RESSALVA

Atendendo solicitação do autor,
o texto completo desta tese será
disponibilizado somente a partir
de 25/03/2026

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de São José do Rio Preto

Adilson Roberto Locali Pereira

Resilient, sustainable, and nutritious:

pigeon pea protein fractions as high-potential food ingredients

São José do Rio Preto

2024

Adilson Roberto Locali Pereira

Resilient, sustainable, and nutritious:

pigeon pea protein fractions as high-potential food ingredients

Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-Graduação em Alimentos, Nutrição e Engenharia de Alimentos, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

Financiadora: CAPES

Orientadora: Profª. Drª. Vânia Regina Nicoletti

Orientadora francesa: Claire Berton-Carabin

Coorientador: Adeline Boire

São José do Rio Preto

2024

Pereira, Adilson Roberto Locali

P436r Resilient, sustainable, and nutritious: : pigeon pea protein fractions as high-potential food ingredients / Adilson Roberto Locali Pereira. -- São José do Rio Preto, 2024

251 p.

Tese (doutorado) - Universidade Estadual Paulista (UNESP), Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto

Orientadora: Vânia Regina Nicoletti
Orientadora estrangeira: Claire Berton-Carabin
Coorientadora estrangeira: Adeline Boire

1. Feijão guandu. 2. Globulinas. 3. Produtos plant-based. 4.Fracionamento de proteínas. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Universidade Estadual Paulista (UNESP), Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

Adilson Roberto Locali Pereira

Resilient, sustainable, and nutritious:

pigeon pea protein fractions as high-potential food ingredients

Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-Graduação em Alimentos, Nutrição e Engenharia de Alimentos, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

Financiadora: CAPES

Comissão Examinadora

Profª. Drª. Vânia Regina Nicoletti
Unesp – Câmpus de São José do Rio Preto
Orientadora brasileira

Profª. Drª Claire Berton-Carabin
INRAE/BIA
Orientadora francesa

Profª. Drª Adeline Boire
INRAE/BIA
Co-orientadora francesa

Prof. Dr. Pedro Esteves Duarte Augusto
CentraleSupélec
Examinador externo, avaliador prévio e membro da banca

Prof. Dr. Rémi Saurel
L'InstitutAgro
Examinador externo, avaliador prévio e membro da banca

Prof. Dr. Guilherme Miranda Tavares
Universidade Estadual de Campinas
Membro da banca

Profª. Drª Miriam Dupas Hubinger
Universidade Estadual de Campinas
Membro da banca

Prof. Dr. Bernard Offmann
Universidade de Nantes
Membro da banca

São José do Rio Preto
25 de março de 2024

Para Adilson, Maria Rosa, Anelize,

Bruno e João Vitor.

Agradecimentos

Às minhas orientadoras de tese, Vânia, Adeline e Claire, por todo o conhecimento partilhado comigo e pela dedicação a este projeto;

Aos meus professores da Universidade Estadual Paulista (Unesp), que contribuíram para meu desenvolvimento pessoal e profissional ao longo dos últimos 11 anos;

À equipa ISD do INRAE/BIA, que me acolheu muito bem durante a minha estadia na França e contribuiu grandemente para a realização deste trabalho;

À Véronique Solé-Jamault e Joëlle Davy, por suas contribuições muito importantes para os estudos relativos à purificação de proteínas;

Aos membros da banca, pela disponibilidade e contribuições para este trabalho;

À Empresa Brasileira de Pesquisa Agropecuária (Embrapa), especialmente Renata Tieko Nassus e Rodolfo Godoy, pela doação de sementes de feijão guandu.

Ao Ministério dos Negócios Estrangeiros Francês e ao Campus France pelo financiamento e gestão da minha bolsa de doutoramento na França;

À minha família e amigos por fazerem parte desta jornada nos últimos 4 anos;

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

Resumo

O feijão guandu (*Cajanus cajan*) é uma leguminosa nativa de países emergentes da África e da Ásia, apresentando alta resistência a ambientes secos. Seu alto teor de proteínas, que varia de 18 a 28%, a torna uma leguminosa potencial para o desenvolvimento de novos produtos vegetais a partir de fontes alternativas ainda pouco exploradas. Contudo, a produção de feijão guandu está limitada aos pequenos produtores. Uma forma de expandir a produção de feijão guandu em larga escala é compreender melhor a sua composição proteica e a sua funcionalidade nos sistemas alimentares. Neste trabalho frações proteicas do feijão guandu foram obtidas, caracterizadas e avaliadas quanto às suas propriedades funcionais. A globulina 7S foi a proteína presente em maior abundância nas frações proteicas do feijão guandu. Esta tese propôs um protocolo para a purificação da globulina 7S do feijão guandu em escala preparativa, visando a recuperação de rendimento suficiente para a caracterização das propriedades físico-químicas e funcionais das frações proteicas. Dados experimentais obtidos em laboratório para diferentes parâmetros físico-químicos mostraram boa concordância com valores calculados a partir de estudos preditivos utilizando a sequência e composição de aminoácidos do feijão guandu. Além do foco em proteínas, esta tese também apresenta uma extensa caracterização da fração lipídica e de carboidratos de ingredientes à base de feijão guandu, além da investigação de compostos minoritários, como os inibidores de tripsina. Para avaliar a funcionalidade, foram avaliadas as propriedades espumantes das frações proteicas. As frações protéicas do feijão guandu apresentaram propriedades espumantes semelhantes aos agentes espumantes de origem animal, como isolado proteico de soro de leite e albumina sérica bovina. Os resultados obtidos nesta tese mostram que as frações proteicas do feijão guandu apresentam alto potencial para aplicação como ingredientes alimentícios na indústria.

Palavras–chave: Feijão guandu. Globulinas. Produtos plant-based. Fracionamento de proteínas.

Abstract

Pigeon pea (*Cajanus cajan*) is a pulse native to emerging countries in Africa and Asia, presenting high resistance to dry environments. Its high protein content, which varies from 18 to 28%, makes it a potential pulse crop for the development of new plant-based products from alternative sources that are still little explored. However, pigeon pea production is limited to small producers. One way to expand pigeon pea production to a large scale is to better understand its proteins composition and, its functionality in food systems. In this work, pigeon pea protein fractions were obtained, characterized and evaluated for their functional properties. The 7S globulin was the protein present in greatest abundance in pigeon pea protein fractions. This thesis proposed a protocol for the purification of pigeon pea 7S globulin on a preparative scale, aiming the recovery of a sufficient yield for the characterization of physico-chemical and functional properties of the protein fractions. Experimental data obtained in the laboratory for different physicochemical parameters showed good agreement with values calculated from predictive studies using the sequence and amino acids composition of pigeon pea. In addition to the focus on proteins, this thesis also presents an extensive characterization of the lipid and carbohydrate fraction of pigeon pea-based ingredients, in addition to the investigation of minor compounds such as trypsin inhibitors. To evaluate functionality, the foaming properties of the protein fractions was evaluated. Pigeon pea protein fractions showed foaming properties similar to foaming agents of animal origin, such as whey protein isolate and bovine serum albumin. The results obtained in this thesis show that pigeon pea protein fractions have high-potential for application as food ingredients in the industry.

Keywords: Pigeon pea. Globulins. Plant-based products. Protein fractionation.

Résumé

Le pois d'Angole (*Cajanus cajan*) est une légumineuse originaire des pays émergents d'Afrique et d'Asie, présentant une grande résistance aux environnements secs. Sa teneur élevée en protéines, variant de 18 à 28 %, en fait une légumineuse prometteuse pour le développement de nouveaux produits d'origine végétale à partir de sources alternatives encore peu explorées. Cependant, la production de pois d'Angole est limitée à de petits producteurs. Une manière d'augmenter la production de pois d'Angole est de mieux comprendre sa composition protéique et, ses propriétés techno-fonctionnelles dans les matrices alimentaires. Dans ce travail, des fractions protéiques variées de pois d'Angole ont été obtenues, caractérisées et évaluées pour leurs propriétés fonctionnelles. La globuline 7S est la protéine la plus abondante dans les fractions protéiques du pois d'Angole. Cette thèse a développé un protocole pour la purification de la globuline 7S du pois d'Angole à une échelle préparative, visant à obtenir un rendement suffisant pour la caractérisation des propriétés physico-chimiques et fonctionnelles des fractions protéiques. Les données expérimentales obtenues en laboratoire pour différents paramètres physico-chimiques ont montré une bonne concordance avec les valeurs calculées à partir d'études prédictives utilisant la séquence et la composition en acides aminés du pois d'Angole. En plus de se concentrer sur les protéines, cette thèse présente également une caractérisation approfondie de la fraction lipidique et glucidique des ingrédients à base de pois d'Angole, en plus de l'étude de composés minoritaires tels que les inhibiteurs de trypsine. Enfin, les propriétés moussantes des fractions protéiques ont été évaluées. Les fractions protéiques du pois d'Angole ont montré des propriétés moussantes similaires à celles de protéines d'origine animale, tels que l'isolat de protéines de lactosérum et l'albumine de sérum bovin. Les résultats obtenus dans cette thèse montrent que les fractions protéiques du pois d'Angole ont un fort potentiel d'application en tant qu'ingrédients alimentaires dans l'industrie.

Mots clés: Pois d'Angole. Globulines. Produits à base de plantes. Fractionnement des protéines.

Table of Contents

Introduction

The world population is expected to reach around 9.8 billion people by 2050. Rethinking food production for a growing number of people while reducing negative impacts on the environment is a key issue to be considered by producers, researchers and government agencies in the coming decades (CLARK *et al.*, 2019). One of the strategies for more sustainable diets involves reducing excessive consumption of proteins of animal origin and increasing the intake of vegetable protein sources (ROSENFELD, 2018). The 2030 Agenda for Sustainable Development, launched by the Food and Agriculture Organization of the United Nations (FAO), includes encouraging projects that promote the sustainable and large-scale production of pulses for human consumption that contribute to reducing greenhouse gas emissions, increased soil fertility and better use of agricultural areas (FAO, 2016)

Private companies have shown accelerated investment in alternative protein sources to recent years, with amounts reaching around 5 billion dollars in 2021 (GFI, 2022). Over the past two decades, several new brands of plant-based meat or dairy products have been founded, the majority in the United States (34%) and the United Kingdom (8%) (GFI, 2023). Among the main ingredients used in the formulation of new plant-based products are soy, pea and wheat proteins, species widely studied to produce plant-based protein concentrations and isolates (AIMUTIS, 2022). However, it is estimated that the production of soybeans and peas, for example, will not be sufficient to meet the demand for plant-based proteins expected in the coming years (FOSCHIA *et al.*, 2017, RAHAMAN *et al.*, 2016), so it is necessary to investigate new protein sources.

Among leguminous seeds, there are several species that are still underutilized – called *orphan crops* or *minor crops* – that have great potential for agronomic development, nutritional requirements and food security (SAMAL *et al.*, 2023). Despite being species well known in their native countries for their socioeconomic impact, these crops are considered underutilized because they are still neglected by policymakers, mainstream agricultural researchers, plant breeders, extension services, technology providers and consumers in general (POPOOLA *et al.*, 2020, VERBEECKE *et al.*, 2023). Furthermore, they comprise species whose grain supply networks are non-existent or not yet well developed (ALI & BHATTACHARJEE, 2023). Another important aspect is that these species are highly resistant to different environmental conditions, being able to withstand dry environments and extreme temperatures (SAMAL *et al.*, 2023).

Pigeon pea (*Cajanus cajan L. Millsp*.) is a highly drought-resistant pulse native to emerging countries in Asia and Africa (TAPAL *et al.*, 2019). World production of pigeon pea is around 5.5 million tons, corresponding to around 6% of world pulse production. The FAO's *The Global Economy of Pulses* includes pigeon peas among the most important pulses in terms of amount of consumption and global production (FAO, 2019). India is the largest consumer and producer of pigeon pea, accounting for around 90% of global

production of this pulse (TAPAL *et al.*, 2019). It's high nutritional value, with protein content ranging from 18-28% of its seed composition (SUN *et al.*, 2020), makes pigeon pea a potential source to produce protein-rich ingredients. In most part of the world, however, pigeon pea are still little used in human consumption. When it was introduced to America, it was used to fed pigeons on the island of Barbados, in the Caribbean Sea, where the name in English came from (VAN DE MAESEN, 1990). In Brazil, for many years the species was mainly used to feed livestock, so that only in the last two decades has it been the focus of studies to develop cultivars more suitable for human consumption (GODOY *et al.*, 2005, MIANO *et al.*, 2020). Its production, however, remains restricted to small producers. In fact, pigeon pea is one of the pulses whose production is almost entirely restricted to small-scale farmers. One of the ways to boost its large-scale production is to overcome possible difficulties in processing the seeds and a better understanding of their protein composition.

Some difficulties in processing pulses such as pigeon pea involve the hard-to-cook phenomenon and the presence of antinutritional factors. Antinutritional factors such as trypsin inhibitors, phytates, tannins, saponins, among others, can hinder the action of digestive enzymes on proteins, compromising their digestibility and absorption by the body (SAHNI *et al.*, 2020, SUN *et al.*, 2020). Pre-treatments such as soaking and boiling are among the primary technological methods to reduce the effects of antinutritional factors in pulses (CODA *et al.*, 2015, ZHOU *et al.*, 2023). Although such pre-treatments have already showed efficient in reducing anti-nutritional compounds in pigeon pea (DEVINDRA *et al.*, 2012, ONWUKA, 2006), it can affect protein structure and the functionality of ingredients based on pre-treated seeds, being a topic that still need further investigation.

The protein composition of pigeon pea is also a topic that needs further studies. Globulins account for around 60% of pigeon pea proteins, with 7S β-conglycinin being its main protein (SINGH & JAMBUNATHAN, 1982). Pigeon pea seeds also contain 11S glycinin, 2S albumin and other proteins in a smaller proportion (KRISHNAN *et al.*, 2017). Studies have investigated the production of pigeon pea protein concentrates and isolates, with the evaluation of their functional properties (ADENEKAN *et al.*, 2018, FERNÁNDEZ SOSA *et al.*, 2021, 2023, MWASARU et al., 1999a, MWASARU et al., 1999b). The alkaline solubilization method followed by isoelectric precipitation is the most used extraction method to obtain protein concentrates and isolates. Such methods enable the recovery of protein extracts rich in globulins, but which also contain other contaminants such as: other protein groups, sugars, phenolic compounds, phospholipids, among others (GRAVEL & DOYEN, 2023). To understand the role of pigeon pea 7S globulin in such complex mixtures, purification by chromatographic techniques can be employed. Krishna & Bhatia (1985) were the first to propose the isolation and purification on a laboratory scale of 7S β-conglycinin from pigeon pea. This study allowed to identify that pigeon pea 7S β-conglycinin is a glycoprotein and has a subunit around 70 kDa and two subunits around 57 kDa, with a molecular weight of 180 kDa in its native state. This pioneering

study presented fundamentals about the composition of the main protein in pigeon pea. However, to also evaluate the functionality of these proteins it is necessary to obtain them in sufficient quantities by migrating the laboratory-scale purification process to a preparative-scale process.

Therefore, the objective of this thesis was to extract, purify, characterize and evaluate the functional properties of different protein fractions from pigeon pea. Firstly, the impact of soaking and boiling pre-treatments on the functionality of pigeon pea protein concentrates obtained by alkaline solubilization followed by isoelectric precipitation was investigated. Then, pigeon pea 7S globulin was purified on a preparative scale using a mild extraction process and characterized for its physicochemical properties. The data of the investigated parameters were compared with theoretical values obtained by predictive studies using pigeon pea sequences and their amino acid composition. Finally, the air/water interfacial and foaming properties of purified 7S globulin and crude globulin extract were investigated. To investigate the use of pigeon pea proteins as possible alternative sources to proteins of animal origin, in studies of foaming properties, whey protein isolate and bovine serum albumin were used as controls.

The manuscript will be divided into five chapters:

Chapter 1 consists of a review of the state of the art regarding the use of pigeon pea seeds to produce food ingredients with a focus on seed pre-treatments and protein extraction methods.

Chapters 2, 3 and 4 present the experimental results. Chapter 2 focuses on the impact of seed pre-treatments on the composition and functionality of pigeon pea-based ingredients. Chapter 3 is focused on the purification and physicochemical characterization of pigeon pea 7S globulin. Chapter 4 presents the behavior at the air/water interface and foaming properties of pigeon pea protein fractions.

Finally, chapter 5 presents a general discussion of the results obtained in this thesis. Also, a general conclusion and perspectives for future work are presented.

Part II. General conclusions and perspectives

This PhD thesis had as its main goal the extraction, characterization and evaluation of the functional properties of protein fractions from pigeon pea. The study carried out was divided into three parts:

• Evaluation of the effect of seed pre-treatment on the composition and functional properties of pigeon pea seeds ingredients;

• Purification on a preparative scale of 7S globulin from pigeon pea using a mild protein extraction method;

• Assessment of air/water interfacial and foaming properties of pigeon pea protein fractions;

In the first part of the study, soaking and boiling pre-treatments modified the composition of pigeon pea flour and protein concentrates. From a nutritional point of view, pre-treatments reduced the levels of trypsin inhibitors, raffinose family oligosaccharides and soluble phenolic compounds in flours and protein concentrates, making a positive contribution to the better digestibility of these ingredients. In terms of functionality, protein concentrates obtained from soaked-boiled seeds had greater water holding capacity, least gelation concentration and emulsion stability than protein concentrate obtained from seeds without any treatment. We further demonstrated that lipids and carbohydrates, together, account for around a fifth of the composition of protein concentrates, playing a significant role in the functionality of these ingredients.

The second part of this thesis is focused on the first study on the preparative-scale purification of 7S globulin from pigeon pea. Only purification studies on an analytical scale had been conducted for the purification of 7S globulin from pigeon pea and in this work purification on a larger scale was proposed, optimized and performed, allowing the recovery of this protein in sufficient quantities for also probe its functional properties. Throughout the large-scale process, however, we observed a partial hydrolysis of 7S globulin, probably caused by the action of some endogenous protease. Replacing the dialysis step with injection into a gel filtration column to eliminate salts and minor compounds showed to be a faster and more efficient alternative to preserve the native state of 7S globulin. We demonstrated that partially hydrolyzed 7S globulin has lower foaming capacity and lower foam stability when compared to native 7S globulin. In terms of physicochemical parameters, native 7S globulin presented values similar to those obtained in predictive studies using pigeon pea sequences and their amino acid composition. On the other hand, partially hydrolyzed 7S globulin presented unconventional values, reiterating the importance of the native state of proteins to accurately determine their physicochemical properties.

In the third part of this thesis, we demonstrate the different behavior that a crude protein extract and a purified 7S globulin present at the air/water interface and its implications for foaming properties. Purified 7S globulin exhibits a stiffer interfacial film and better foaming properties than crude protein extracts. A decrease in the rigidity of interfacial films stabilized by crude protein extracts can be explained by the loss of the 7S

globulin domain at the interface and the greater contribution of other adsorbent compounds, such as sugars, phospholipids, phenols, among others. The type of seed used in the production of protein extracts also had an impact on the properties evaluated, probably due to different levels of minor compounds present. Finally, we also demonstrated that fractions rich in 7S globulin from pigeon pea perform similarly or even better than foaming agents of animal origin, such as whey protein isolate and bovine serum albumin.

The perspectives for the work carried out in this thesis involve investigating other functional properties of pigeon pea protein fractions, such as emulsifying and gel-forming properties. The identification and quantification of minor compounds, especially those that interfere with the functionality of the ingredients, must also be investigated. Finally, other protein fractionation processes, such as dry fractionation, can be explored and compared with classical aqueous fractionation processes to better understand how different processes for obtaining protein fractions can impact the functionality of ingredients obtained from pigeon pea.

References

BARLOW, S. M., GAUNT, J. K. The metabolism of α-tocopherol by plants, **Phytochemistry**, v. 11, n. 7, p. 2161–2170, jul. 1972. DOI: 10.1016/S0031-9422(00)88371-5.

CARTER, C. E., MANTHEY, F. A. Seed treatments affect milling properties and flour quality of black beans (Phaseolus vulgaris L.), **Cereal Chemistry**, v. 96, n. 4, p. 689–697, 1 jul. 2019. DOI: 10.1002/cche.10165.

CHIGWEDERE, C. M., NJOROGE, D. M., VAN LOEY, A. M., *et al.* Understanding the Relations Among the Storage, Soaking, and Cooking Behavior of Pulses: A Scientific Basis for Innovations in Sustainable Foods for the Future. **Comprehensive Reviews in Food Science and Food Safety**, Blackwell Publishing Inc. , 2019.

DEVINDRA, S., SREENIVASA, R. J., BHASKAR, V., *et al.* Effect of heat treatment on α-galactoside content of red gram seeds (Cajanus cajan, L), **Journal of Food Processing and Preservation**, v. 36, n. 2, p. 97–103, abr. 2012. DOI: 10.1111/j.1745-4549.2011.00556.x.

FERNÁNDEZ SOSA, E. I., CHAVES, M. G., QUIROGA, A. V., *et al.* Comparative Study of Structural and Physicochemical Properties of Pigeon Pea (Cajanus cajan L.) Protein Isolates and its Major Protein Fractions, **Plant Foods for Human Nutrition**, v. 76, n. 1, p. 37–45, 1 mar. 2021. DOI: 10.1007/s11130-020-00871-7.

FLEURIET, A. ; M. J. J., Phenolic acids in fruits and vegetables. **Flavonoids in Health and Disease**, Boca Raton, 2003. p. 1–42.

GRAHAM, D. E., PHILLIPS, M. C. Proteins at liquid interfaces, **Journal of Colloid and Interface Science**, v. 70, n. 3, p. 403–414, jul. 1979. DOI: 10.1016/0021-9797(79)90048-1.

HENDRICKS, N. G., JULIAN, R. R. Two-step energy transfer enables use of phenylalanine in action-EET for distance constraint determination in gaseous biomolecules, **Chemical Communications**, v. 51, n. 64, p. 12720–12723, 18 ago. 2015. DOI: 10.1039/c5cc03779d.

KRISHNA, T, G.., MITRA, R. K., BHATIA, C. R. Seed globulins of Cajanus cajan, **Qualitas Plantarum Plant Foods for Human Nutrition**, v. 27, n. 3–4, p. 313–325, dez. 1977. DOI: 10.1007/BF01092324.

KRISHNA, T.G., BHATIA, C. R. Vicilin from cajanus cajan seeds, **Phytochemistry**, v. 24, n. 10, p. 2201–2203, jan. 1985. DOI: 10.1016/S0031-9422(00)83010-1.

KRISHNAN, H. B., NATARAJAN, S. S., OEHRLE, N. W., *et al.* Proteomic Analysis of Pigeonpea (Cajanus cajan) Seeds Reveals the Accumulation of Numerous Stress-Related Proteins, **Journal of Agricultural and Food Chemistry**, v. 65, n. 23, p. 4572–4581, 14 jun. 2017. DOI: 10.1021/acs.jafc.7b00998.

LOCQUET, N., AÏT-KADDOUR, A., CORDELLA, C. B. Y., 3D Fluorescence Spectroscopy and Its Applications. **Encyclopedia of Analytical Chemistry**, Chichester, UK, John Wiley & Sons, Ltd, 2018. p. 1–39. DOI: 10.1002/9780470027318.a9540.

MORITA, S., FUKASE, M., HOSHINO, K., *et al.* A Serine Protease in Soybean Seeds That Acts Specifically on the Native a Subunit of /S-Conglycinin. Plant Cell Physiol. [S.l: s.n.], 1994.

MULLER, M., MILORI, D. M. B. P., DÉLÉRIS, S., *et al.* Solid-phase fluorescence spectroscopy to characterize organic wastes, Waste Management, v. 31, n. 9–10, p. 1916–1923, set. 2011. DOI: 10.1016/j.wasman.2011.05.012.

MURILLO, E., PLUMPTON, S. E., GAUNT, J. K. The Properties and Distribution of α-Tocopherol Oxidase in Plants, Biochemical Society Transactions, v. 4, n. 3, p. 486–487, 1 jun. 1976. DOI: 10.1042/bst0040486.

ONIGBINDE, A. O., AKINYELE, I. O. Oligosaccharide Content of 20 Varieties of Cowpeas in Nigeria, Journal of Food Science, v. 48, n. 4, p. 1250–1251, 25 jul. 1983. DOI: 10.1111/j.1365-2621.1983.tb09203.x.

ONIMAWO, I. A., AKPOJOVWO, A. E. Toasting (dry heat) and nutrient composition, functional properties and antinutritional factors of pigeon pea (Cajanus cajan) flour, Journal of Food Processing and Preservation, v. 30, n. 6, p. 742–753, 2006. DOI: 10.1111/j.1745-4549.2006.00093.x.

PACE, C. N., VAJDOS, F., FEE, L., *et al.* How to measure and predict the molar absorption coefficient of a protein, Protein Science, v. 4, n. 11, p. 2411–2423, 1995. DOI: 10.1002/pro.5560041120.

POTIN, F., LUBBERS, S., HUSSON, F., *et al.* Hemp (Cannabis sativa L.) Protein Extraction Conditions Affect Extraction Yield and Protein Quality, Journal of Food Science, v. 84, n. 12, p. 3682–3690, 1 dez. 2019. DOI: 10.1111/1750-3841.14850.

RAKSHIT, M., SHARMA, A., SAHA, J., *et al.* Optimization of soaking condition of blackgram to minimize flatogenic sugar content in blackgram-based products, LWT- Food Science and Technology, v. 63, n. 2, p. 814–820, out. 2015. DOI: 10.1016/j.lwt.2015.04.026.

RASHWAN, A. K., OSMAN, A. I., ABDELSHAFY, A. M., *et al.* Plant-based proteins: advanced extraction technologies, interactions, physicochemical and functional properties, food and related applications, and health benefits, Critical Reviews in Food Science and Nutrition, p. 1–28, 15 nov. 2023. DOI: 10.1080/10408398.2023.2279696.

XIA, Z. W., ZHANG, J. G., NI, Z. J., *et al.* Functional and emulsification characteristics of phospholipids and derived o/w emulsions from peony seed meal, Food Chemistry, v. 389, 30 set. 2022. DOI: 10.1016/j.foodchem.2022.133112.

YANG, J., YANG, Q., WATERINK, B., *et al.* Physical, interfacial and foaming properties of different mung bean protein fractions, Food Hydrocolloids, v. 143, p. 108885, out. 2023. DOI: 10.1016/j.foodhyd.2023.108885.