calcium in the sample, according to the AOAC method (1990), with adaptations:

$$\text{mg oxalic acid/100g} = \frac{\mu g\ Ca/ml \times [FC \times (\text{final volume} \times FD) \times 100]}{\text{sample weight (g)}}$$

Where:

FC = 2.246/1000 (conversion of micrograms calcium to mg oxalic acid)
FD = dilution factor

Table 2
M1 and M3 test in UV-vis spectrophotometry

	Oxalic acid concentration			
	purslane - juice (g/100g WM)	purslane - whole plant (g/100g DM)	purslane (leaves, g/100g dry matter)	spinach - whole plant (g/100g DM)
M1	3.883 ± 0.121[a]	33.21 ± 0.5354[a]	32.47 ± 0.53[a]	40.11 ± 1.16[a]
M3	0.9176 ± 0.03769[b]	12.26 ± 0,091[b]	13.56 ± 0.7448[b]	13.75 ± 0.92[b]

* Different letters indicate statistically different values ($p<0.05$) for Tukey Test.

The comparison between the methods using the UV-vis spectrophotometry revealed that M1 (without the precipitation step) showed higher oxalic acid content compared to M3 UV-vis spectrophotometry operates by selecting a wavelength where the substance of interest exhibits maximum absorption. However, other substances may absorb at the same wavelength, potentially causing errors in the result. Therefore, precipitation is employed to isolate the compound of interest (calcium oxalate) and reduce analytical interferents. The formation of calcium oxalate during the precipitation step is illustrated in Figure 3.

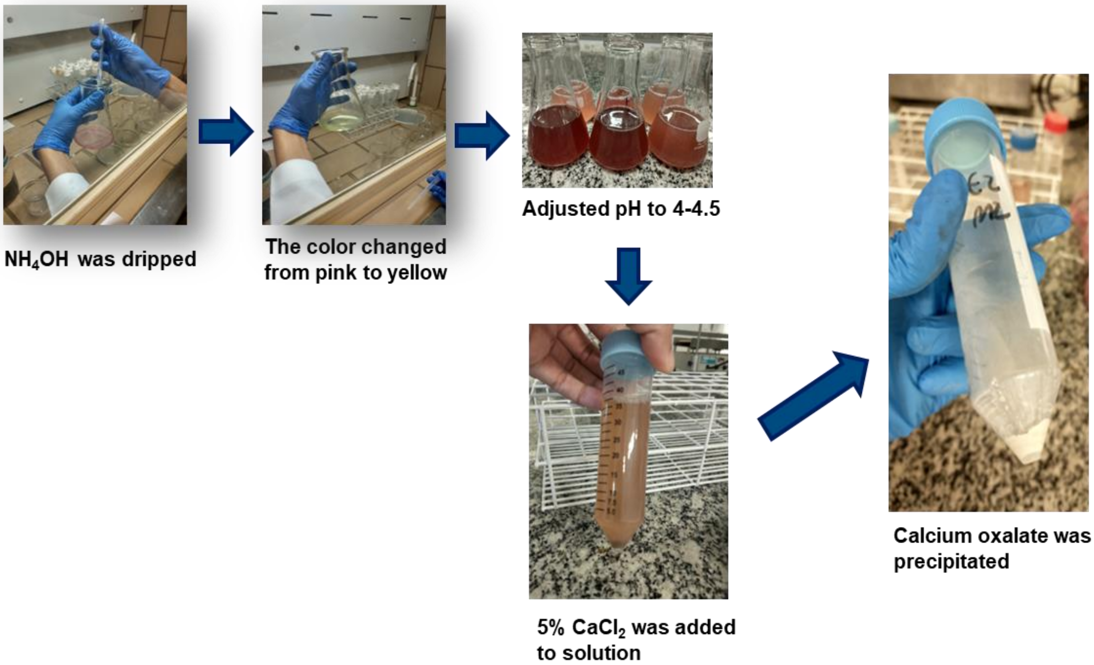


Figure 3. Precipitation of calcium oxalate.

Table 3
Comparison between UV-vis spectrophotometry and FAAS

	Oxalic acid concentration			
	purslane - juice (g/100g wet matter)	purslane - whole plant (g/100g dry matter)	purslane - leaves (g/100g dry matter)	spinach - whole plant (g/100g dry matter)
M2 (UV-vis spectrophotometry spectrophotometer)	0,67 ± 0,011[a]	9,51 ± 0,75[a]	28,73 ± 2,30[a]	22,01 ± 1,71[a]
M3 (UV-vis spectrophotometry)	0,91 ± 0,037[b]	11.96 ± 0,09[b]	13.01 ± 0,74[b]	11.87 ± 0,82[b]
M2 (FAAS)	0,24 ± 0,01[c]	1,62 ± 0,11[c]	7,613 ± 0,73[c]	10,40 ± 0,12[c]
M3 (FAAS)	0,37 ± 0,072[d]	5,55 ± 0,49[d]	6,56 ± 0,28[c]	10,52 ± 0,40[c]

* Different letters indicate statistically different values ($p<0.05$) for Tukey Test.

Naik et al., (2014) evaluated the oxalic acid content in purslane leaves using UV-vis spectrophotometry (M1). The coefficient of determination (R^2) for the oxalic acid standard curve reported by the authors was 0.983, close to the value of 0.9806 observed in the present study. Additionally, the authors found oxalic acid levels of 6.36 g/100g in dry weight for Portulaca oleracea. They also measured oxalic acid by titration with $KMnO_4$, obtaining values of 6.34 g/100g, similar to those obtained by UV-vis spectrophotometry. The oxalic acid levels found by Naik et al. (2014) were lower than those found in M1 of this study. This difference may be attributed to variations in the location where the purslane was harvested. Factors such as climate, temperature, humidity and the stage of maturation can influence the composition of the plant.

In the UV-vis spectrophotometry analysis, M3 showed higher contents for the juice and purslane–whole plant, but this trend was not observed for purslane-leaves and spinach-whole plant, where M2 demonstrated significantly higher contents. Each matrix can present many differences in its constituents, which can result in interference during spectrophotometric analysis. One of the disadvantages of using the Uv-vis spectrophotometry is the interference of colored compounds present in the sample [14].

Regarding the method employed by Adeniyi et al. (2009), oxalate extraction from Nigerian food samples involved a digestion step (10 mL 6M HCl for 1 hour), followed by precipitation as detailed in M2 of this study. However, oxalate quantification was performed using titration with standardized KMnO4 solution until reaching a light pink coloration. According to the study, leaves of Amaranthus sp. exhibited 91.9 mg/100g of fresh weight. Instrumental techniques offer higher sensitivity for quantifying target analytes compared to titration methods, leading many studies to utilize more advanced techniques such as UV-vis spectrophotometry, Flame Atomic Absorption Spectrometry (FAAS), and High-Performance Liquid Chromatography (HPLC).

The quantification technique of oxalic acid using HPLC has been widely employed in the literature [1, 15, 16, 17, 6, 18, 19, 20, 21]. Extraction parameters (pH, temperature, and time) for determining oxalic acid in spinach via HPLC were investigated by Kusuma et al. (2016), who found levels ranging from 424.95 to 856.57 mg/100g of fresh matter sample. Savage et al. (2000) also determined total oxalate content in spinach using the HPLC method, reporting levels of 329.6 mg/100g of fresh weight sample. Elevated levels of oxalic acid in spinach samples were also found in the current study (10.52 – 22.01 mg/100g of dry matter) for M2 and M3. The quantification of oxalic acid by AAS was described in AOAC (1990), while the determination of this antinutrient content by FAAS was conducted in the present study. As for the analysis by FAAS, the M3 presented higher contents for the purslane-juice and purslane-whole plant samples, not differing significantly from the M2 when compared with purslane-leaves and spinach-whole plant. The results of the FAAS analysis indicate a minor difference between the evaluated extraction methods, suggesting less interference when using this technique compared to UV-vis spectrophotometry.

In addition, M3 can be a better option due to the use of more dilute acid and shorter extraction time compared to M2, making it safer for analysts and contributing to Green Chemistry.

Conclusions

The comparison of oxalic acid extraction and quantification methods described in the literature and modified in this research can help to obtain more reliable results on the contents of these antinutrients in matrices of purslane (*Portulaca oleracea* L.) and spinach (*Spinacea oleracea*). The precipitation step was essential in reducing the influence of matrix interferents during oxalic acid quantification. Extraction using diluted acid (0.25N HCl) for a short time (15 min) resulted in lower result fluctuations. Additionally, the absence of interference from colored compounds in matrices is an advantage of using FAAS. Therefore, employing more dilute acid for a shorter duration and quantifying by FAAS, as demonstrated in M3, resulted in better performance for determining oxalic acid content, besides contributing to Green Chemistry.

Limitations

Not applicable**.**

Ethics statements

Not applicable.

CRediT author statement

Pâmela Souza: Conceptualization, Methodology, Software Prisma, Data curation, Writing- Original draft preparation.: **Juliana Pallone:** Supervision, Writing-Reviewing.: **Eduardo Orlando.:** Methodology, Investigation, Data curation.: **Augusto Costa-Santos:** Data curation, Writing-Reviewing and Editing.: **Ellen Ayres:** Visualization, Writing-Reviewing.: **Amauri Rosenthal:** Visualization, Writing-Reviewing.: **Anderson Teodoro:** Supervision, Writing-Reviewing.

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Declaration of Competing 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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CONCLUSÃO GERAL

O presente trabalho demonstrou os resultados das características físico-químicas, potencial bioativo e características sensoriais das formulações de suco misto de laranja, cupuaçu e beldroega desenvolvidas. A formulação com 60% de laranja, 30% de cupuaçu e 10% de beldroega apresentou bom potencial antioxidante e aceitabilidade sensorial, sendo uma boa escolha para a produção do suco misto.

Os testes realizados para a determinação de ácido oxálico possibilitaram encontrar o melhor método de extração e de quantificação, dentre os testados, deste antinutriente, sendo a Espectrometria de Absorção Atômica com Chama uma boa alternativa, com menor interferência de compostos presentes na matriz.

O processamento por Alta Pressão Hidrostática 400 MPa/10 min foi uma boa alternativa ao método de pasteurização, com a preservação do potencial bioativo e redução de ácido oxálico nas bebidas processadas.

A Homogeneização à Alta pressão, além de preservar o potencial antioxidante e ser eficiente na redução de ácido oxálico, melhorou a bioacessibilidade do magnésio, sendo uma boa técnica de processamento a ser aplicada para o produto desenvolvido.

O desenvolvimento de sucos mistos contendo frutas nativas brasileiras e PANC em suas formulações, aliado a processamentos por tecnologias de alta pressão contribuem para produções mais sustentáveis.

CONSIDERAÇÕES FINAIS

A partir do trabalho produzido, outras pesquisas poderão ser realizadas como o estudo de estabilidade microbiológica e física da formulação de suco misto eleita (60% de laranja, 30% de cupuaçu e 10% de beldroega), a caracterização físico-química e estudo de bioacessibilidade de minerais do resíduo obtido do suco de beldroega (que se encontra em andamento), e estudos dos consumidores sobre a incorporação de PANC em sucos mistos. Dessa forma. uma pesquisa completa sobre o produto desenvolvido poderá impulsionar a comercialização de sucos mistos com PANC e espécies de frutos nativos, contribuindo para o fortalecimento da agricultura familiar e a valorização da biodiversidade brasileira.

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APÊNDICE

Para fins de cumprimento de pré-requisito da disciplina Elaboração de Artigos, segue anexado à tese a seguinte publicação:

Food neophobia, risk perception and attitudes associations of Brazilian consumers towards Non-Conventional Edible Plants and research on sale promotional strategies

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Abstract

Non-conventional edible plants (NCEP) are plants or parts of plants that are not usually consumed by the population and have limited geographic distribution. This study investigated the consumption of NCEP, the influence of Food Neophobia and risk perception on Brazilian consumers attitudes as well as purchase preferences to determinate the best sale promotional strategy. Participants (n=271) answered the online questionnaire that consisted of socio-demographic questions, items about consumption (open questions), Food Neophobia Scale, risk perception (to assign the level risk) and attitudes towards NCEP (using 5-point Likert scale). Fisher's exact test was used to investigate possible associations. Task purchase choice was evaluated using the Best-Worst scale.The most consumed conventional leafy vegetables were collard greens (*Brassica oleracea* L. var. *acephala* D.C) (95.6%) and lettuce (*Lactuca sativa*) (88.5%). As for NCEP, taioba (*Xanthosoma taioba* E.G) (26.7%), bertalha (*Basela alba* L.) (23.3%) and beldroega (*Portulaca oleracea* L.) (14.1%) were the most cited leafy vegetables. High food neophobia individuals demonstrated to try NCEP if its nutritional value and safety are proven, showing a demand for such strategy, this would increase the consumption of these vegetables. The perception of a high risk was also associated with the inclusion of NCEP in the diet. The inclusion of NCEP at the moment of purchase, even at a higher price, presented higher scores when compared to the purchase of only conventional vegetables, at lower prices. Despite this, the price can still be a limiting factor and the search for promotional strategies should be reinforced to increase the commercialization of NCEP at street markets.

Keywords: non-conventional leafy vegetables, sustainable eating, healthy eating, food neophobia, risk perception, purchasing choice, best-worst scaling.

Footnote

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1. Introduction

Brazil has the greatest biodiversity on the planet, exhibiting more than 46,000 plant species in its territory (MMA, 2017). Even in the face of the vast Brazilian biodiversity, only a few species, most of them exotic (from other countries), are used in diet (Tuler, Peixoto & Silva, 2019).

The valorization of biodiversity can be seen as a sustainable solution for reducing hunger and malnutrition, as well as for mitigating the environmental impacts generated by the monoculture system. In 2015, the United Nations created the Sustainable Development Goals, which include an agenda with 17 goals to be achieved by 2030, with the aim of appealing worldwide to actions for poverty eradication, protection of the environment, climate and reaching peace and prosperity of the population. Goal # 2 is aimed at eradicating hunger, achieving food and nutrition security, as well as promoting sustainable agriculture (Brazil, 2021).

Non-conventional edible plants (NCEP) are plants or parts of plants that have restricted use and limited geographic distribution, not being organized in the production chain such as conventional vegetables (potatoes, tomatoes, cabbage, among others) (Brazil, 2010).

This term is used in Brazil, but some NCEP such as beldroega is used and studied in other coutriens. This plant is used in Medicine Traditional Chinese for diuretic, febrifuge, antiseptic, and antispasmodic treatments (Chan et al., 2000; Xiang et al., 2005). Pharmacological effects of this plant such as anticancer and anti-inflamatory are related in literature (Zhao et al., 2013; Zhao et al., 2015; Souza et al., 2022). In Brazil many studies about NCEP have been developed with themes related to food product development, evaluation of nutritional composition, bioactive compounds and pharmacological effects (Teixeira et al., 2019; Machado et al., 2021; Hoff et al., 2022).

The cultivation and commercialization of NCEP contribute to the creation of healthy and sustainable food systems, coupled with the nutritional quality of the species and easy production, being an alternative that promotes greater sustainability and may contribute to achieving some of the objectives of the 2030 Agenda, regarding food and sustainable production, and food and nutrition security (Mariutti et al., 2021).

In this sense, the use of NCEP as an eating habit has several advantages for the health of both the planet and consumers aiming the strength of biodiversity and diversification of foods on the plate, in addition to many species of NCEP having high nutritional value and functional properties (Kinupp & Lorenzi, 2014; Mehwish et al., 2020; Oliveira, Wobeto, Zanuzo, & Severgnini, 2013; Petropoulos et al., 2016; Viana et al., 2015).

The increase in the consumption of NCEP by the population can be strengthened through the implementation of strategies such as the attribution of values of healthy and sustainability for these underused species. Indeed, the attribution of values of healthy and sustainability are extrinsic factors that can affect consumer's sensory perception and positively impact the purchasing choice (Barbosa et al., 2021; Pambo et al., 2018; Silva, Bioto, Efraima, & Queiroz, 2017; Vidigal et al., 2011).

Price is an important variable in consumers' purchase decision (Lima-Filho et al., 2008). However, the importance of the price factor for the consumer is associated with the perception of value and quality of the products (Lima-Filho et al., 2013). Another factor that can influence the consumption of NCEP is the degree of consumers' neophobia. The term "non-conventional" referring to non-conventional food plants, as well as the unfamiliarity of a food can influence the perception of neophobic consumers by association with the new or unknown (Barbosa et al., 2021). In fact, the concept of food neophobia refers to the resistance to try out new or unfamiliar foods, which can be associated with each individual's knowledge and cultural experiences, so that exposure to different cultures can reduce the levels of neophobia (Flight, Leppard, & Cox, 2003; Pliner & Hobden, 1992).

Among the parameters that affect the purchase decision, risk perception can also be pointed out as an important factor (Andrade, Deliza, Yamada, & Galvão, 2013). Antinutrients are defined as compounds or classes of compounds that can reduce the nutritional value of foods, interfering with digestibility, absorption or use of nutrients, and have a toxic effect if ingested in high concentrations (Santos, 2006). Methods such as blanching, dehydration, grinding, milling, soaking, cooking, germination, fermentation, roasting, frying, extrusion, peeling and genetic improvement have been used to eliminate or reduce antinutrients in food matrices (Higashijima, Lucca, Rebizzi, & Rebizzi, 2020).

The fear of trying new foods may be associated with a lack of information about safety and toxicity. For this, it is necessary to know the perception of risk attributed to the consumption of NCEP, which are considered non-conventional foods, besides the fact that information concerning their possible toxicity or methods aiming to reduce their toxic substances and antinutrients may still be unknown by consumers.

Thus, this study aimed to evaluate the associations between food neophobia degree, risk perception and attitudes towards NCEP to identify the factors that may limit the consumption of these vegetables. Besides, provide contributions to find strategies to increase the comsumption of NCEP, such as sustainability and healthiness claims and safety. Furthermore, this the influence of price on purchase choices and the promotional strategies were also investigated and whether it may help family farmers to sell NCEP and promote the consumption of these plants by Brazilian consumers.

2. Material and Methods

2.1 Participants

This study was approved by the Research Ethics Committee (REC) of the Federal University of the State of Rio de Janeiro (UNIRIO), CAAE: 15336719.8.0000.5285, and the respondents expressed their agreement to participate through the Free and Informed Consent Term (FICT).

The questionnaire was available for response from December 2020 to January 2021. In total, 275 participants over 18 years old fully answered the survey questions. As a criterion for participation, only Brazilians residents were accepted. Four individuals were excluded. So, finally there was a total of 271 participants. Respondents were recruited through social media (Facebook, Instagram or WhatsApp) when they received a survey invitation with a link to access the questionnaire. At end, after the acknowledgments, the participant was asked to forward them to other known people following the snowball method (Bienarcki & Waldorf, 1981) for sampling the consumers.

2.2. Questionnaire

The online questionnaire was created on the Google Forms platform, and covered socio-demographic questions, items about the consumption of dark green leafy and NCEP, food neophobia scale, risk perception, sentences created to investigate possible associations between food neophobia, risk perception and attitudes about NCEP. Lastly, purchase choice task using the best-worst scale was contemplated.

2.2.1. Comsumption of dark greens leafy and NCEP

Open and randomized three sentences created by the authors about the consumption of dark greens leafy and NCEP were developed. The sentences were: 1- “Which dark green leafy vegetables do you usually eat? Select all the options that apply, and if you select "other", please describe the name(s) of the leafy in the blank.” These answers were used to identify which vegetables was conventional or non-conventional and the difference of consumption among them. 2- “Which one is your favorite?” 3- “How often do you consume dark green leafy?” Sentences about the knowledge of the NCEP term and their consumption were also asked in this section: 1 – “Have you heard about the term Non-Conventional Edible Plants?”. 2 – Have you already consumed Non-Conventional Edible Plants?”. 3 – “If yes, which one(s)?”. 4- “Where did you buy it?”.

2.2.2. Food Neophobia Scale

To assess the degree of participants' neophobia, the Food Neophobia Scale (FNS) (Pliner & Hobden, 1992), translated into the Brazilian Portuguese version by Previato and Behrens (2014), was used in this study. The FNS is composed of 10 items presented as statements, which were evaluated using a 7-point Likert scale (1=totally disagree; 2=moderately disagree; 3=slightly disagree; 4=neither disagree; nor agree; 5=slightly agree; 6=moderately agree; 7=totally agree). The total score was calculated for each participant, then classified as low Food Nephobia (sum ≤ 25) and as a high Food Neophobia (sum ≥35) (Flight, Leppard, & Cox, 2003), whith the theorical possible scores ranging from 10 - 70 points (Pliner & Hobden, 1992).

2.2.3. Risk perception

Participants were asked to assign the risk level when consuming NCEP (dark green leaves). For this, risk to health, the following response options were risky: "high to health, "moderate to health", "indifferent", "poor to health" and "none to health".

2.2.4. Consumers' attitudes towards NCEP

Six attitudinal sentences were presented to participants: Sentence 1: "If the nutritional value was proven, I would try a non-conventional edible plant.", Sentence 2: "I would certainly include non-conventional edible plants in my eating habits.", Sentence 3: "I prefer to consume conventional vegetables to non-conventional edible plants.", Sentence 4: "I would like to learn more about non-conventional edible plants.", sentence 5: "Heat treatment (such as cooking) can be used to reduce the level of toxic substances in dark green vegetables." and Sentence 6: "If safety was proven, I would try a non-conventional edible plant.". The Sentences 2, 3 and5 were created by the authors while the Sentences 1, 4 and 6 were elaborated by Marques et al. (2022) (unpublished data). The 5-point Likert scale (1 = totally disagree; 2= disagree; 3 = neither disagree; nor agree; 4= agree; 5 = totally agree) ranging from strongly disagree (1) to strongly agree (5) was used in this task (Likert, 1932).

The use of sentences created by the authors is due to the possibility of investigating participants' attitudes about NCEP, using words that could be understood easily.

2.3.5 Food purchase choice task

In the food purchase choice task using the best-worst scaling method (Jaeger et al., 2008; Adamsen et al., 2013), consumers were presented with some priced plant choice options and were asked to select their best and worst purchase option, simulating a real situation at a point of sale (for example, at a farmers' market).

A complete experimental design with two variables was carried out totaling 6 possible prototypes (options). The variables investigated were: familiarity of the plant (familiar "conventional" or non-familiar "non-conventional" or both) and price (low price US$0.70 or high price US$1.14). The six prototypes used were (P1 = conventional -

US$0.70/ bunch; P2 = non-conventional - US$0.70/ bunch; P3 = conventional + non-conventional - US$0.70/ bunch; P4 = conventional + non-conventional - US$1.14/ bunch; P5 = non-conventional - US$1.14/ bunch; P6 = conventional - US$1.14/ bunch). A prototype corresponded to a text and a value (for example, P3 = conventional + non-conventional and US$0.70/ bunch). The use of text was preferred than image, because the selection of the image could impact the answer depending on the chosen plant, the quality of the photo, the angle/framing of the photo, or another reason. A particular plant was also not chosen because it could influence in consumer preference. Therefore, only the “conventional” or “non-conventional” text was used.

Table 1. Complete experimental design and protypes

Participants received a set of three prototypes at a time for evaluation in the task. Each consumer performed eight evaluations of eight sets of prototypes, which were presented in a balanced way (Macfie & Bratchell, 1989). One example of a prototype set evaluated by the respondents can be seen in Figure 1. In total, 134 consumers out of the 271 evaluators completed this task correctly and were considered for this part of study

Figure 1. Example of a prototype set assessed in the food purchasing choice task.

2.3. Statistical analyses

2.3.1 Fischer's exact test

Contingency tables and Fisher's exact test were performed to verify the associations between Food Neophobia, risk perception and consumers attitudes towards NCEP. Other variables also considered for associations. The Significance level was set at $p < 0.05$. All data analyses were performed using Xlstat software (Paris, França, 2020.5.1).

2.6.2 Best-worst scaling

For the statistical analysis of data obtained from the best-worst scaling, the calculation of the difference between best (B) and worst (W) scores for each prototype was used, and the average was calculated from the number of times each attribute (variable) was chosen as the “best” and “worst” option, the number of respondents (n) and the frequency of each prototype in the design of the set of choices (options) (r) (Adamsen, 2013; Liu, Li, Steele, & Fang, 2018). The formula is expressed as the following equation:

$$\text{Average} = \frac{\text{best - worst score}}{r \times n}$$

B-W scores were also indexed as an index, with the preferred prototype being related to the index 100, and the remaining prototypes receiving indices relative to the preferred prototype (Adamsen 2013; Cohen, 2009; Auger, Devinney, & Louviere 2007). Coefficients resulting from these prototypes measured the probability of choice compared to the preferred prototype.

3. Results and discussion

From 271 survey participants, 79% (n=214) were women and 20.3% (n=55) men, all aged over eighteen years old. The representative participation of women in this study may be associated with greater concern and gender responsibility in the activities related to the choice and purchase of food for the family. Socio-demographic characteristics are showed in Table 2. Possible associations between food neophobia and socio-demographic data were evaluated. Regarding age, young adults tended to show low neophobia, Individuals aged 21 to 30 years old had an association with low (p=0.042) and medium neophobia (p=0.032), while for individuals aged 31-40 years, there was an association with low neophobia (p=0.032). This result may indicate that 21-40 years old is less resistant to knowing and consuming new foods, while more younger individuals may be more selective in terms of consumption and food choice. These results agree with the data already predicted in the literature that demonstrate that food neophobia tends to decrease with increasing age (Pliner & Hobden, 1992). Participants in high school and post-graduate studies had and association with low (p=0.003 and p=0.027, respectively) and high neophobia (p=0.013 and p=0.001, respectively). These results can depend on the area and the topics covered in the education of these individuals. High neophobia (p=0.002) associations was also found to individuals with income equal to or above ≥ US$ 1.185.

Table 2. Socio-demographic characteristics of the participants (n=271).

Regarding risk perception, there was an association (p=0.000) between individuals aged 21 to 30 years old with the attribution “no health risk”, while for individuals aged 60 years old or older, there was an association (p=0.034) with the option “little health risk”. However, there was an association (p=0.04) between “no” answers when participants were asked if they had ever consumed NCEP and the attribution of “moderate health risk”. From these results we can infer that the lack of knowledge about the safety of these non-conventional leafy vegetables can result in an obstacle for the consumption of NCEP by the population. Scientific dissemination on nutritional information, as well as safety and toxicity, must be strongly present so that the population can gain more knowledge about the species and methods that can be used to reduce the level of possible toxic substances.

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3.1 Consumption of conventional dark green leafy vegetables and NCEP

Most participants (36.7%) of the survey reported the consumption of dark green leafy vegetables 3-4 times a week, 24.4% on a daily basis, while 26.7% of them consumed 1-2 times a week, 7% on every 15 days, 2% once a month, 1% consumed 5 days a week and 1% never consumed. Regular and sufficient consumption of fruits and vegetables is recommended by the Food Guide for the Brazilian population, and it is associated with the prevention of non-communicable chronic diseases (Brazil, 2014; WHO, 2004). Considering that the present study only evaluated the frequency of consumption of dark green leafy vegetables, among the universe of fruits and vegetables, it can be observed that ca. 61.1% of the respondents consume them more than twice a week, with most of this percentage being responsible for daily consumption. Thus, these results showed that the research volunteers are regular consumers of dark green leafy vegetables, which was desirable to get the potential consumer public of NCEP dark green leafy. In addition, certain factors may be associated with an adequate consumption of vegetables. Culinary skills, emotional well-being, being female, having better socioeconomic conditions, as well as a higher level of education, and not drinking or smoking, are associated with greater chances of having healthy eating habits, with higher consumption of vegetables (Gomes et al. al., 2022; Jomori et al., 2022; Rower et al., 2017). Therefore, the profile of the participants (mostly women and with a high level of education) may have contributed to these findings of frequency of consumption of vegetables in the respondents' diet.

Among species of dark green leafy vegetables normally consumed by participants, 37 are NCEP (being 8 species with the most frequency of citation) and 14 are conventional leafy vegetables (being 7 species with the most frequency of citation). In fact, the most cited dark green leafy vegetables (above 5% of the frequency of citations), consumed on a regular basis, are shown in Table 3. Participants could cite more than one option to this sentence. The most cited conventional leafy vegetables were collard greens (*Brassica oleracea* L. var. *acephala* D.C) (95.6%) and lettuce (*Lactuca sativa*) (88.5%). According to a study carried out by Canella et al. (2018), the green leafy vegetables with the highest share of household purchases in Brazil were cabbage, followed by lettuce. Regarding the NCEP, among non-conventional species, taioba (*Xanthosoma taioba* E.G) (26.7%), bertalha (*Basela alba* L.) (23.3%) and beldroega (*Portulaca oleracea* L.) (14.1%) were the most cited ones. When subjects that participated in the survey were asked about their favorite leafy vegetables and 21.8% of them cited *Brassica oleracea* L. (conventional vegetable). Among the 22 leafy vegetables reported as preferred, 8 species were the most cited ones (above 2% of the citation frequency). The citation frequency of leafy vegetables indicated as favorite(s) by consumers is shown in Table 4.

Table 3. Mostly consumed conventional and non-conventional edible leafy among participants (n=271).

Table 4. Conventional and non-conventional edible leafy indicated as favorite (n=271).

The participants' level of knowledge about the meaning of the term NCEP was measured, and it was observed that 83% of them knew it, while 17% responded they did not know it. Results of a study realized in Brasília (Distrito Federal, Brazil) showed that 53,5% of respondents did not know the term NCEP, while 46.2% stated to have knowledge (Silva et al., 2022). This difference observed for a local survey may be associated with cultural and socioeconomic factors that are more restricted to the region.

In the present study, 189 participants reported to consume NCEP and informed which already consumed. When participants were asked if they have ever consumed NCEP, 72% of them answered “yes”, 10% replied “no” and 18% reported “I do not know”. As the concept of NCEP is relative, that is, for some regions the consumption of certain species may be usual, while these same species may be underused in other areas, doubts may have arisen when questions about the consumption of NCEP were asked. Interestingly, regarding knowledge on and consumption of NCEP, among residents of Brasília (Federal District), 53.5% of the respondents answered they did not know the term, while 46.2% answered positively. There was an association (p=0.04) between “no” answers when participants were asked if they had ever consumed NCEP and the attribution of “moderate health risk”. From these results we can infer that the lack of knowledge about the safety of these non-conventional leafy vegetables can result in an obstacle for the consumption of NCEP by the population.

The consumption of different NCEP varies according to the cultural aspects of each region and the favorable conditions (environmental factors such as climate and soil) for the cultivation of certain species. Thus, some NCEP will possibly be consumed only in some regions. Questions only about NCEP (not just for leafy vegetables, but for any non-conventional edible vegetable part), among these, it asks which NCEP have already been consumed by the participants. From results direct to sentences about NCEP, among the 138 species of NCEP already consumed by participants in this study, 11 were cited more often - above 5% of the citation frequency (Table 5).

Table 5. Non-conventional edible plants already consumed among participants (n=189).

Among the most cited NCEP, *Xanthosoma tai*oba E. G. Gonç. presented the highest citation frequency (46.0%), followed by *Pereskia aculeata* Mill. (44.9%), *Portulaca oleracea* L. and *Stachys byzantina* K. Koch - both with 26.5%, *Tropaeolum majus* L. (23.3%), *Amarathus deflexus* L. (14.8%) and *Rumex acetosa* L. (11.11%). Different results can be found in different regions in the country. In Silva et al. (2022), from 170 respondents in the capital of Brazil (Brasília, Distrito Federal, Brazil), the majority declared to consume Apium graveolens (42.9%), folowed by *Pereskia aculeata* Mill. (42,35%), *Xanthosoma taioba* E. G. Gonç. (29.4%), *Stachys byzantina* K. Koch (14.7%), Physalis pubescens L. (12.3%), *Anredera cordifoli* (9.4%), *Tropaeolum majus* L. (8.23%), *Portulaca oleracea* L. (5.29%) and *Sonchus oleraceus* L (3.5%).

In table 6, data show where and how NCEP are acquired by consumers. Only 182 from participants indicated the sites where they did so. Seven consumers that declared consuming NCEP did not answer this question. According to the results obtained, most of these non-conventional vegetables are cultivated at home (vegetable garden using vases) or in family farms, with a citation frequency of 48.9%, followed by purchase in organic farmers market (26.4%). The other places mentioned by the participants are presented in Table 6. The category “others” refers to citations such as: garden, website, organic baskets, community vegetable gardens and establishments that sell natural products.

Organic trade fairs still do not represent a high percentage for the purchase of NCEP by consumers. These results emphasize the need for strategies to promote the commercialization of NCEP at these sales sites or at in agroecological fairs, providing a short sales channel.

3.2 Food Neophobia level

According to results obtained from FNS, 40% (n=108) of the participants had low neophobia, while 34% (n=92) and 26% (n=71) of the individuals presented medium and high neophobia, respectively. In reality, respondents with high neophobia, those who could hence show greater resistance to the introduction of NCEP into their diet, represented only a small proportion of the participants.

3.3 Risk perception

Regarding the results assessed for risk perception, 44.6% (n= 122) and 33.2% (n=89) of the subjects attributed the consumption of NCEP to no or little health risk, respectively. Only a small part of the participants related the consumption of NCEP to indifferent (16.6%; n=45), moderate (5.2%; n=14) and high health risk (0.4%; n=1).

3.4 Influence of Food Neophobia and risk perception on consumers attitudes towards NCEP

No associations (p=0.17) were found between risk perception and food neophobia. Participants (n=271) answered the attitudinal sentences depicted in sub-item 2.2.4 using the 5-point Likert scale. According to results obtained (Table 7), most participants (77.46%) strongly agreed with Sentence 1 - “If the nutritional value was proven, I would try a NCEP”. After investigating the association of the level of food neophobia with the sentences about consumers' attitudes towards NCEP. Participants classified as having a high degree of neophobia showed a statistically significant association with the attributions "Agree" (p<0.0001) and “Totally agree” (p=0.005) given for the Sentence 1. Thus, the diffusion of nutritional information about NCEP can be used as a strategy to reduce the resistance of individuals with a high degree of neophobia to try out NCEP. The use of the term “NCEP” can have a negative effect on individuals who are unfamiliar with a non-conventional food and the implementation of strategies can complement the use of the term, such as the dissemination of information about the ecological, social and healthiness, increase expectative to prove by individuals who are unaware of NCEP or have a high degree of neophobia (Barbosa et al., 2021). There was no association between this sentence and the risk perception (Table 8).

Table 7. Associations of Food Neophobia and consumers attitudes towards NCEP.

Table 8. Associations of risk perception and consumers attitudes towards NCEP.

Regarding the Sentence 2 – “I would certainly include Non-Conventional Edible Plants in my eating habits.”, 77.86% of participants strongly agreed. Some participants (n=53) classified with high neophobia showed an association with the attribution “Totally agree” (($p<0.0001$) and “Agree” ($p=0.021$) to this sentence (Table 7). Investment in more strategies to increase NCEP consumption should be implemented to increase adherence by these individuals. Individuals with low neophobia are also associated with the “Totally agree” to this sentence ($p=0.000$). The attribution of the term “None health risk” had an association ($p=0.02$) for the term “Totally agree” while was observed association for attribution “High heath risk” for the term “disagree” to this sentence. Thus, the inclusion of NCEP in the diet by individuals that attribute high health risk to these vegetables will be more difficult.

Individuals that attributed “none health risk” and “poor health risk” had an association ($p=0.000$ and $p=0.025$, respectivelly) for the term “totally disagree” to Sentence 3 – “I prefer to consume conventional vegetables to non-conventional edible plants” (Table 8). However, 39.11% of respondents answered “neither disagree, nor agree” to this sentence and had an association ($p=0.026$), which infers that consumers are not willing to have a greater intake of NCEP than of conventional vegetables. It is expected that by improving the acquisition of knowledge, there will be no preferences between the two types of vegetables.

Furthermore, for Sentence 4 – “I would like to learn more about non-conventional edible plants.”, 79% of the participants answered they strongly agreed regarding their interest in learning more about NCEP. Even for individuals who attribute a low degree of risk to NCEP, there is an association ($p=0.028$) with an interest in learning more about these vegetables. This may be associated with several motivations, such as culinary preparations, acquisition of more species for food, among other reasons. This sentence had no association ($p=0.07$) with food neophobia.

Most participants (ca. 70%) reported they agreed with Sentence 5-“Heat treatment (such as cooking) can be used to reduce the level of toxic substances in dark green vegetables.” (Table 7). These answers demonstrate they have consciousness at some level about the risk on its consumption regarding food security and culinary knowledge (or a way to) on how to diminish such risk. Some methods such as cooking, boiling, high isostatic pressure, enzymatic treatments, among others, are used to reduce the level of anti-nutritional factors and toxic substances in vegetables (Higashijima et al., 2020). Associations were observed between attributions to “none health risk” and “totally disagree” ($p=0.031$) to Sentence 5. This result shows that there is still a lack of knowledge, although by a small number of participants, about the methods of reducing toxic substances in dark green vegetables. It is important to bring this knowledge to consumers so that scientifically sound information can be widely disseminated regarding the effects of exposure to toxic substances present in food, which, depending on the amounts, can confer toxic effects, often in the long term deadline.

Regarding Sentence 6 – “If safety was proven, I would try a non-conventional edible plant, the vast majority of participants (approximately 90%) agreed with the statement, which indicates that the inclusion of NCEP in the diet by the respondents depends on the availability of proof(s) that these foods are safe indeed. Most participants showed concern about the security before trying the NCEP (accordingly this sentence). In reality, NCEP comprise a group of highly diverse species and varieties, and those containing toxic compounds must undergo appropriate domestic or technological processing, so that they can be considered safe for human consumption. Thus, it is important to stress out that cooking food, for instance, is a possible alternative to make them suitable for consumption by inactivation of microorganisms and/or toxic substances. Individuals with high degree of neophobia showed associations with the attributions of the terms "Totally agree" (p=0.000) and "Agree" (p=0.003) for Sentence 6. This result is very interesting, as it is a sentence of attitude conditional on the consumption of NCEP, and which shows the intention to try these vegetables by individuals with food neophobia. The result reinforces the importance of proof of safety to increase adherence. In addition, proof of safety is also an important fator by individuals with low degree of neophobia and is associated (p=0.013) with the condition to try a NCEP. There is a clear indication that respondents could overcome their resistance and even increase their interest towards the consumption of these vegetables. In terms of strategy, it is important to bring this knowledge to consumers about which vegetables have toxic effects and which methods can be used to reduce these compounds.

Participants who attributed “Indifferent” for the risk level had an association (p=0.04) for the agreement to Sentence 6. Scientific dissemination on nutritional information, as well as safety and toxicity, must be strongly present so that the population can gain more knowledge about the species and methods that can be used to reduce the level of possible toxic substances.

3.5. Best-Worst scaling task outcomes: a trade on what is more relevant?

The preference results of participants (n=271) obtained from the best-worst scaling task are shown in Table 9. According to our results, prototype 3 (conventional and non-conventional for low price) was the preferred one, with a B-W score of 417, followed by prototypes 4 (conventional and non-conventional for high price) with a B-W scores of 123 and followed by prototype 2 (non-conventional for low price) with a (B-W score of 97). Furthermore, prototypes 1, 5 and 6 exhibited B-W scores of -54, -214 and -369, respectively. A positive result indicate that a prototype was chosen more frequently as the best, while a negative result means that a prototype was selected as the worst (Adamsen, 2013). Prototypes 4 and 2 had the same probability of being chosen.

Among the options with only one type of vegetable, the option encompassing NCEP presented the highest M-P scores, for low and high prices, inferring a greater preference for the acquisition of NCEP over conventional vegetables at the moment of purchase. Thus, it would be interesting to create promotional strategies to increase the commercialization of NCEP by producers. In fact, a study carried out in Australian markets showed that promotional strategies are made to favor the purchase of unhealthy foods (Grigsby-Duffy et al., 2020). The authors also highlighted the need for promotional strategies to strengthen the commercialization of healthy and sustainable foods. The present study demonstrates that the possibility to purchase NCEP sold combined with conventional vegetables ("combo" mode) can be attractive to consumers at the time of purchase, both at a low and high price.

Accordingly with the results, this can be a guideline to assist family farmers in the commercialization of NCEP in agroecological or organic fairs, as an initial tactic to present the NCEP to the public and later have the commercialization only NCEP and work with pricing strategies.

The increased dissemination on different media of the nutritional and toxicity properties of NCEP for consumers, as well as the importance of introducing these non-conventional foods into the eating habit for the preservation of the biodiversity must be increasingly reinforced, aiming to increase the level of knowledge and interest in the acquisition of these foods. Therefore, the use of informational communication resources for consumers in the NCEP's sales outlets or using the Internet of Things (IoT) can be a means of awakening or interest in consumption by the population. A survey was carried out with to evaluate the main offer elements that have the greatest impact on consumers' preference and acceptance of organic food products (Melovic et al., 2020). According to these authors, price, distribution channel – such as specialized organic stores or the internet –, and modern media as an instrument of promotion have an important impact on consumers' perception and purchase decisions. In the current study, prototypes 4 and 2 had the same probability of being chosen after prototype 3. Subsequently, prototype 1 stood out, followed by prototypes 6 and 5.

4. Limitations

The online research was launched during the covid-19 pandemic. Despite, the fact that most of the population in Brazil was still under social isolation restrictions, there was a low adherence of consumers on participating in the study. Besides, respondents were not homogenously distributed nationwide (data not shown), meaning that regionality was not able to be assessed nor emphasize the country`s diverse cultural and eating habits. NCEP in the Southeast might not be a NCEP in the North region of the country, for instance. Regardless such critical concept of NCEP, the study was preliminary and the first to focus on purchase choice task to assess how NCEP may be a stimulus when shopping vegetables. Despite data demonstrated that there are different profiles of the population with diverse motivations of consumption of NCEP, the research relevance is on proposing strategies to promote NCEP commercialization and consumption encouraging consumers to eat them, but that still require further studies.

5. Conclusion

This study allowed evaluate the best promotional strategies that can be used by family farmers in agroecological fairs to increase the commercialization of PANC. The knowledge about the consumer's perception of the sustainability and health attributes of these non-conventional vegetables can help to understand the influence on the purchase decision of these species by consumers. The dissemination of informations about these attributes as well as the safety and the methods that can reduce the toxic substances and anti-nutritional factors specific to each species can encourage an increase in purchases.

. The high degree of neophobia was associated with the condition of trying a non-conventional leafy vegetable if the nutritional value and safety were proven, according to survey results, what highlights the importance of disseminating information about NCEP to overcome that resistance, leading to higher levels of consumption of these food products by neophobic individuals. Moreover, participants who declared not to consume NCEP were associated with the attribution of moderate health risk – according to the risk perception scale –, which means that, as they are unfamiliar foods, may cause some resistance by the participants due to the lack of knowledge about the safety of these vegetables, reinforcing the importance of disseminating information about safety and processing methods for the reduction of toxic compounds as a sales strategy. Individuals with a high degree of risk were associated with disagreement about including these vegetables in their diet, which also reinforces the importance of more scientific disclosure about safety.

The associations in this study for food neophobia, risk perception and consumer attitudes towards NCEP will help to promote strategies to increase the consumption of these vegetables. Proof of nutritional value and safety are important factors and could be used in strategies to increase consumer adherence.

The promotional strategy to bring two familiarity options (conventional + unconventional), for a relatively higher price, represented by prototype 4, to the detriment of a single option (conventional or unconventional) for a low price, such as prototypes 1 and 5, suggests that consumers are willing to pay a slightly higher price to have both options when buying vegetables. These results will provide guidance for the development of pricing strategies that aim to increase consumer adherence and the consequent improvement in the commercialization of these non-conventional vegetables.

CRediT authorship contribution statement

Pâmela Gomes de Souza: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Denise Rosane Perdomo Azeredo:** Conceptualization, Investigation, Methodology, Supervision Writing – view & editing. **Thadia Turon Costa da Silva:** Formal analysis and Supervision. **Carla da Silva Carneiro:** Formal analysis and Supervision. **Anderson Junger Teodoro:** Formal analysis and Supervision. **Ellen Mayra Menezes Ayres:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – view & editing.

Declaration of Competing 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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Appendix

Table A.1

Table 1. Complete experimental design and protypes.

Prototype	**Familiarity of the leaf bunch**	**Price**
P1	Conventional	US$0.70
P2	non-conventional	US$0.70
P3	conventional + non-conventional	US$0.70
P4	conventional + non-conventional	US$1.14
P5	non-conventional	US$1.14
P6	conventional	US$1.14

Fig. A.1

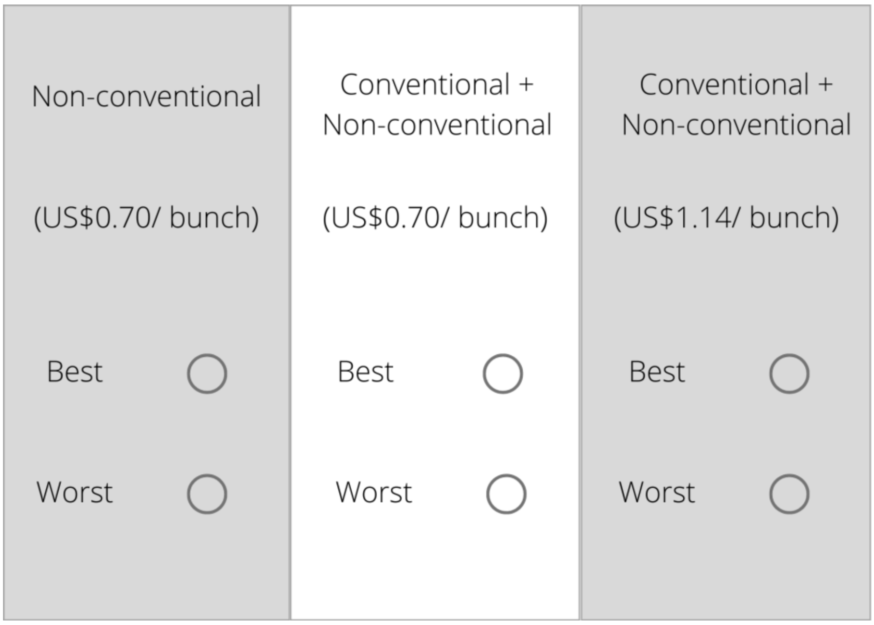

Figure 1. Example of a prototype set assessed in the food purchasing choice task.

Table A. 2.

Table 2. Socio-demographic characteristics of the participants (n=271).

Characteristic	Number of participants			
	Total sample	Food Neophobia		
	(n=271)	Low (n=108)	Medium (n=92)	High (n=71)
Gender				
Female	214 (79%)	93	73	48
Male	55 (20.3%)	19	15	21
I prefer not to declare	2 (0.7%)	-	1	1
Age (years)				
18 - 20	7 (3%)	3	2	2
21-30	63 (23%)	19*	28*	16
31-40	55 (20%)	30*	16	9
41-50	46 (17%)	21	15	10
51-59	56 (21%)	26	13	17
>60	44 (16%)	13	15	16
Education				
High school	52 (19.2%)	12*	19	21*
University degree	91 (33.6%)	38	25	28
Post-graduate studies	128 (47.2%)	62*	45	21*
Income (monthly)				
≤ US$ 237	19 (7%)	4	7	8
US$ 237 - 474	24 (9%)	11	8	5
US$ 474 - 711	49 (18%)	19	12	18
US$ 711 – 948	27 (10%)	11	7	9
US$ 948 – 1.185	32 (12%)	12	10	10
≥ US$ 1.185	120 (44%)	55	45	20*

Note. *p<0.05 for Fisher's exact test.

Tabela A.3

Tabela 3. Mostly consumed conventional and non-conventional eadible leafy among participants (n=271).

Popular names			
Conventional leaves	**Species name**	**Number of Citations**	**Frequency (%)**
Collard gress	*Brassica oleracea* L. var. *acephala* D.C	258	95.6
Lettuce	*Lactuca sativa*	239	88.5
Spinach	*Spinacia oleracea*	187	69.3
Rocket	*Eruca sativa*	185	68.5
Watercross	*Nasturtium officinale*	168	62.2
Chicory	*Cichorium endivia* var. *latifolium*	86	31.9
Endive	*Cichorium intybus* var. *intybus*	79	29.3
NCEP			
Taioba	*Xanthosoma taioba* E. G. Gonç.	72	26.7
Bertalha	*Anredera cordifolia* (Ten.) Steenis	63	23.3
Beldroega	*Portulaca Oleracea* L.	38	14.1
Ora-pro-nobis	*Pereskia aculeata* Mill.	28	10.4
Mostarda	*Brassica juncea* L. Czern	15	5.6

Tabela A.4

Table 4. Conventional and non-conventional eadible leafy indicated as favorite (n=271).

Popular names	**Species name**	**Number of citations**	**Frequency (%)**
Collard gress	*Brassica oleracea* L. var. *acephala* D.C	59	21.8
Lettuce	*Lactuca sativa*	48	17.7
Rocket	*Eruca sativa*	31	11.4
Spinach	*Spinacia oleracea*	21	7.7
Taioba	*Xanthosoma taioba* E. G. Gonç.	15	5.5
Watercress	*Nasturtium officinale*	13	4.8

Table A.5

Table 5. Mostly cited non-conventional eadible plant already consumed among participants (n=189).

Popular names	**Species name**	**Number of citations**	**Frequency (%)**
Taioba	*Xanthosoma taioba* E. G. Gonç.	87	46.0
Ora-pro-nóbis	*Pereskia aculeata* Mill.	85	44.9
Beldroega	*Portulaca Oleracea* L.	50	26.5
Peixinho da horta	*Stachys byzantina* K. Koch	50	26.5
Capuchinha	*Tropaeolum majus* L.	44	23.3
Caruru	*Amarathus deflexus* L.	28	14.8
Azedinha	*Rumex acetosa* L.	21	11.1
Hibisco	*Hibiscus rosa-sinensis* L.	12	6.3
Almeirão	*Chicorium intybus*	11	5.8
Chaya	*Cnidoscolus aconitifolius* (Mill.) I. M. Johnst.	10	5.3
Umbigo da bananeira	*Musa* spp.	10	5.3

Table A.6

Table 6. Origin of the NCEP acquired by consumers (n = 182).

Sites	Number of citations	Frequency(%)
Own production at home/farm	89	48.9
Organic farmers' market	48	26.4
Gift given by friends/family	36	19.8
Streets and sidewalks	19	10.4
Supermarket/hortifruti	16	8.8
Researches and events	10	5.5
Family farming site	8,9	4.9
Restaurants	8,0	4.4
Vegetable gardens and restaurants of Universities and Research Institutes	6.9	3.8
Community gardens	6.0	3.3
Others	11	6.0

Table A.7.

Table 7. Associations of Food Neophobia and consumers attitudes towards NCEP.

Sentences	Likert Scale	Food Neophobia (number of participants)			
		Total sample (n=271)	Low (n=108)	Medium (n=92)	High (n=71)
Sentence 1					
If the nutritional value was proven, I would try a non-conventional edible plant.	Totally disagree	4	-	1	-
	Disagree	4	1	2	1
	Neither disagree, nor agree	30	12	9	9
	Agree	23	2*	6	15*
	Totally Agree	210	93	71	45*
Sentence 2					
I would certainly include non-conventional edible plants in my eating habits.	Totally disagree	2	-	1	1
	Disagree	1	-	1	2
	Neither disagree, nor agree	22	4*	5	14*
	Agree	35	9	11	15*
	Totally Agree	211	99*	71	38*
Sentence 3					
I prefer to consume conventional vegetables to non-conventional edible plants.	Totally disagree	53	33*	14	9
	Disagree	24	11	11	3
	Neither disagree, nor agree	106	41	38	30
	Agree	46	15	14	17
	Totally Agree	42	12	12	11
Sentence 4					
I would like to learn more about non-conventional edible plants.	Totally disagree	3	-	2	1
	Disagree	2	1	-	1
	Neither disagree, nor agree	9	-	3	6*
	Agree	44	18	13	13
	Totally Agree	213	93	71	49
Sentence 5					
Heat treatment (such as cooking) can be used to reduce the level of toxic substances in dark green vegetables.	Totally disagree	15	4	5	6
	Disagree	6	3	2	2
	Neither disagree, nor agree	67	25	26	17
	Agree	58	23	19	16
	Totally Agree	125	57	37	29
Sentence 6					
If safety was proven, I would try a non-conventional edible plant.	Totally disagree	1	2	2	1
	Disagree	3	-	-	-
	Neither disagree, nor agree	15	3*	6	8*
	Agree	33	9	8	16*
	Totally Agree	219	98*	74	45*

Note. *p<0.05 for Fisher's exact test.

Table A.8.

Table 8. Associations of Risk perception and consumers attitudes towards NCEP.

Sentences	Likert scale	Risk perception (number of participants)					
		Total sample (n=271)	High to health	Moderate to health	Indifferent	Poor to health	None to health
Sentence 1							
If the nutritional value was proven, I would try a non-conventional edible plant.	Totally disagree	4	-	-	1	-	4
	Disagree	4	-	-	1	2	1
	Neither disagree, nor agree	30	1	2	8	7	12
	Agree	23	-	2	4	7	10
	Totally Agree	210	-	10	31	73	95
Sentence 2							
I would certainly include non-conventional edible plants in my eating habits.	Totally disagree	2	-	-	1		1
	Disagree	1	1*	-	1		1
	Neither disagree, nor agree	22	-	-	10*		6
	Agree	35	-	4	6		12
	Totally Agree	211	-	10	27*		69*
Sentence 3							
I prefer to consume conventional vegetables to non-conventional edible plants.	Totally disagree	53	-	1	6	11*	38*
	Disagree	24	-	1	2	10	12
	Neither disagree, nor agree	106	-	7	25*	37	40*
	Agree	46	-	2	6	21	17
	Totally Agree	42	1	3	6	10	15
Sentence 4							
I would like to learn more about non-conventional edible plants.	Totally disagree	3	-	-	3*	-	-
	Disagree	2	-	-	1	-	1
	Neither disagree, nor agree	9	-	1	5*	1	2
	Agree	44	-	3	11	11	19
	Totally Agree	213	1	10	25*	77*	100
Sentence 5							
Heat treatment (such as cooking) can be used to reduce the level of toxic substances in dark green vegetables.	Totally disagree	15	-	-	2	2	11*
	Disagree	6	-	1	2	2	2
	Neither disagree, nor agree	67	-	2	16	20	30
	Agree	58	1	6	9	23	19*
	Totally Agree	125	-	5	16	42	60
Sentence 6							
If safety was proven, I would try a non-conventional edible plant.	Totally disagree	1	-	1	1	1	1
	Disagree	3	-	1	1	-	-
	Neither disagree, nor agree	15	1	5	5	9	9
	Agree	33	-	26	12*	10	10
	Totally Agree	219	-	12	26*	102	102

Note. *p<0.05 for Fisher's exact test.

Table A.9

Table 9. Relative importance and Index score of six prototypes obtained in the Best-Worst Scaling task.

Prototype	**Tipo**	**Price**	**Total Best**	**Total Worst**	**B-W (score)**	**Avarage (B-W)**	**Data Source**	**Index**
P3	Conventional + non-conventional	US$0.70	425	8	417	0.78	7.3	100
P4	Conventional + non-conventional	US$1.14	244	121	123	0.23	1.4	19
P2	Non-conventional	US$0.70	186	89	97	0.18	1.4	19
P1	Conventional	US$0.70	136	190	-54	-0.10	0.8	11
P5	Non-conventional	US$1.14	53	267	-214	-0.40	0.2	3
P6	Conventional	US$1.14	28	397	-369	-0.69	0.3	4

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