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A comprehensive study of cowpea protein:

Techno-functional properties, rheological behavior, emulsification and film-forming capacity

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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.

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“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis.”

José de Alencar (n.d.)

RESUMO

O feijão-caupi (*Vigna unguiculata* (L.) Walp), uma leguminosa versátil e adaptável, nativa da África, destaca-se pelo seu alto teor de proteínas, variando entre 20 e 30%. Essa característica faz do feijão-caupi uma fonte promissora de proteína vegetal, ainda pouco explorada na indústria alimentícia. Neste estudo, a proteína concentrada do feijão-caupi (CPC) foi extraída por precipitação isoelétrica, caracterizada e avaliada quanto às suas propriedades funcionais e reológicas, sendo aplicada no desenvolvimento de emulsões e filmes comestíveis. Inicialmente, as características e propriedades do CPC tanto em pó quanto em solução foram investigadas. As globulinas foram as proteínas mais abundantes no CPC em pó, que apresentou um perfil proteico com proporção adequada de aminoácidos essenciais. O CPC, predominantemente amorfo, apresentou alta temperatura de desnaturação (131,86 °C) e na estrutura secundária de suas proteínas houve prevalência de folhas- β . Em solução, o pH representou um fator crítico sobre as propriedades tecno-funcionais do CPC, incluindo solubilidade, turbidez, formação de espuma e capacidade emulsificante, as quais melhoram em condições alcalinas. Soluções aquosas de CPC também foram analisadas quanto aos efeitos combinados de pH, concentração e temperatura sobre suas propriedades reológicas. Os resultados indicaram sensibilidade à concentração de proteína e à taxa de cisalhamento, especialmente em concentrações acima de 10%. O pH influenciou significativamente a tensão de cisalhamento e a viscosidade. O pH alcalino induziu alterações estruturais que afetaram as temperaturas de desnaturação e gelificação da proteína. O estudo também explorou a relação entre pH (3-11), concentração de óleo (2-10%) e estabilidade de emulsões, com foco na eficácia do CPC como agente emulsificante natural. O pH e a concentração de óleo impactaram significativamente a distribuição do tamanho das partículas, sendo mais uniforme em pH 9. Emulsões estabilizadas com CPC exibiram comportamento de fluido pseudoplástico, intensificado com maiores concentrações de óleo. Após tratamento térmico, emulsões em pH 7 e 9 sofreram desestabilização, enquanto em pH 11 permaneceram estáveis. Emulsões em pH 9 e 11 mostraram os maiores graus de estabilidade durante 30 dias de armazenamento. A CPC também foi avaliada como potencial matéria-prima para o desenvolvimento de filmes comestíveis. Filmes preparados em diferentes pHs (7, 9 e 11) revelaram que o pH 9 foi favorável a estruturas secundárias mais ordenadas dessas proteínas, microestrutura mais homogênea e redução da permeabilidade ao oxigênio (PO_2) no filme em relação aos outros pHs. Solubilidade, resistência à tração e deformação na ruptura não demonstraram dependência estrita do pH da solução formadora de filme. Por fim, a incorporação de emulsão nas soluções formadoras de filme

também foi investigada. A presença de emulsão melhorou a resistência à tração e o alongamento na ruptura e afetou significativamente o índice de cristalinidade relativa dos filmes, que aumentou nas formulações com 0.8 e 1% de emulsão. A formulação com 1% de emulsão apresentou a menor PO_2 e filmes sem rachaduras nas imagens de microestrutura. Filmes com mais de 0,2% de emulsão apresentaram maior opacidade, enquanto o filme controle foi mais transparente. Os resultados desta tese demonstram que o CPC possui alto potencial para aplicação como ingrediente alimentício na indústria.

Palavras-chave: Proteína vegetal, Propriedades funcionais, Propriedades reológicas, Emulsões, Filmes comestíveis

ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp), a versatile and adaptable legume native to Africa, stands out for its high protein content, ranging between 20 and 30%. This characteristic makes cowpeas a promising source of plant protein, still underexplored in the food industry. In this study, cowpea protein concentrate (CPC) was extracted by isoelectric precipitation, characterized, and evaluated for its functional and rheological properties, being applied in the development of emulsions and edible films. Initially, the study investigated the characteristics and properties of CPC both in powder and solution forms. Globulins were the most abundant proteins in CPC powder, which showed a protein profile with an adequate proportion of essential amino acids. The predominantly amorphous CPC had a high denaturation temperature (131.86 °C), and the secondary structure of its proteins was dominated by β -sheets. In solution, pH proved to be a critical factor in the techno-functional properties of CPC, including solubility, turbidity, foaming, and emulsifying capacity, which improved under alkaline conditions. Aqueous solutions of CPC were also analyzed for the combined effects of pH, concentration, and temperature on their rheological properties. The results indicated that the rheological properties were sensitivity to protein concentration and shear rate, especially above 10%. The pH significantly influenced shear stress and viscosity. Alkaline pH induced structural changes that affected the protein's denaturation and gelation temperatures. This study also explored the relationship between pH (3-11), oil concentration (2-10%), and emulsion stability, focusing on the effectiveness of CPC as a natural emulsifying agent. pH and oil concentration significantly impacted droplet size distribution, which was more uniform at pH 9. Emulsions stabilized with CPC exhibited shear- fluid behavior, intensified with higher oil concentrations. After thermal treatment, emulsions at pH 7 and 9 became unstable, while those at pH 11 remained stable. Emulsions at pH 9 and 11 showed the highest degrees of stability over a 30-day storage period. Additionally, CPC was evaluated as a raw material for developing edible films. Films prepared at different pHs (7, 9, and 11) revealed that pH 9 was favorable to more ordered secondary structures of these proteins, more homogeneous microstructure, and reduced oxygen permeability (PO₂) in the film compared to the other pHs. Solubility, tensile strength, and elongation at break did not show a strict dependence on the pH of the film-forming solution. Finally, the incorporation of emulsion into the film matrix was also investigated. The presence of emulsion improved tensile strength and elongation at break and significantly affected the relative crystallinity index of the films, which increased in the formulations with 0.8 and 1% emulsion. The formulation with 1% emulsion showed the lowest PO₂ and films without cracks

in the microstructure images. Films with more than 0.2% emulsion exhibited higher opacity, while the control film was more transparent. The results of this thesis demonstrate that CPC has high potential for application as a food ingredient in the industry.

Keywords: Plant-based protein, Functional properties, Rheological properties, Emulsions, Edible films

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INTRODUCTION

By 2050, the global population is projected to reach approximately 9.8 billion people (United Nations, 2024). In addition to population growth, changing behaviors, beliefs, and lifestyle preferences demand the development of modern technologies that can provide high-quality and sustainable food (Ahmed et al., 2022; Aiking, 2011; Alexander et al., 2019). Addressing the challenge of feeding this growing population while minimizing environmental impacts is imperative for the food industry. One strategy for achieving more sustainable diets is to reduce excessive consumption of animal proteins while simultaneously increasing the intake of plant-based proteins.

Plant-based proteins offer a viable alternative to animal proteins, as they are low-cost and obtained from abundant resources, with sustainable cultivation systems that require less capital and energy consumption for production (Bessada et al., 2019; De Boer et al., 2014; Stone et al., 2018). Although soy is one of the primary sources of vegetable protein (Singh et al., 2008), particularly in Brazil, it is assumed that soy production will not be sufficient to meet the demand for plant-based proteins in the coming years. Therefore, the partial replacement of animal proteins by plant proteins can be driven by the diversification of protein sources, with the inclusion of legumes that are still underutilized in human nutrition.

The cowpea (*Vigna unguiculata* (L.) Walp), but also known around the world as “black-eyed peas” or “southern peas” (all used in the USA), “frijol caupí” (Spanish-speaking countries in America), “lobia” (India), “caupi” (Brazil), “caupí” and “carilla” (Spain), “niébé” (French-speaking countries in Africa) and “feijão-frade” (Portugal) (Carvalho et al., 2017), is one of the most adaptable and versatile legumes, capable of adapting to a wide range of soil types, high temperatures and drought (Carvalho et al., 2017; Ehlers & Hall, 1997). Africa is responsible for 95.3% of the world's cowpea production (FAO, 2022), while Brazil has historically been the world's third largest producer, preceded by Nigeria and Niger, and the largest producer and consumer in Latin America (Freire-Filho et al., 2023). Currently, this crop has been acquiring greater economic expression, and its cultivation is carried out by small, medium and large producers (Freire-Filho et al., 2012;2023).

Cowpeas have been the subject of research as a potential alternative source of plant-based protein. These studies have investigated the extraction of proteins and the evaluation of their properties, including solubility, water and oil retention capacity, foam formation, emulsification, as well as thermal and rheological characteristics and amino acid profiles (Ge et al., 2021; Oliveira et al., 2024; Schlangen et al., 2022; Tang et al., 2021; Vasconcelos et al., 2010). Despite the existing literature on the technological properties of cowpea protein (CP),

there is still a lack of information in the published works regarding all those properties in an integrated manner. These properties confer a broad range of applications for the proteins in question, including use in enriched products, ingredient replacement, edible films, microcapsules, and emulsions (Campbell et al., 2016; de Almeida et al., 2021; Hewage & Vithanarachchi, 2009; Pereira et al., 2009). The functionality, nutritional value and structural role of proteins are essential for their usefulness. Therefore, the present study concentrates on two main applications of cowpea protein: the stabilization of emulsions and the production of edible films.

Proteins can function as natural emulsifiers due to their amphiphilic properties, allowing them to self-aggregate and form a continuous and uniform layer around the oil droplets (Dickinson et al., 1997). The key properties of cowpea protein, including its ability to adsorb and interact with water and oil, coupled with a solubility exceeding 80%, underscore its exceptional performance as an emulsifier and stabilizer (Ge et al., 2021; Ragab et al., 2004; Tang et al., 2021; Thompson et al., 2023). To date, a single study has evaluated the emulsifying capacity of cowpea protein isolates (Thompson et al., 2023). This scarcity of research on the topic suggests that there is still a significant need for further investigation in this area.

One promising solution to the excessive use of synthetic plastics is the development of edible films. In the literature, only two studies have focused specifically on edible films based on cowpea protein (Almeida et al., 2021; Hewage & Vithanarachchi, 2009). This indicates that the potential of cowpea protein remains unexplored even though evidence of its favorable techno-functional properties. While the available literature is limited, it suggests that cowpea protein can form films with satisfactory properties. Nevertheless, the development of films with distinctive properties using this unexplored raw material may prove to be a considerable challenge.

Therefore, the objective of this thesis was to extract, characterize, and evaluate the techno-functional properties of cowpea protein with a view to its potential application in the stabilization of emulsions, and in the development of edible films. The initial step involved the extraction of cowpea protein through the application of the isoelectric precipitation technique. The resulting protein concentrate from cowpea (CPC) was then subjected to characterization and evaluation for its functionality across a range of pH values. Subsequently, the concentrate was employed in the formation of stable emulsions, which were developed at varying pH levels and oil concentrations. The emulsions were subjected to analysis in order to ascertain their droplet size and stability. Edible films based on the CPC were then obtained and characterized

at varying pH levels. Using a selected emulsion formulation, films were developed by adding different concentrations of this emulsion to the matrix, aiming to modify the film properties.

GENERAL CONCLUSIONS

The primary objective of this thesis was to extract, characterize, and apply cowpea protein concentrate (CPC), to establish its potential as a versatile biopolymer with a wide range of possible applications.

The SDS-PAGE analysis of the CPC revealed a spectrum of protein sizes, with pronounced bands around 50 kDa, indicative of 7S globulins (vicilins). From a nutritional perspective, the extraction of CPC resulted in a reduction in trypsin inhibitor levels. Furthermore, the conversion of raw cowpea into CPC resulted in proportions of both essential and non-essential amino acid levels that meet the recommendations set forth by the FAO. Its high concentration of lysine, an essential amino acid often limiting in most cereals, endows CPC with significant nutritional value, rendering it a highly suitable option for dietary supplementation. In terms of functionality, protein concentrate solutions under alkaline pH conditions demonstrated enhanced solubility, as well as superior emulsifying and foaming capabilities, in comparison to protein concentrates under acidic pH conditions. These functional characteristics are essential for the optimal application of plant proteins in food processing.

Further elucidation was made regarding the rheological and thermal aspects of aqueous CPC solutions under different concentration and pH conditions. The rheological analysis demonstrated a high degree of sensitivity to protein concentration and shear rate, particularly at elevated concentrations. The apparent viscosity at low shear rates for concentrations up to 10% indicated a shear thinning behavior, becoming similar to Newtonian fluids at higher shear rates. At a 20% concentration, shear thinning behavior was observed across all shear rates. The pH had a significant impact on the observed behavior: acidic conditions near the isoelectric point affected shear stress, viscosity, and elastic behavior, while neutral and alkaline pH levels maintained consistent behavior. During the thermal cycle, changes in molecular interactions and protein structure led to progressive changes in viscosity. The pH also influenced viscosity and elastic properties, with alkaline pH causing significant structural changes and a reduction in denaturation and gelation temperatures. The reduced viscosity of cowpea protein suspensions, particularly at concentrations up to 5%, offers substantial benefits in process engineering. Their lower resistance to flow enhances efficiency in industrial operations like mixing, homogenization, and pumping, leading to energy savings and easier handling. This study also extends the rheological analysis of cowpea solutions to higher pH ranges, a relatively unexplored area in the literature. The findings could be valuable for food science, particularly

in developing new systems and materials, such as emulsions (Chapter 4) and films (Chapters 5 and 6).

Based on the previous results, CPC demonstrated promising potential for emulsion stabilization, especially at neutral to alkaline pH levels, where proteins exhibit greater solubility and repulsion, reducing aggregation and flocculation, resulting in improved stability. Furthermore, its low viscosity, particularly at low concentrations, enhances its suitability for emulsions without significantly affecting system viscosity. Considering this evidence, the third part of this thesis investigated the behavior of CPC-stabilized emulsions under different pH conditions and oil concentrations. The results indicated a broader droplet size distribution, with enhanced homogeneity observed in the emulsions at pH 9, in comparison to pH 7 and 11. Emulsions with higher oil concentrations demonstrated a more pronounced shear thickening behavior, while pH variations exerted a significant influence on the consistency index and flow behavior index, underscoring the emulsions' responses to shear forces. Heating and cooling led to changes in viscosity, which were attributed to modifications in the emulsion's microstructure and intermolecular interactions. Emulsions prepared at pH 11 exhibited enhanced stability and resistance to thermal fluctuations. The physical stability analysis indicated that emulsions prepared at pH 9 and 11 exhibited enhanced stability of the cream layer, with a comparable stability profile.

The final two chapters were built on the previous one to establish guidelines for film formulation. An optimal pH range of 7 to 11 was identified, ensuring high solubility and stable charge density. The rheological analysis showed that low viscosities in protein solutions and emulsions are favorable for film production. Therefore, in the fourth and fifth parts of this study, the CPC was evaluated for its film-forming capacity under different pH conditions and subsequently with the incorporation of varying emulsion concentrations into its composition. The results demonstrated that pH 9 emerged as a critical point for the formation of the edible films studied, exhibiting a notable reduction in the incidence of cracks on the surface and within the thickness of the films. This condition also had a significant impact on oxygen permeability (O_2P) of the material. The films formed at the different pH levels under study exhibited structures with a low degree of crystallinity. This was accompanied by a reduction in the prevalence of disordered structures when the pH was decreased from 11 to 7. The CPC films demonstrated excellent UV barrier properties. However, the variation in pH did not significantly affect the water vapor permeability, solubility, tensile strength, or elongation at break of the films. In response to the addition of emulsions at varying concentrations, the film microstructure exhibited an uneven distribution of emulsion droplets on the lower surface in

contact with a mold. Nevertheless, the films presented a cohesive structure, particularly at 1%, without apparent cracks. The emulsion may have acted as a filler, which resulted in an increase in the films' tensile strength and elongation at break. The addition of the hydrophobic component did not compromise the barrier and solubility properties. Furthermore, there was a significant reduction in oxygen permeability in films with the highest emulsion concentration (1%). These findings demonstrate the efficacy of the methodology for incorporating lipidic substances into CPC films and their potential for future modifications, including the incorporation of lipophilic compounds such as bioactive substances.

The central argument of this thesis is that CPC is a significant and multifaceted material with a wide range of applications. The thesis demonstrates how the findings of each chapter are interconnected, providing a comprehensive understanding of CPC. The study indicates that CPC is a highly versatile biopolymer, with the potential for enhanced effectiveness through adjustments in pH and concentration conditions. The integration of the findings provides a comprehensive understanding of the nutritional and functional properties of CPC, as well as its applications in emulsions and edible films. This work generates a significant dataset that reveals the advantages and limitations of different compositions and pH levels tested, thereby providing a solid foundation for future research. These findings contribute to our understanding of CPC and provide valuable preliminary evidence for its potential applications in both technological and food-related fields.

REFERENCES

- AHMED, N., ALI, A., RIAZ, S., AHMAD, A., & AQIB, M. (2022). Vegetable Proteins: Nutritional Value, Sustainability, and Future Perspectives. In *Vegetable Crops - Health Benefits and Cultivation*. IntechOpen. <https://doi.org/10.5772/intechopen.100236>
- AIKING, H. Future protein supply. **Trends in Food Science & Technology**, v. 22, n. 2-3, p. 112-120, 2011.
- ALENCAR, J. (S.D.). Frase atribuída. Não há fonte específica publicada.
- ALEXANDER, P., BROWN, C., DIAS, C., MORAN, D., & ROUNSEVELL, M. D. A. Sustainable Proteins Production. In *Proteins: Sustainable Source, Processing and Applications* (pp. 1–39). Elsevier. (2019). <https://doi.org/10.1016/B978-0-12-816695-6.00001-5>
- ALMEIDA, F. C., DE SOUZA, C. O., PHILADELPHO, B. O., FRANÇA LEMOS, P. V., CARDOSO, L. G., SANTANA, J. S., ALVES DA SILVA, J. B., CRUZ CORREIA, P. R., CAMILLOTO, G. P., DE SOUZA FERREIRA, E., & DRUZIAN, J. I. Combined effect of cassava starch nanoparticles and protein isolate in properties of starch-based nanocomposite films. **Journal of Applied Polymer Science**, v. 138, n. 18, p. 50008, (2021). <https://doi.org/10.1002/app.50008>
- BESSADA, S. M., BARREIRA, J. C., & OLIVEIRA, M. B. P. Pulses and food security: Dietary protein, digestibility, bioactive and functional properties. **Trends in Food Science & Technology**, v. 93, p. 53-68, 2019.
- CAMPBELL, L., EUSTON, S. R., & AHMED, M. A. Effect of addition of thermally modified cowpea protein on sensory acceptability and textural properties of wheat bread and sponge cake. **Food Chemistry**, v. 194, p. 1230-1237, 2016.
- CARVALHO, M., LINO-NETO, T., ROSA, E., & CARNIDE, V. Cowpea: a legume crop for a challenging environment. **Journal of the Science of Food and Agriculture**, v. 97, n. 13, p. 4273-4284, 2017.
- DE BOER, J., SCHÖSLER, H., & AIKING, H.. “Meatless days” or “less but better”? Exploring strategies to adapt Western meat consumption to health and sustainability challenges. **Appetite**, v. 76, p. 120-128, 2014. <https://doi.org/10.1016/j.appet.2014.02.002>
- DICKINSON, E., GOLDING, M., & POVEY, M. J. Creaming and flocculation of oil-in-water emulsions containing sodium caseinate. **Journal of colloid and interface science**, v. 185, n. 2, p. 515-529, 1997.
- EHLERS, J. D.; HALL, A. E. Cowpea (*Vigna unguiculata* L. walp.). **Field crops research**, v. 53, n. 1-3, p. 187-204, 1997.
- FAO. *Production Quantities of Cow Peas Dry by Country*. Accessed July 21, 2024. <http://faostat.fao.org/>
- FREIRE FILHO, F. R., RIBEIRO, V. Q., FREIRE FILHO, F. R., & RIBEIRO, V. Q. Feijão-Caupi na Embrapa Meio-Norte: melhoramento, cultivares lançadas, genealogias e base genética. Embrapa, 2023.

- FREIRE, F. F. R., RIBEIRO, V. Q., & ROCHA, M. D. M. Production, breeding and potential of cowpea crop in Brazil. Embrapa, 2012.
- GE, J., SUN, C. X., MATA, A., CORKE, H., GAN, R. Y., & FANG, Y. Physicochemical and pH-dependent functional properties of proteins isolated from eight traditional Chinese beans. **Food Hydrocolloids**, v. 112, p. 106288, 2021.
- HEWAGE, S., & VITHANARACHCHI, S. M. Preparation and characterization of biodegradable polymer films from cowpea (*Vigna unguiculata*) protein isolate. **Journal of the National Science Foundation of Sri Lanka**, v. 37, n. 1, 2009.
- OLIVEIRA, M. M. G., FESSORI, A. G. B. W., HUAMANÍ-MELÉNDEZ, V. J., & MAURO, M. A. (2024). Exploring the rheological and thermal behavior of cowpea protein concentrate: Impact of pH and concentration. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 694, p. 134106, 2024. <https://doi.org/10.1016/j.colsurfa.2024.134106>
- PEREIRA, H. V. R., SARAIVA, K. P., CARVALHO, L. M. J., ANDRADE, L. R., PEDROSA, C., & PIERUCCI, A. P. T. R. (2009). Legumes seeds protein isolates in the production of ascorbic acid microparticles. **Food Research International**, v. 42, n. 1, p. 115-121, 2009. <https://doi.org/10.1016/j.foodres.2008.10.008>
- RAGAB, D. D. M., BABIKER, E. E., & ELTINAY, A. H. (2004). Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and/or salt concentration. **Food chemistry**, v. 84, n. 2, p. 207-212, 2004. [https://doi.org/10.1016/S0308-8146\(03\)00203-6](https://doi.org/10.1016/S0308-8146(03)00203-6)
- SCHLANGEN, M., TAGHIAN DINANI, S., SCHUTYSER, M. A. I., & VAN DER GOOT, A. J. (2022). Dry fractionation to produce functional fractions from mung bean, yellow pea and cowpea flour. **Innovative Food Science & Emerging Technologies**, v. 78, p. 103018, 2022. <https://doi.org/10.1016/j.ifset.2022.103018>
- SINGH, P., KUMAR, R., SABAPATHY, S. N., & BAWA, A. S. (2008). Functional and edible uses of soy protein products. **Comprehensive reviews in food science and food safety**, v. 7, n. 1, p. 14-28, 2008.
- STONE, A. K., WANG, Y., TULBEK, M., & NICKERSON, M. T. (2018). Plant protein ingredients. In *Encyclopedia of Food Chemistry* (pp. 229–234). Elsevier. <https://doi.org/10.1016/B978-0-08-100596-5.21601-6>
- TANG, X., SHEN, Y., ZHANG, Y., SCHILLING, M. W., & LI, Y. (2021). Parallel comparison of functional and physicochemical properties of common pulse proteins. **Lwt**, v. 146, p. 111594, 2021.
- THOMPSON, C. M. B., ACEVEDO, B. A., AÑÓN, M. C., & AVANZA, M. V. (2023). Emulsifying Capacity of Cowpea Protein Isolates. Effect of Thermal and Hydrolytic Treatment. **Plant Foods for Human Nutrition**, v. 78, n. 2, p. 366-374, 2023.
- UNITED NATIONS, Population Division. *World Population Prospects*. Accessed July 21, 2024. <https://population.un.org/wpp/>
- VASCONCELOS, I. M., MAIA, F. M. M., FARIAS, D. F., CAMPELLO, C. C., CARVALHO, A. F. U., De AZEVEDO MOREIRA, R., & De OLIVEIRA, J. T. A. (2010). Protein fractions,

amino acid composition and antinutritional constituents of high-yielding cowpea cultivars. **Journal of food composition and analysis**, v. 23, n. 1, p. 54-60, 2010. <https://doi.org/10.1016/j.jfca.2009.05.008>