I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIOPRODUCTS ENGINEERING

PROTEIN RECOVERY FROM BROKEN BLACK BEAN: IMPACT ON FUNCTIONAL AND NUTRITIONAL PROPERTIES

Renata F. Teixeira[1*], Laís Benvenutti[1], Débora de Oliveira[1] & Acácio A. F. Zielinski[1]

[1] *Chemical and Food Engineering Department, Federal University of Santa Catarina, EQA/UFSC CEP 88040-900, Florianópolis, SC, Brazil.*
** Corresponding author's email address: renatafialho_t@gmail.com*

ABSTRACT

Protein extraction methods significantly impact protein fractions' purity, solubility, nutritional properties, and functionality. The pressurized liquid extraction (PLE) method to extract protein from broken black beans (BBB) using water as solvent resulted in the lowest levels of antinutritional compounds. In alkaline conditions, it exhibited the highest protein purity and yield, better solubility, and *in vitro* protein digestibility. Conversely, heating-stirring extraction (HSE) with alkali resulted in lower protein contents and digestibility, while ultrasound-assisted extraction (UAE) employing water as solvent showed reduced solubility and emulsification properties. Extraction by PLE induced protein structural alterations, favoring β-turn and α-helix conformations, enhancing solubility and emulsification capacity. The water and oil holding capacities varied among extraction methods, impacting food products' texture and sensory attributes. Overall, PLE emerged as a promising approach for recovering protein from BBB, offering potential applications in protein supplements, meat substitutes, and baked goods, meeting the rising demand for sustainable protein sources in the food industry.

Keywords: Emerging technologies. Antinutritional factors. IVPD. Sustainable protein sources.

1 INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) are one of the most important pulses for human nutrition, cultivated across various regions worldwide, with Brazil being the second-largest global producer. During bean processing, mechanical damage generates a by-product with low commercial value. This by-product, particularly broken black beans (BBB), is notable for its high protein content, offering a valuable opportunity to recover and utilize high-quality proteins. Emerging technologies have demonstrated significant potential in protein recovery, enhancing their purity, functionality, and nutritional properties[1,2]. Among these technologies, ultrasound-assisted extraction (UAE) facilitates the release of proteins from plant matrices, potentially increasing process efficiency and improving protein characteristics[3]. More recently, the gradual adoption of pressurized liquid extraction (PLE) has been increasingly utilized for protein recovery from plant-based sources, showing promising results[4,5]. However, its specific application to recovering proteins from beans or their by-products remains underexplored in the current literature.

Therefore, this study aimed to evaluate the impact of conventional heating-stirring extraction (HSE) alongside UAE and PLE technologies on the recovery of proteins, conformational changes, functionality, and nutritional properties (such as *in vitro* protein digestibility and antinutritional factors) of broken black bean protein concentrate (BBBPC) using water and alkaline solution as solvents.

2 MATERIAL & METHODS

Broken black bean protein concentrates (BBBPC) were recovered using the conventional heating-stirring extraction (HSE) method and emerging technologies such as ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE). Two solvents were utilized: water and water under alkaline conditions (adjusted to pH 9.0 with 0.1 M NaOH) in extraction conditions with a solid-liquid ratio of 1:20 (w/v) at a temperature of 60 °C for 15 min (PLE operated at 100 bar with a flow rate of 4 $mL.min^{-1}$, and UAE at a power of 300 W and an ultrasonic frequency of 20 kHz). The extracts were then subjected to isoelectric protein precipitation steps (pH adjustment to 4.5), followed by centrifugation, neutralization, and lyophilization. Protein purity and yield were determined based on protein content using the Kjeldahl method. The antinutritional factors (ANF) evaluated were phytic acid, trypsin inhibitor activity, and tannins. *In vitro* protein digestibility (IVPD) analysis was performed using a multi-enzymatic method (peptidase, trypsin, and α-chymotrypsin)[6]. The solubility of the proteins was determined at neutral pH[7]. The techno-functional properties of BBBPC evaluated included emulsifying activity index (EAI), emulsion stability index (ESI), foaming capacity (FC), foam stability (FS), oil holding capacity (OHC), and water holding capacity (WHC)[8].

3 RESULTS & DISCUSSION

The results regarding protein purity and yield, solubility, techno-functional characteristics (water holding capacity – WHC, oil holding capacity – OHC, emulsifying activity index – EAI, emulsion stability index – ESI, foaming capacity – FC, and foam stability – FS) of BBBPC are presented in Table 1.

Protein fractions recovered by the PLE (water) and HSE (alkaline) methods showed the highest protein purity (86.7 % and 86.1 %, respectively), while UAE and HSE using water as a solvent resulted in the lowest protein contents (79.3 % and 79.5 %, respectively). PLE (alkaline) showed the highest protein yield (23.9 %), followed by HSE (22.2 %) against the 17.9 % yield of UAE.

Using an alkaline solvent led to higher yield values than water (8 % to 17 % higher) due to the breakdown of the cell wall in concentrated alkaline solutions, which facilitates the release of proteins. The highest solubility of BBBPC was obtained with the PLE method under alkaline conditions (83.3 %), surpassing UAE and HSE by approximately 27 % and 55 %, respectively. Employing water as a solvent resulted in protein solubility of 75.6 % with PLE, compared to 67.6 % with UAE and 64.4 % with HSE. The increased protein solubility obtained through the application of PLE can be attributed to the fragmentation of large insoluble protein aggregates into smaller soluble proteins, as well as the balancing of hydrophobic and hydrophilic sites on the molecular surface of the proteins, resulting from unfolding and structural rearrangement under high-pressure conditions[2,9].

Proteins' water- and oil-holding capacities are crucial for products' sensory stability and shelf life when used in beverage, meat, and bakery formulations. WHC and OHC directly influence texture, contributing to the quality and acceptability of these foods. The application of PLE with both solvents and UAE (alkaline) resulted in the highest OHC values (11.8-12.9 g/g), while the highest WHC values (2.2-2.4 g/g) were obtained with UAE and HSE using water as the solvent. Furthermore, the highest OHC values coincided with the lowest WHC values, except for PLE (alkaline). This effect occurs due to the greater exposure of hydrophobic groups, which trap oil and reduce WHC[10]. A high OHC is associated with flavor retention and juiciness in foods. On the other hand, high WHC values, related to the dissociation and partial denaturation of the protein, contribute to the attributes of tenderness and mouthfeel moisture in foods. The characteristics influencing foam formation and emulsification, such as protein solubility, molecular structure, and intermolecular interactions, are crucial for food product development.

Table 1 Purity and protein yield, solubility, and techno-functional characteristics of BBBPC recovered by different solvents and extraction methods.

Method	Purity (%)	Yield (%)	Solubility (%)	WHC (g/g)	OHC (g/g)	EAI (m^2/g)	ESI (min)	FC (%)	FS (min)
PLE (water)	86.7^{a}±1	19.9^{c}±3	75.6^{b}±3	1.2^{d}±0.1	$11.8^{a.b}$±1	6.5^{b}±0.3	30.2^{a}±2	106.7^{b}±5	91.2^{d}±1
PLE (alkaline)	$82.7^{b.c}$±2	23.9^{a}±2	83.3^{a}±1	$2.0^{a.b}$±0.1	$11.9^{a.b}$±0	8.4^{a}±0.4	31.5^{a}±2	125.5^{a}±4	95.2^{b}±4
UAE (water)	79.3^{c}±1	16.5^{f}±2	67.6^{c}±6	2.4^{a}±0.2	$9.9^{b,c}$±2	1.6^{e}±0.2	25.1^{b}±3	$103.3^{b,c}$±3	96.3^{a}±2
UAE (alkaline)	$82.5^{b.c}$±1	17.9^{e}±1	$65.8^{c.d}$±2	$1.6^{c,d}$±0.1	12.9^{a}±1	2.0^{d}±0.2	14.9^{d}±0.2	96.7^{c}±3	96.6^{a}±1
HSE (water)	79.5^{c}±1	18.8^{d}±1	$64.4^{d.e}$±3	$2.2^{a.b}$±0.3	$9.4^{b,c}$±1	3.0^{c}±0.1	17.9^{c}±1	83.3^{d}±2	92.5^{c}±1
HSE (alkaline)	$86.1^{a.b}$±1	22.2^{b}±2	53.6^{e}±1	$1.9^{b,c}$±0.1	$10.2^{b,c}$±1	2.7^{c}±0.4	17.7^{c}±2	86.7^{d}±5	91.1^{d}±1

Consequently, the increased solubility of BBBPC recovered by PLE, especially under alkaline conditions, significantly improved EAI, ESI, and FC, with enhancements of 320 %, 111 %, and 30 %, respectively, compared to UAE (alkaline), as it is the soluble protein fraction that contributes to emulsification and foam formation capacity. HSE technology (water and alkaline) yields intermediate EAI and ESI values, which results in the lowest FC. However, all treatments maintained above 90 % of the initially formed foam after 60 min, demonstrating high stability. The good foam stability is ideal for application in food products such as mousses and toppings. Proteins with superior emulsification capabilities are valuable for producing hamburgers, sausages, and meat substitutes.

In Table 2, greater spectral changes were observed in the protein concentrates recovered by PLE, which exhibited a predominant β-turn conformation (>44 %) and high proportions of intramolecular β-sheet (>35 %). Additionally, an α-helix conformation (11 %) was identified exclusively using the PLE (alkaline) method, indicating that applying high pressure induces partial unfolding of the secondary protein structure of BBB. Conversely, the UAE and HSE technologies were primarily composed of random coil (>46 %) and β-turn (>29 %) conformations, regardless of the solvent used. It is important to note that the unfolding of protein structure, particularly the transition of β conformations, plays a crucial role in determining the digestibility, solubility, and interfacial properties of proteins[11].

Table 2 Relative proportion of secondary structures in BBBPC recovered by different extraction technologies and solvents.

Conformation (wave number)	Intermolecular β-sheet (%)	β-turn (%	Intramolecular β-sheet (%)	No order-Random coil (%)	α-helix (%)	Intermolecular β-sheet (%)
	1620-1630 cm^{-1}	1660-1690 cm^{-1}	1620-1640 cm^{-1}	1640-1649 cm^{-1}	1650-1660 cm^{-1}	1690-1700 cm^{-1}
Method						
PLE (water)	3.3	53.5	44.2	-	-	-
PLE (alkaline)	3.2	44.1	34.8	-	11.2	7.1
UAE (water)	3.1	37.4	-	48.9	-	11.3
UAE (alkaline)	3.2	33.4	-	52.4	-	12.4
HSE (water)	2.4	41.8	-	47.5	-	9.3
HSE (alkaline)	2.3	34.9	-	50.8	-	12.4

Protein concentrates recovering using PLE (water) exhibited the lowest levels of phytic acid, tannins, and trypsin inhibitory activity (TIA) compared to other extraction technologies (Table 3). In contrast, the highest concentrations of these compounds were observed when HSE was employed. Specifically, BBBPC recovered via HSE (water) showed elevated tannin content, showcasing significant variations among the solvents and techniques investigated. While using the alkaline solvent during UAE led to an increase in tannins in the BBBPC, a reduction in these compounds was noted with HSE technology. The intricate interactions between tannins and protein extraction methods may be influenced by the medium's pH, where differing charges can facilitate tannin-protein complex formation, impacting nutrient availability.

On the other hand, using HSE (alkaline) resulted in higher levels of phytates and TIA, potentially hindering protein digestibility. PLE (water) application led to a substantial reduction of over 48 % in these compounds compared to HSE (alkaline) technology. Regardless of the method, using water-alkaline as the solvent resulted in the highest levels of these antinutritional factors (ANFs).

High-pressure techniques were observed to disrupt the protein-antinutrient bonds, converting them into their free forms and consequently lowering their levels in the concentrates. Additionally, the highest IVPD values (~71 %) were obtained using the PLE (alkaline) and UAE (water) technologies. These extraction approaches facilitate the breakdown of complex protein structures, enhancing the accessibility of proteolytic enzymes to their active sites. Conversely, the lowest digestibility (67 %) was recorded for proteins recovered by HSE (alkaline). This highlights the significance of extraction methods in influencing the levels of ANFs in protein concentrates, with PLE showing promise in reducing these compounds effectively and improving the IVPD.

Table 3 *In vitro* protein digestibility and antinutritional factors concentration of BBBPC recovered by different solvents and extraction methods.

Method	IVPD (%)	Phytic acid (mg PAE/g)	TIA (TIU/mg)	Tannins (mg TAE/g)
PLE (water)	$69.9^{a.b}$±1	15.3^{f}±0.6	21.2^{e}±1	19.4^{d}±0.1
PLE (alkaline)	70.8^{a}±1	18.6^{e}±0.3	35.2^{c}±1	20.1^{d}±0.1
UAE (water)	70.9^{a}±1	20.2^{d}±0.4	26.4^{d}±4	20.9^{c}±0.3
UAE (alkaline)	68.8^{b}±1	21.9^{c}±0.2	36.8^{b}±2	21.9^{b}±0.1
HSE (water)	68.5^{b}±1	25.1^{b}±0.5	35.5^{b}±4	23.7^{a}±0.4
HSE (alkaline)	66.8^{c}±1	29.7^{a}±0.3	47.4^{a}±3	22.4^{b}±0.8

4 CONCLUSION

Using PLE (water) resulted in the lowest concentrations of ANFs (phytic acid, tannins, and trypsin inhibitors). At the same time, PLE (alkaline) enhanced the solubility, IVPD, and techno-functional characteristics of BBBPC, ascribed to alterations in the protein structure's conformation, such as unfolding under high-pressure fluid conditions. UAE (water) exhibited elevated IVPD and WHC levels but diminished EAI and solubility, potentially linked to modifications in protein surface behavior. Conversely, apart from WHC and EAI attributes, the application of HSE, utilizing both solvents, yielded protein concentrates with reduced functionality. The adoption of PLE technology for protein retrieval demonstrates promise for utilization in protein supplements, meat substitutes, confectioneries, and baked goods, consequently elevating the worth of bean by-products. Furthermore, this strategy addresses the increasing demand for substitute protein sources by employing an eco-friendly, reliable, and effective approach. The outcomes derived from this investigation could unveil novel perspectives for advancing the food industry and various technological implementations.

REFERENCES

[1] TEIXEIRA, R. F. *et al.*, 2023, Prospects on emerging eco-friendly and innovative technologies to add value to dry bean proteins. Crit. Rev. Food Sci. Nutr., 1-25.

[2] TEIXEIRA, R. F. *et al.*, 2024. Unveiling the potential of pressurized liquid extraction for recovering protein fractions from broken black beans: Insights into thermal and structural properties. Food Hydrocoll**.** 149, 109649.

[3] ZHAO, C. *et al.*, 2022. Ultrasound-induced red bean protein–lutein interactions and their effects on physicochemical properties, antioxidant activities and digestion behaviors of complexes. LWT**.** 160, 113322.

[4] GUZMÁN-LORITE, M.; MARINA, M. L.; GARCÍA, M. C. 2022. Successive extraction using natural deep eutectic solvents and pressurized liquids for a greener and holistic recovery of proteins from pomegranate seeds. Food Res. Int., 161,111862.

[5] TORRES, T. M. S. *et al.*, 2022. Protein valorization from ora-pro-nobis leaves by compressed fluids biorefinery extractions. Innov. Food Sci. Emerg. Technol. 76, 102926.

[6] SÁ, A. G. A. *et al.*, 2021. Oilseed by-products as plant-based protein sources: Amino acid profile and digestibility. Future Foods. 3, 100023.

[7] WANG, Q. *et al.*, 2021. Ultrasound-assisted alkaline proteinase extraction enhances the yield of pecan protein and modifies its functional properties. Ultrason. Sonochem., 80, 105789.

[8] GUNDOGAN, R.; KARACA, C. A., 2020. Physicochemical and functional properties of proteins isolated from local beans of Turkey. LWT. 130, 109609.

[9] XIA, N. *et al.*, 2012. Preparation and characterization of protein from heat-stabilized rice bran using hydrothermal cooking combined with amylase pretreatment. J. Food Eng.110 (1), 95-101.

[10] RAHMAN, M. M.; LAMSAL, B. P. 2021. Ultrasound-assisted extraction and modification of plant-based proteins: Impact on physicochemical, functional, and nutritional properties. Compr. Rev. Food Sci. Food Saf, 20 (2),1457-1480.

[11] ZHAO, M. *et al.*, 2020. Enhancing the solubility and foam ability of rice glutelin by heat treatment at pH12: Insight into protein structure. Food Hydrocoll, 103,105626.

ACKNOWLEDGEMENTS

The authors are grateful to CAPES (Grant number 88887.495308/2020-00, RFT scholarship).