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Scientific Research

Chemical and Functional Properties of Hard-to-Cook Bean (*Phaseolus vulgaris*) Protein Concentrate

Maira R. Segura-Campos, Jimena Cruz-Salas, Luis Chel-Guerrero, David Betancur-Ancona*

Facultad de Ingeniería Química, Universidad Autónoma de Yucatán,
Mérida, México
Email: *bancona@uady.mx

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Abstract

The objective of this research was to evaluate the chemical and functional properties of hard-to-cook (HTC) bean (*Phaseolus vulgaris*) protein concentrate to determine their potential practical applications. The respective protein concentrate was obtained from the flour using isoelectric precipitation and the protein content was 73.03%. Proximate composition and *in vitro* digestibility were measured to evaluate the chemical properties, and nitrogen solubility, emulsifying capacity, emulsion stability, foaming capacity, foam stability and viscosity were measured to evaluate its functional properties. The proximate composition of the HTC bean (*P. vulgaris*) flour and protein concentrate registered values of moisture, ash, protein, fat, fiber and NFE of 8.92, 4.52, 21.71%, 4.41%, 4.11% and 65.25% for flour and of 2.68%, 2.54%, 73.03%, 2.77%, 1.31% and 20.35% for protein concentrate. The *in vitro* digestibility was of 76.7%. The hard-to-cook bean protein concentrate exhibited good functional properties suggesting its use as additive. This concentrate registered solubility values that are ranging from 2.5% to 71.81%. The emulsifying (EC) and foaming capacity (FC) registered values of 89% - 97% and of 7% - 53% at different pH levels, respectively as well as an emulsion (ES) and foaming stability (FS) pH- and time-dependent. The HTC bean (*P. vulgaris*) protein concentrate registered a viscosity profile dependent of shear rate. The results suggest that HTC bean (*P. vulgaris*) protein concentrate is a valuable food ingredient or additive.

Keywords

Hard-to-Cook Bean, *Phaseolus vulgaris*, Protein Concentrate, Chemical and Functional Properties

*Corresponding author.

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1. Introduction

Inadequate postharvest handling and storage techniques (*i.e.* temperature > 25˚C and relative humidity > 65%) produce the HTC defect in beans. This defect is the result of physical and chemical changes at the intercellular level during storage, which causes increased stability of the middle lamella during cooking. Insolubilization of pectic substances by enzyme phytase is the most widely accepted explanation. Although other possible enzymatic reactions may contribute to hardening, such as removal of methyl groups from pectins by pectinesterases, hydrolysis of storage proteins byproteases, polyphenol oxidation assisted by peroxidases or polyphenolases and, less likely, lipids oxidation by lipoxygenases. The HTC defect in beans and other legumes is considered a fundamental textural quality, and occurs when they absorb sufficient water during cooking but fail to soften. The longer required cooking time and consequently higher energy requirements for preparation of HTC seeds negatively affect their nutritional quality and make them less acceptable and marketable [1].

The rapidly growing food industry demands new ingredients. This has drawn the attention of researchers to legume components suited for wet-fractionation. At the moment, there is a strong public interest in food ingredients from natural sources. Some technological alternatives have been proposed for use of hardened beans (HTC), including extrusion [2] dry fractionation, alkaline heat treatment and soaking in saline solutions [3]. In response, the wet-fractionation process has been proposed as a means of detoxification of beans, but has also been proven as a viable technology for integral use of this seeds. Wet-fractionation produces protein concentrates, fiber rich fractions and starch fractions [4]. Alkaline extraction is a technological alternative for protein isolation from HTC beans. The concentrated protein has low trypsin inhibitor activity and meets Food and Agriculture Organization recommendations for essential amino acids content in diets for adults, meaning they are potentially useful as a supplementary vegetable protein source in food manufacturing [5].

Given the demand of the food industry for new functional ingredients, it is worthwhile to characterize the hard-to-cook bean (*Phaseolus vulgaris*) protein concentrate with a view toward establishing its possible uses and adding values to this legume seed. For the above mentioned, the objective of this research is to evaluate the chemical and functional properties of hard-to-cook bean (*Phaseolus vulgaris*) protein concentrate.

2. Materials and Methods

2.1. Seeds and Chemicals

Common black beans (*Phaseolus vulgaris* L.) var. Jamapa used in this study were obtained from local market in Mérida, Yucatán, México. Two lots of 1 kg each were used to determine cooking time and hardness following methods in applicable Mexican regulations [6]. All chemicals were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Flour Preparation

Selected seeds were processed in a disk mill (model 4-E Quaker, Mill Straub Co., Philadelphia, PA) until producing flour. The flour was then sifted through 4.76 and 2.38 mm screens and the hulls removed with a fluidizing air-bed. It was then milled in a Cyclotec mill (Tecator, Hoganas, Sweden) until passing through a 0.841-mm screen.

2.3. Protein Concentrate

A single extraction was done with 10 kg of HTC bean flour using a wet fractionation method [7], with some modifications. Briefly, whole flour was suspended in distilled water at a 1:10 (w/v) ratio, pH adjusted to 8.0 with 1.0 M NaOH, and the dispersion stirred at room temperature for 1 h at 400 rpm with a mechanical agitator (Caframo Rz-1, Heidolph Schwabach, Germany). This suspension was wet-milled with a Kitchen-Aid mill and the fiber solids separated from the starch and protein mix by straining through 0.177-mm screens and washing the residue five times with distilled water. The protein-starch suspension was allowed to sediment for 30 min at room temperature to recover the starch and protein fractions. The pH of the separated solubilized proteins was adjusted to the isoelectric point (4.3) with 1.0 N HCl. The suspension was then centrifuged at 1317 × g for 12 min (Mistral 3000i, Curtin Matheson Sci., Houston, TX), the supernatants discarded and the precipitates freeze-dried at −47C and 13×10^{-3} mbar until use (Free Zone 4.5, Labconco. Kansas City, MO).

2.4. Chemical Properties of Hard-to-Cook Bean (*Phaseolus vulgaris*) Protein Concentrate

2.4.1. Proximate Composition

Proximate composition of the HTC bean flour and the protein concentrate was calculated using official Association of Official Analytical Chemists (AOAC) procedures [8]: nitrogen (method 954.01), fat (920.39), ash (923.03), fiber (962.09) and moisture (925.09). Protein content was calculated as nitrogen × 6.25 and carbohydrate content was estimated as nitrogen-free extract.

2.4.2. *In Vitro* Protein Digestibility

This characteristic was determined according to Hsu *et al*. method [9], using a multi enzymatic solution containing 1.6 mg trypsin (Type IX Sigma T-0303 with 13,000 - 20,000 BAEE units/mg protein), 3.1 mg chymotrypsin (Type II Sigma C-4129 with ≥40 units/mg powder) and 1.3 mg peptidase (III grade Sigma P-7500 with 50 - 100 units/g powder) per millilitre. Changes in pH were measured with a potentiometer after 10 min. Apparent *in vitro* digestibility (*Y*) was measured with the follow Equation:

$$Y = 210.464 - 8.103X$$

where, X = protein suspension pH immediately after digestion with multi-enzymatic solution for 10 min.

2.5. Functional Properties of Hard-to-Cook Bean (*Phaseolus vulgaris*) Protein Concentrate

2.5.1. Nitrogen Solubility

The nitrogen solubility was determined following the procedure of Were *et al.* [10]. A total of 125 mg of protein concentrate was dispersed in 25 mL of distilled. The pH was adjusted at values of 2, 4, 6, 8 and 10 with NaOH 0.1 M or HCl 0.1 M; the solutions were stirred for 30 min at room temperature and centrifuged at 4320 × *g* for 30 min. The supernatant was analyzed for nitrogen using the AOAC (1997) method 954.01. The solubility defined as the amount of soluble nitrogen from the total nitrogen, was calculated as follows:

$$\text{Nitrogen solubility}(\%) = \frac{\text{Supernatant nitrogen concentration}}{\text{Sample nitrogen concentration}} \times 100$$

2.5.2. Emulsifying Capacity (EC) and Emulsion Stability (ES)

The EC was measured by an oil titration method similar to that of Jiménez-Colmenero and García-Matamoros [11]. A total of 300 mg of protein concentrate was dissolved in 300 mL of distilled water with 3.0% of NaCl. The pH was adjusted at values of 2, 4, 6, 8 and 10 with NaOH 0.1 M or HCl 0.1 M. The solutions were placed in the baker of a blender. A burette filled with 100% pure maize oil was placed above the beaker. A pair of electrodes connected to a multimeter was fixed to the baker to measure the electrical resistance (in ohms) of the emulsion. The solution was first stirred at 60% output of a 120 V rheostat for 45 s to make a homogenized solution and to get a constant resistance reading. The output was then increased to 100%, and the oil was immediately dispensed from the burette into the beaker at 0.5 mL/s, generating an oil-in-water emulsion at room temperature. A sudden increase in resistance was observed when the oil capacity of the sample emulsion reached a maximum value and the emulsion collapsed to form a water-in-oil emulsion. At that point, oil delivery was stopped. EC was measured in control solution 3% NaCl to subtract the value of the sample. The EC was expressed in mL of oil per mL of protein dispersion according to the following relationship:

$$\%\ \text{EC} = \frac{\text{mL of oil expended in the test sample}}{\text{mL of dispersion employed}} \times 100$$

ES was determined according Dagorn-Scaviner *et al.* [12]; to a 30 mL dispersion of protein (3 mg/mL) in water with 3.0% of NaCl were added 10 mL of 100% pure maize oil. The pH was adjusted at 5.5 with NaOH 0.1 M. The solution was stirred for 30 s to make a homogenized solution. The emulsion was placed in a graduated test tube. Simultaneously were recorded the time and the phase separation. The volume (mL) of the aqueous phase was determined at 30 s, 5, 30 and 120 min.

2.5.3. Foam Capacity (FC) and Foaming Stability (FS)

The FC and FS were determined according to the method described by Chau *et al.* [13]. 100 mL of a suspension of 1.5% hydrolyzed was prepared; the pH was adjusted at values of 2, 4, 6, 8, and 10. The suspension was stirred

at low speed in a blender for 5 min. The suspension was transferred to a 250 mL graduated test tube and recorded the volume of foam after 30 s; the FC was expressed as the percentage increase in foam volume after 30 s. The foam was allowed to stand and the volume (mL) was measured after 5, 30 and 120 minutes. The FS was determined as the volume of foam remaining after 5, 30 and 120 min.

2.5.4. Viscosity

Viscosity was evaluated using an adaptation of the Li and Chang (1997) method [14]. The protein concentrate was dispersed in water to 4% (w/v, db) and it was homogenized during 30 min at 25˚C. The viscosity was measured in a digital rheometer (AR2000, TA Instruments, New Castle, DE) with concentric cylinders geometry using a scanning speed from 0 to 1000 Hertz at 25˚C. The results were reported in Pa·s.

2.6. Statistical Analysis

All results were analyzed using descriptive statistics with a central tendency and dispersion measures. One-way ANOVAs were run to evaluate chemical properties. A LSD multiple range test was used to determine differences between treatments. All analyses were done according to Montgomery [15] and processed with the Statgraphics Plus version 5.1 software.

3. Results and Discussion

3.1. Chemical Properties of Hard-to-Book Bean (*Phaseolus vulgaris*) Protein Concentrate

The moisture, ash, protein, fat, fiber and NFE contents are shown in **Table 1**. Crude protein content in the HTC bean flour (21.71%)was similar to those reported for HTC of *P. vulgaris* (21.7%) and *P. sativum* (21.4%), but lower than this reported for HTC of *Vigna unguiculta* (25.64%) [16]. Using alkaline extraction and isoelectric precipitation, HTC bean protein concentrate crude protein content (73.03%) was similar to the 71.9% reported by Morales de León *et al.* [5]. However, protein recovery under the studied conditions was 13.65%, much less than the 36.15% reported for HTC bean [5]. Recovery was probably low due to the more neutral pH used in the present study. Values between pH 10.0 and 12.0 are more efficient, but run the risk of protein denaturation, structure modifications and destroying some relevant amino acids.

The *in vitro* digestibility of the HTC bean protein concentrate (76.7%) was notably better than that reported for raw HTC bean varieties (25% - 29%) [17], and similar to HTC *V. unguiculata* protein concentrate (75.25%) [16]. However, it was lower than for freshly harvested *P. lunatus* (79.8%) [18] or *V. unguiculata* (78.5%) [19]. Protein digestibility in common beans is inhibited by changes in the protein structure and formation complexes between the protein and starch, hemicelluloses, minerals and other proteins during storage. This implies that the factors, which control protein digestibility, are similar to those responsible for increased cooking time in HTC beans. Storage at high temperature and relative humidity increases endogenous protease activity and consequent hydrolysis of bean storage protein. This would apparently increase overall protein digestibility but this also causes increased interactions with digestibility-limiting agents such as high molecular weight tannins, which are 1.64 times greater in HTC beans [16].

Table 1. Proximate composition (% d.b.) of hard-to-cook bean flour and protein concentrate.

Component	Flour	Protein concentrate
Moisture	8.92 ± 0.47^{b}	2.68 ± 0.29^{a}
Protein	21.71 ± 0.13^{a}	73.03 ± 0.21^{b}
Fat	4.41 ± 0.21^{b}	2.77 ± 0.12^{a}
Crude fiber	4.11 ± 0.44^{b}	1.31 ± 0.12^{a}
Ash	4.52 ± 0.05^{b}	2.54 ± 0.01^{a}
NFE	65.25 ± 0.48^{b}	20.35 ± 0.20^{a}

$^{a-b}$Different superscript letters in the same row indicate statistical difference ($p < 0.05$). Data are the mean, n = 3.

3.2. Functional Properties of Hard-to-Cook Bean (*P. vulgaris*) Protein Concentrate

Solubility is one of the most important functional properties of proteins. Many of the other functional properties such as emulsification and foaming are affected by solubility. The solubility for HTC bean (*P. vulgaris*) protein concentrate ranging from 2.5% to 71.81% is shown in **Figure 1**. The nitrogen pH-solubility profile of HTC bean (*P. vulgaris*) protein concentrate showed three general regions: one of minimum solubility (pH5-6) essentially the isoelectric pH range and two of solubility maxima at pH 2 and 8. Minimum solubility in the HTC bean (*P. vulgaris*) protein concentrate was around pH 5, a level similar to that reported for minimum solubility in the protein isolates of *P. calcaratus* (5%), *Dolichoslablab* (5.08%) and *Glycine max* (5.26%) [13]. HTC bean (*P. vulgaris*) protein concentrate had good nitrogen solubility at both extremes of the pH range (acid and alkaline). A similar behavior has been reported for Chel-Guerrero *et al.* [4] in *P. lunatus* and *C. ensiformis* protein isolates. This makes the HTC bean (*P. vulgaris*) protein concentrate potentially useful in applications where high solubility profiles could have a widespread application in formulation of food systems. Possible uses as ingredient in baby food, baked products or additive in carbonated drinks, diet drinks, and desserts [4].

Emulsion capacity measures the ability of a protein to help dispersion of an oil phase into an aqueous medium [20]. The HTC bean (*P. vulgaris*) protein concentrate exhibited good EC values (89% - 97%) at different pH levels (**Figure 2**), with values higher to those of *P. lunatus* and *C. ensiformis* protein isolates (41.78% - 56.46%) [4]. The results obtained in the current study and previous studies tend to indicate that the responses of the emulsification functionality to extraction technique and conditions are dependent on the botanical source of the proteins. According to Mwasaru *et al.* [21] differences in the emulsifying activity of protein may be related to their solubility and conformational stability. Paredes-Lopez *et al.* [22] observed that the sample with the lowest

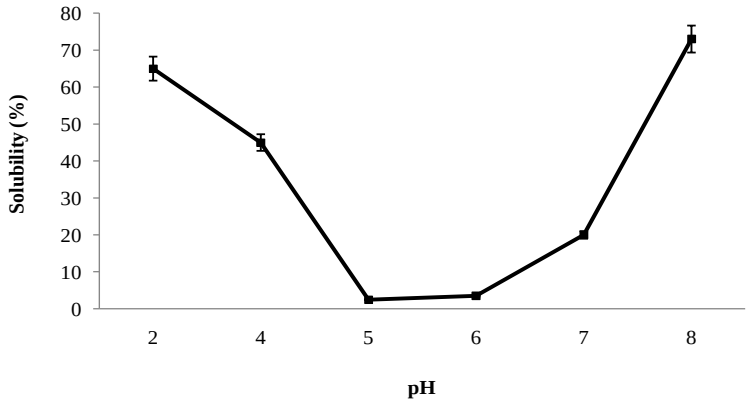

Figure 1. Solubility curve of hard-to-cook bean (*P. vulgaris*) protein concentrate. Protein solubility is expressed as percentage of soluble nitrogen at various pH values. Results are means of 3 replicates.

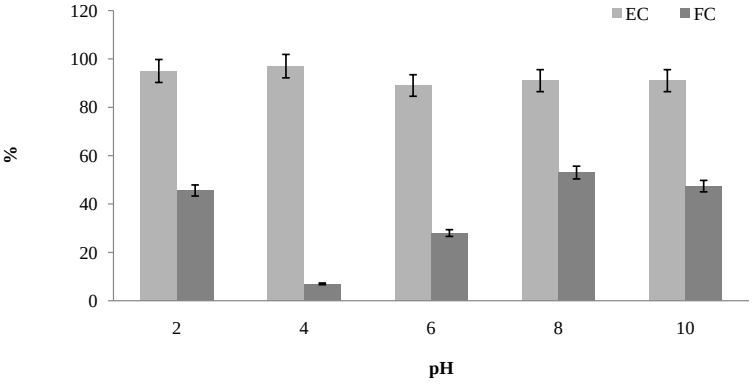

Figure 2. Emulsifying and foaming capacity of hard-to-cook bean protein (*P. vulgaris*) concentrate at different pH values. Results are means of 3 replicates.

solubility exhibited the lowest emulsifying activity and the highest emulsion stability, an observation partly consistent with the results obtained in the present study sinceHTC bean (*P. vulgaris*) protein concentrate at pH 5-6 exhibited the lowest solubility and the lowest emulsifying activity but was not the highest in emulsion stability. According to Mwasaru *et al.* [21], hydrophobicity of proteins has also been reported to influence their emulsifying properties The results obtained here may partially result of the high hydrophobic amino acids content in the HTC bean (*P. vulgaris*) protein concentrate, which allows the protein-protein interaction in the interface. Emulsion stability for HTC bean (*P. vulgaris*) protein concentrate was pH- and time-dependent reaching values of near 100% at acid pH (**Figure 3**). Interactions between proteins and lipids are common in many food systems, and thus, the ability of proteins to form stable emulsions is important. Thus, considering these emulsifying properties, HTC *P. vulgaris* protein concentrate could be used as ingredient and stabilizer in emulsion-based food formulations such as salad dressing and mayonnaise.

Foaming reflects the capacity of proteins to form stable layers surrounding gas droplets in a liquid phase. Proteins with good foaming properties should be soluble in the aqueous phase, diffuse and concentrate at the air/water interface, partially unfold to form a cohesive layer around the gas bubbles, and possess sufficient viscosity and mechanical strength to prevent rupture and coalescence of the droplets [20]. Some food proteins are capable of forming good foams, and their capacity to form and stabilize foams depends on the type of protein, degree of denaturation, pH, temperature and whipping methods. Foaming properties for HTC bean (*P. vulgaris*) protein concentrate were measured based on their whippability at pH 2.0, 4.0, 6.0, 8.0 and 10.0. Foaming capacity (FC) was pH-dependent, with the lowest value at pH 4 and the highest at pH 8. The high FC at alkaline pHs may be due to an increase in the net charge of the protein which weakens hydrophobic interactions and increases protein flexibility, allowing them to spread to the air-water interface more quickly, encapsulating air particles, and increasing foam formation. Foaming stability (FS) diminished through time (30 s, 5, 30 and 120 min) (**Figure 4**). This property was lowest at neutral pH for the established times, but higher at acid and alkaline pHs. Given these results, the relationship of hydrophilic versus hydrophobic properties is a key factor in balancing FC and FS. The poor foaming stability of HTC bean (*P. vulgaris*) protein concentrate was probably due to its protein denaturation, which would hinder formation of a stable film around the gas bubbles. Although the HTC bean (*P. vulgaris*) protein concentrate was capable of forming films with the air interface, the films were not strong enough to maintain their integrity.

The viscosity profile of HTC bean protein concentrate is shown in **Figure 5**. The results showed that to higher shear rate a higher viscosity was registered. The HTC bean (*P. vulagris*) protein concentrate can be used in food systems as thickening agents, such as in dry foods and in soup mixes, to obtain a certain viscosity when reconstituted with water.

The use *Phaseolus vulgaris* beans and protein derivatives such as flour, concentrates and isolates depends on their capacity to absorb water and soften sufficiently during soaking and cooking. Products derived from fresh beans are widely used in industry, however hard-to-cook bean they are poorly used food processors. The good

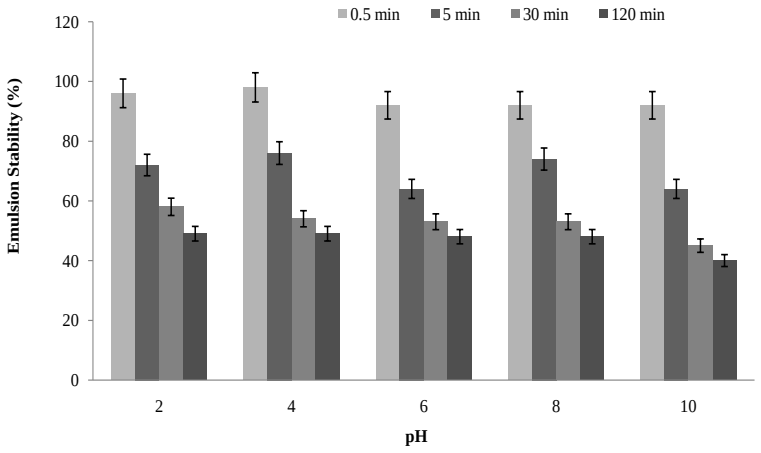

Figure 3. Emulsion stability of hard-to-cook bean protein (*P. vulgaris*) concentrate at different pH values (2 - 10).

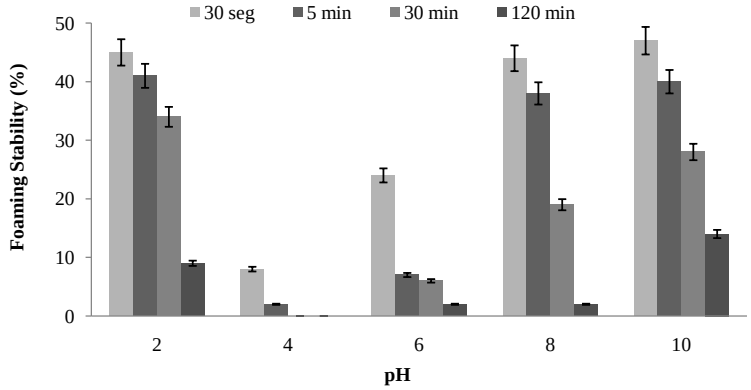

Figure 4. Foaming stability of hard-to-cook bean protein (*P. vulgaris*) concentrate at different pH values (2 - 10).

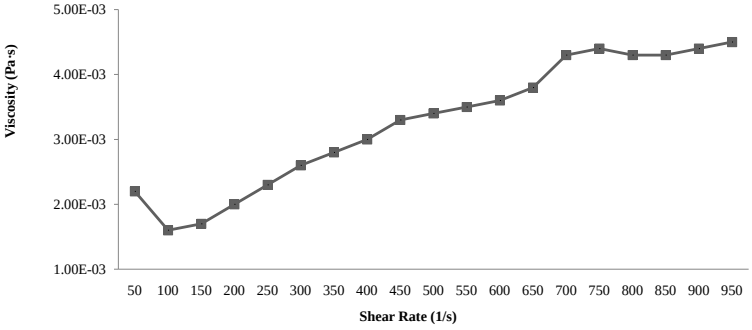

Figure 5. Viscosity profiles of HTC bean (*P. vulgaris*) protein concentrate.

properties of water interactions by the protein concentrate on study permit its use in the development of food systems with nutritional and functional quality.

4. Conclusion

The HTC bean (*P. vulgaris*) protein concentrate exhibits good functional properties. The low cost of HTC *P. vulgaris* as a substrate represents the revalorization of an agricultural product with reduced acceptability and marketability that may be transformed into a highly valuable food ingredient or additive. Because of its functional properties, the protein concentrate of HTC bean is very attractive as the functional ingredient in food systems. They can be incorporated into products such as bakery products, seasonings, and sausages among others. But sensory and texture analyses of the products are necessary.

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