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ORIGINAL PAPER

Physical, techno-functional and antioxidant properties of black cumin seeds protein isolate and hydrolysates

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Abstract

The present work aims to study physical, techno-functional and antioxidant properties of black cumin seeds protein isolate (BCSPI) and its hydrolysates (BCSPH). BCSPI and BCSPH were characterized by high contents of protein (78.4–83.5%). Thermal characterization by DSC showed that the denaturation temperature (T_d) increased from 90.7 to 131.9 °C. BCSPI was characterized by the lowest T_d. The highest T_d was observed for BCSPH with a hydrolysis degree (DH) of 14.74%. The enzymatic hydrolysis was found to enhance protein solubility. BCSPH (lowest DH = 12.58%) exhibited significantly higher emulsifying and foaming properties than BCSPI. The above observation was probably relating to the decrease of interfacial tension from 28.50 to 25.67 mN/m (BCSPH).Antioxidant activities of BCSPI and BCSPH were assessed in vitro through different antioxidant tests. At a concentration of 1 mg/mL, BCSPH with higher DH (19.63%) exhibited significantly the greatest scavenging activity (90.82%) towards DPPH radical. Antioxidant activities were improved with the increase of the degree of hydrolysis. The obtained data suggest that the choice of the hydrolysis degree can improve the functional and the antioxidant activities of protein isolate. Overall, BCSPH have peculiar techno-functional and antioxidant properties which promote their use in food, cosmetic or pharmaceutical products as natural dietary proteins.

Keywords Black cumin seeds · Protein isolate · Protein hydrolysates · Techno-functional properties · Antioxidant activities

Introduction

Vegetables proteins are increasingly used in the food formulations [1] thanks to their techno-functional and nutritional properties. Usually, protein isolate has extracted with alkaline conditions followed by an isoelectric precipitation step (pH between 4 and 5) [2]. Various parameters such as pH, temperature, solvent, extraction time and solvent to meal ratio have an important effect on protein recovery [3]. The techno-functional properties of proteins can be affected by the chemical and enzymatic treatments. Enzymatic hydrolysis has been used more than the chemical treatments on account of either through easier control of reaction, or higher specificity and or minimal formation of by-products [4]. However, the degree of hydrolysis should be controlled because excessive enzymatic modification may damage proteins techno-functional properties in some plant protein cases [5, 6]. In this context, Jamdar et al. [7] and Mulla and Ahmed [6] have reported the above observation for peanut and *Lepidium sativum* seed meal, respectively. Further hydrolysis of proteins has a negative effect on the techno-functional properties [6]. The abovementioned data is probably attributed to the presence of small peptides, which can reduce the interfacial tension [6]. Generally, enzymatic hydrolysis induces a decrease of molecular weight and an increase of ionisable and hydrophobic groups, which affect the supramolecular structure and the techno-functional properties of proteins [6, 8, 9]. Such modifications of the structure–function configuration of proteins were also influenced their antioxidant activities [7, 10]. The techno-functional properties and the biological activities of protein hydrolysates from grain or legumes have attracted much

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attention [11, 12]. The functional and antioxidant properties of protein hydrolysates were affected by the treatment conditions (pH, time, enzyme concentration and temperature) and also by the enzyme type [4]. Previous studies proved Alcalase and Flavourzyme were the best enzymes to produced protein hydrolysates, which were characterized by interesting biological activities [4, 13].

Oxidative stress leads to many undesirable impacts on food, because it damages quality. Indeed, it can affect the flavour, texture and colour. Moreover, oxidation of biomolecules induces the occurrence of diseases such as cardiovascular and Alzheimer's diseases as well as cancer [14] because it causes the oxidation of cellular molecules, proteins, DNA, and lipids. Thus, it is absolutely necessary to produce safe and natural antioxidants as alternatives to synthetic ones with low cost and high activity. In the light of literature, the proteins from plants can be a potential source of natural antioxidants which might be used as protection agents against oxidative damage to human [15, 16]. Researchers reported the potential of proteins isolate form vegetable source to act as effective natural antioxidants such as chickpea [15] and black cumin seeds [16] isolates. Furthermore, several antioxidative peptides have been obtained by hydrolysis of proteins from plants such as maize kernels [17], *Moringa oleifera* seed [18], Pigeon pea [19] and black bean [11]. Nevertheless, there are few reports in the literature studding the structural, functional and biological properties of black cumin (*Nigella Sativa* L.) proteins, despite their potential source of vegetable protein (20–27%) [16]. In fact, the extract of the black cumin seeds has been reported to have a wide array of many beneficial biological activities such as antitumor activity [20], antibacterial activity [21] and antioxidant activity [16, 22]. Coşkun et al. [23] studied the influence of aqueous (alkali extraction-isoelectric precipitation) and organic (n-hexane) extraction conditions on the structure and functionality of protein from black cumin seeds of Turkey cultivates.

To the best of our knowledge, the thermal, techno-functional and antioxidant properties of protein from black cumin seeds of Tunisia cultivates, has not been investigated. Thus, the aim of the present study was firstly to prepare (BCSPI) and its hydrolysates (BCSPH) derived from enzymatic hydrolysis with Savinase®. Secondly, chemical composition, thermal properties (DSC), techno-functional properties and antioxidant activities of black cumin seeds protein isolate (BCSPI) and black cumin seeds protein hydrolysates (BCSPH), with different degree of hydrolysis, were investigated. Some techno-functional properties were evaluated through protein solubility and emulsifying and foaming properties. Antioxidant activities of BCSPI and BCSPH in vitro were assessed, including DPPH radical scavenging activity, reducing power, chelating ability and ß-carotene bleaching method.

Materials and methods

Raw materials and BCSPI preparation

A Tunisian variety of mature black cumin seeds (*N. Sativa* L.) was purchased from an herbal market in Sfax, Tunisia. Seeds sample were milled to obtain black cumin seeds flour and then stored at − 20 °C.

Black cumin seeds flour was defatted by stirring in hexane [(1:5 w/v), flour: hexane] for 4 h. This procedure was repeated twice. The resulting flour was air-dried and stored at − 20 °C prior to the proteins extraction.

Black cumin seeds protein isolate (BCSPI) was prepared according to the method of Papalamprou et al. [24] with slight modifications. Defatted black cumin seeds flour was dispersed with distilled water (0.04 g/mL), the pH of the resulting suspension was adjusted to pH = 11 using 1.0 mol/L NaOH and the mixture was stirred for 2.5 h at 30 °C. The obtained mixture was then centrifuged at 3000×*g* at 25 °C for 20 min to collect the supernatant. The extraction process was repeated two additional times and supernatants were pooled and adjusted to pH 4.5 with 1.0 mol/L HCl to precipitate the protein. The precipitate was recovered by centrifugation at 3000×*g* at 25 °C for 20 min and the supernatant was discarded. The extract was washed with water, freeze-dried and then stored at − 20° C until analysis.

Preparation of black cumin seeds protein hydrolysates (BCSPH)

The protein isolate was hydrolyzed using Savinase®. BCSPI was mixed with distilled water (1:1 w/v) and then cooked at 80 °C for 5 min to inactivate endogenous enzymes. The cooked proteins sample was homogenized at high speed for 2 min. The sample was adjusted to pH 9.0 at 55 °C for optimal Savinase® activity. The enzyme was added at a 1:1 (U/mg) enzyme/protein ratio and the hydrolysis reaction was started. The pH of the solution was maintained at pH = 9.0 by continuous addition of 4.0 mol/L NaOH. After the required digestion time, the solution was heated for 20 min at 80 °C to inactivate enzyme and stop the enzymatic hydrolysis. BCSPI hydrolysates were then centrifuged at 5000×*g* for 20 min. Finally, the soluble fractions referred to proteins hydrolysates were freeze-dried and stored at − 20 °C.

Determination of the degree of hydrolysis

The degree of hydrolysis (DH), is the percent ratio of the number of peptide bonds broken (h) to the total number of peptide bonds in the substrate (h_{tot}), was evaluated from the

quantity of base (NaOH) added to maintain the pH constant during the hydrolysis reaction [25] as follows:

$$\mathrm{DH}(\%) = \frac{h}{h_{\mathrm{tot}}} \times 100 = \frac{B \times Nb}{MP} \times \frac{1}{\alpha} \times \frac{1}{h_{\mathrm{tot}}} \times 100$$

where B is the quantity of NaOH consumed (mL) to maintain the pH constant during the proteolysis. Nb is thenormality of the base, MP is the mass (g) of the protein and α represents the average degree of dissociation of the α-NH_2 groups released during hydrolysis.

The total number of peptide bonds (h_{tot}) in the protein substrate was assumed to be 7.22 mmol/g [26].

Chemical composition

Chemical composition of BCSPI and BCSPH for the moisture content, ash, crude fat and protein content were done according to the methods of AOAC [27]. The total carbohydrate of BCSPI and BCSPH was determined by the difference of mean value, 100 − (sum of percentage of moisture, ash, proteins and fat) [28].

Differential scanning calorimetry (DSC) analysis

The experiments were performed using Differential Scanning Calorimeter-DSC METTLER TOLEDO as described by Wani et al. [29] in the temperature range of 25–200 °C at a rate of 10 °C/min using a 5 ± 0.25 mg sample. A sealed empty pan was used as a reference.

Surface tension measurements

The surface tension (mN/m) of BCSPI and its hydrolysates was evaluated using a TSD 971 TENSIOMETRY SYSTEM DIGITAL (Gibertini Elettronica, Italia). In order to measure the surface tension, 20 mL of sample solution (1 g/100 mL) were poured into glass beaker potash placed into the tensiometer platform. A platinum wire ring was submerged into the prepared solution and then slowly pulled through the water–air interface. Surface tension values represent the average of three independent measurements carried out at room temperature (20 °C).

Techno-functional properties

Protein solubility

The protein solubility profile was studied in the pH range of 2–12. Briefly, the suspensions of the samples (1 g/100 mL) were adjusted to the desired pH value using 0.1 mol/L HCl or 0.1 mol/L NaOH. The mixtures were stirred for 90 min, at 180 rpm, at 25 °C in a shaking incubator and then centrifuged at 3000×*g* for 20 min. Protein contents in the supernatants were evaluated by the Khjeldhal method and the solubility profile was expressed using the following equation:

$$\textit{Solubility}\,(\%) = \frac{\text{Amount of protein in the supernatant}}{\text{Amount of protein in the sample}} \times 100$$

Emulsifying properties

The emulsion activity index (EAI) and the emulsion stability index (ESI) of BCSPI and its hydrolysates were determined by the method of Pearce and Kinsella [30]. The emulsions were prepared by homogenizing 21.25 mL of sample solution at different concentrations (1 to 5 g/100 mL) with 3.75 mL of refined corn oil for 1 min at a speed of 13.500 rpm using an ULTRA-TURAX T 25 basic (IKA WERKE). Aliquots of the emulsion (200 µL) was taken from the bottom of the container into 1.8 mL of 0.1 g/100 mL of sodium dodecyl sulfate (SDS) at 0 and 10 min after homogenization and the absorbance of the diluted solutions was measured at 500 nm.

EAI and ESI were calculated as follows:

$$\mathrm{EAI}\left(\mathrm{m^2/g}\right) = \frac{2 \times 2.303 \times \mathrm{A_0} \times \mathrm{N}}{\mathrm{C} \times \varphi \times 10000}$$

$$\mathrm{ESI(min)} = \frac{\mathrm{A_0}}{\mathrm{A_0} - \mathrm{A_{10}}} \times \mathrm{t}$$

where A_0 is the absorbance of the diluted emulsion immediately after homogenization, N is the dilution factor (N = 100), C is the weight of protein per volume (g/mL), φ is the oil volume fraction of the emulsion, (A_0–A_{10}) is the change in absorbance between 0 and 10 min and t is the time interval, 10 min.

Foaming properties

The foaming capacity (FC) and foaming stability (FS) of BCSPI and BCSPH were determined by the method of Coffman and Garcia [31]. Sample solution of 20 mL at different concentrations (1 to 5 g/100 mL) was homogenized using a homogenizer (ULTRA-TURAX T 25 basic (IKA WERKE)) at 13.500 rpm for 2 min. Volumes were recorded before and after whipping using a graduated cylinder. The foaming stability (FS, min) is defined as the period at which 50% of the foam volume disappears, whereas the foaming capacity (FC, %) was expressed as the volume increased due to whipping and calculated according to the following equation:

$$\%FC = \frac{V_2 - V_1}{V_1} \times 100$$

where V_2 volume of solution after whipping, V_1 initial volume of solution.

Antioxidant activities

DPPH radical-scavenging assay

The DPPH radical-scavenging capacity of BCSPI and BCSPH was determined using the method described by Bersuder et al. [32]. The absorbance of samples (0.2 to 1 mg/mL) was recorded at 517 nm. Butylat-edhydroxyanisole (BHA) was the reference antioxidant. DPPH radical-scavenging capacity was calculated using the following equation:

$$\text{DPPH radical scavenging capacity } (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the sample) and A_{sample} is the absorbance of Bersuder (with the DPPH solution).

Reducing power assay

The potential of BCSPI and BCSPH to reduce iron (III) was performed according to the method of Yıldırım et al. [33]. The absorbance of samples (0.2 to 1 mg/mL) and BHA used as reference were recorded at 700 nm. The highest is the absorbance of the reaction mixture; the highest is the reducing power.

Antioxidant assay using the ß-carotene bleaching method

The capacity of samples to prevent the bleaching of ß-carotene was determined according to Koleva et al. [34]. A solution of 0.5 mg ß-carotene in 1 mL chloroform with 200 µL of Tween-40 and 25 µL of linoleic acid was prepared. The chloroform was entirely evaporated under vacuum (rotary evaporator, Heidolph, Schwabach, Germany) at 40 °C, then 100 mL of bi-distilled water was added and the obtained mixture was vigorously stirred. The resulting emulsion was freshly prepared before each experiment. An aliquot (2.5 mL) of the ß-carotene–linoleic acid emulsion was transferred to test tubes containing 0.5 mL of each sample at different concentrations (0.2 to 1 mg/mL). The tubes were incubated at 50 °C for 2 h and the absorbance of each sample was measured at 470 nm. BHA was used as the reference antioxidant. A control consisted of 0.5 mL of distilled water instead of the sample solution.

Metal chelating assay

The chelating activities of BCSPI and BCSPH (0.2 to 1 mg/mL) for ferrous ion (Fe^{2+}) were determined by the method of Decker and Welch [35]. The absorbance of the Fe^{2+}–ferrozine complex and Ethylene-diaminetetraacetic acid (EDTA) used as reference were read at 562 nm. The chelating antioxidant activity for Fe^{2+} was calculated by the following equation:

$$\text{Chelating activity } (\%) = \frac{\left(A_{\text{control}} - A_{\text{sample}}\right)}{A_{\text{control}}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of samples.

Statistical analysis

Results were expressed as mean values ± standard deviation of three independent determinations. SPSS for Windows, Version 19 (SPSS Institute, Inc., Cary, NC) was used for the statistical analyses determination. Differences were significant at $p < 0.05$.

Results and discussion

Production of BCSPH

The functionality of protein hydrolysates is a crucial factor in their potential as techno-functional food ingredients. In fact, the protein substrate, the specificity of the enzyme, the hydrolysis conditions and the degree of hydrolysis had certainly an effect on the physicochemical and biological properties of protein hydrolysates. The hydrolysis curve of BCSPI, obtained after different times of hydrolysis (15 min, 30 min, 60 min and 120 min) with Savinase®, is shown in Fig. 1. The curve reveals a high rate of hydrolysis during the initial 10 min. The hydrolysates obtained after 15 min, 30 min,60 min and 120 min had DH = 12.58%, 14.74%, 16.90% and 19.63%, respectively, were selected to study their techno-functional properties and antioxidant activity. The curve of hydrolysis is similar to those obtained for hydrolysates from buckwheat [36], chickpea [15] and black bean [11].

Chemical characteristics of BCSPI and BCSPH

The chemical composition of BCSPH was determined and compared with that of BCSPI (Table 1). As shown in Table 1, all BCSPH had a significant ($p < 0.05$) protein content (DH = 12.58%:79.6 g/100 g; DH = 14.74%:80.5 g/100 g; DH = 16.90%:82.6 g/100 g and DH = 19.63%:83.5 g/100 g)

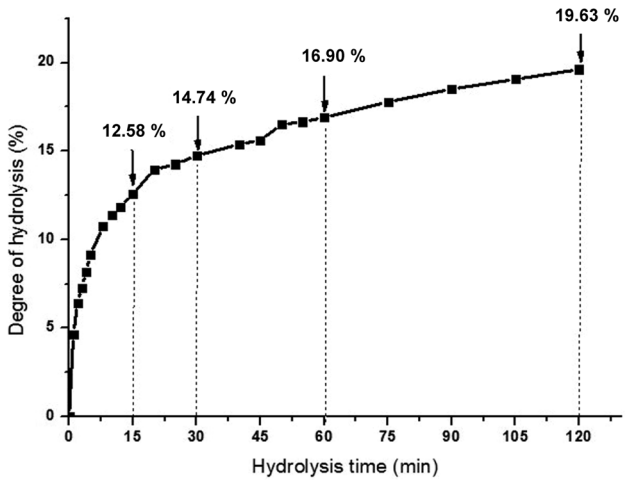

Fig. 1 Hydrolysis curve of black cumin extract treated with Savinase. Hydrolysis was conducted at the following conditions: enzyme (U)/protein (mg) ratio of 1:1, pH 9.0 and at 55 °C

higher than that obtained for BCSPI (78.4 g/100 g). The protein content increases slightly with the increase of degree of hydrolysis and results proved that BCSPH can be an essential source of proteins. The high protein content of BCSPH was a consequence of the removal of insoluble undigested non-protein substances and the high solubilisation of the proteins during hydrolysis. All BCSPH had ($p < 0.05$) relatively low lipid content (5.8–8.7 g/100 g) than BCSPI (10.6 g/100 g). The low lipid and moisture content in the BCSPH might significantly contribute to stability during storage. These results are in accordance with those found by Mokni Ghribi et al. [26]. It was also noted that the ash contents ranged from 4.9 to 5.9 g/100 g for BCSPH and values were higher than those obtained for BCSPI (4.1 g/100 g). This is may be due to the addition of alkali during the proteolysis reaction for keeping the pH constant.

Thermal properties

The thermal stability of BCSPI and its hydrolysates was determined by differential scanning calorimetry (DSC) (Table 1). The DSC thermogram showed that the denaturation temperature of BCSPI was 90.7 °C. It was 118.8 °C, 131.9 °C, 121.9 °C and 95.8 °C, respectively, for BCSPH with a degree of hydrolysis of 12.58%, 14.74%, 16.90% and 19.63%. The result indicated that BCSPH showed significantly ($p < 0.05$) better thermal stability than the BCSPI. The relatively highest stability was observed for sample with DH = 14.74%. Mokni Ghribi et al. [26] reported that denaturation temperature of chickpea protein hydrolysates varied from 43.78 to 60.97 °C. Compared to this result, all BCSPH samples exhibited greater T_d (Table 1) than protein hydrolysates from chickpea [26].

Surface tension

The ability of BCSPI to reduce the interfacial tension between two phases as a function of % DH was performed (Fig. 2). Results reveal that BCSPI and BCSPH were efficiently able to decrease interfacial tension relative to water (72 mN/m). Statistical analyses have indicated that the interfacial tension of BCSPH was significantly lower than that obtained for BCSPI ($p < 0.05$). Our finding could be explained by the reduction of BCSPI molecular size during hydrolysis. This phenomenon ameliorates molecular flexibility and induces conformational changes which offering greater rates of diffusion to the interface [37]. On the other hand, the lowest DH (DH = 12.58%) had significantly ($p < 0.05$) the best potential to decrease interfacial tension.

Table 1 Chemical characteristics and thermal properties of black cumin seeds protein isolate and its hydrolysates (DH = 12.58%, 14.74%, 16.90% and 19.63%) obtained after treatment with Savinase

	BCSPI	DH = 12.58%	DH = 14.74%	DH = 16.90%	DH = 19.63%
Components					
Dry matter (%)	97.1 ± 0.3^{a}	$94.1 \pm 1.1^{b,d}$	$95.3 \pm 0.5^{c,d,e}$	$96.1 \pm 0.3^{a,e}$	$96.7 \pm 0.2^{a,e}$
Protein[A]	78.4 ± 0.1^{a}	79.6 ± 0.7^{b}	80.5 ± 0.3^{c}	82.6 ± 0.5^{d}	83.5 ± 0.4^{e}
Carbohydrate[A]	$6.9 \pm 0.6^{a,d}$	$6.7 \pm 0.7^{a,c}$	7.6 ± 0.5^{a}	$5.8 \pm 0.3^{b,c,d}$	4.8 ± 0.1^{b}
Fat[A]	10.6 ± 0.5^{a}	8.7 ± 0.4^{b}	7.0 ± 0.1^{c}	$6.1 \pm 0.3^{d,f}$	$5.8 \pm 0.1^{e,f}$
Ash[A]	4.1 ± 0.2^{a}	$5.1 \pm 0.4^{b,f,h}$	$4.9 \pm 0.1^{c,f,g}$	$5.6 \pm 0.1^{d,e,g,h}$	5.9 ± 0.4^{e}
Thermal properties					
T_0 (°C)	50.7 ± 0.8^{a}	$87.3 \pm 0.5^{b,f}$	105.0 ± 0.9^{c}	$88.4 \pm 0.8^{d,f}$	56 ± 0.3^{e}
T_d (°C)	90.7 ± 0.2^{a}	118.8 ± 0.3^{b}	$131.9 \pm 1.3^{c,f}$	$121.9 \pm 0.3^{d,f}$	95.8 ± 0.4^{e}

All values given are means of three determinations $(\bar{x} \pm SD)$

Means in row with different small letters are significantly different ($p < 0.05$)

BCSPI black cumin seeds protein isolate, *DH* degree of hydrolysis, *SD* standard deviation, T_0 onset temperature, T_d denaturation temperature

[A]In % dry matter

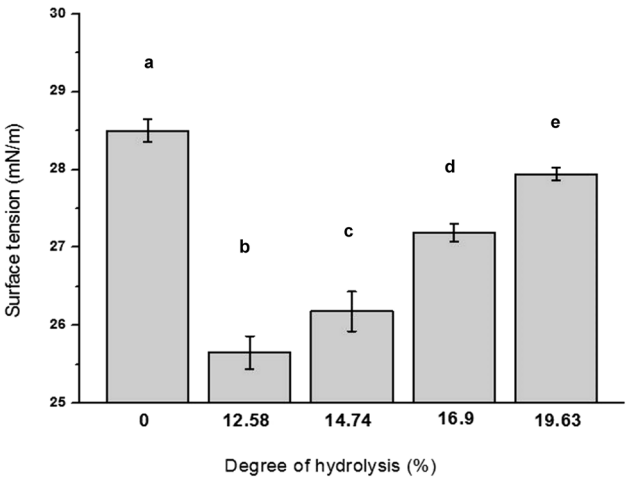

Fig. 2 Evolution of surface tension of BCSPI and its hydrolysates with different DH. Means in each degree of hydrolysis with different letters (**a**, **b**, **c**, **d**, **e**) are significantly different ($p < 0.05$)

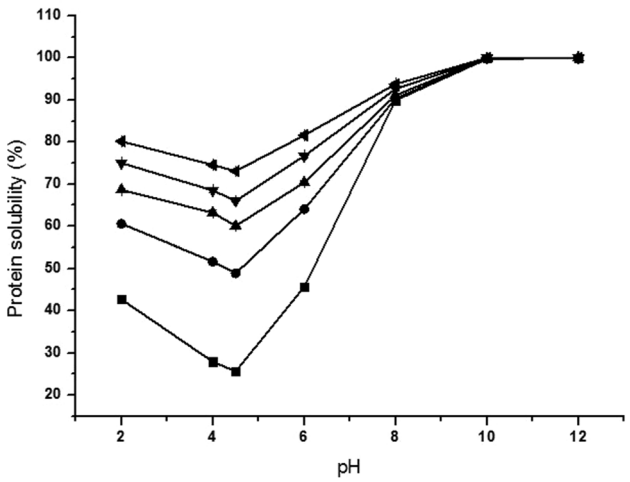

Fig. 3 Protein solubility profile of BCSPI and BCSPH as a function of pH. (■) BCSPI; (●) BCSPH 12.58%; (▲) BCSPH 14.74%; (▼) BCSPH 16.90%; and (◄) BCSPH 19.63%

In fact, excessive enzymatic hydrolysis leads to obtain small peptides which present antagonist behaviour and as a consequence it slows diffusion into the air–water interface.

Techno-functional properties

Protein solubility

Solubility profile of BCSPI and BCSPH as a function of pH is given in Fig. 3. The lowest solubility of samples was obtained at pH range of 4.0–5.0 with a minimum at pH 4.5 (isoelectric point pI) while the highest solubility level was at alkaline pH. Our findings are similar to that obtained for lentil protein [38]. Results show that BCSPH had higher protein solubility than BCSPI values ($p < 0.05$ at $pH < 8$) which are required for food formulation, especially in acidic foods. This improvement in protein solubility is probably a result of the production of smaller peptides during proteolysis and the increase in the ionisable carboxyl and amino groups which enhance the hydrophilicity and the protein solubility. Previous studies have reported similar results [4, 39]. Thus, enzymatic hydrolysis could be used as a potential alternative to ameliorate the solubility of vegetable proteins which is required in many food formulation systems.

Emulsifying properties

Hydrolysis affects the surface characteristics of protein and molecular weight which influence the EAI and ESI. Table 2 exhibits EAI and ESI of BCSPI and BCSPH as a function of concentration. Significant ($p < 0.05$) differences were obtained in EAI and ESI of BCSPI and their hydrolysates at all the concentration tested. BCSPH evinces significantly ($p < 0.05$) higher EAI and ESI than BCSPI only at small degree of BCSPI hydrolysis (DH = 12.58%). Therefore, enzymatic hydrolysis ameliorates considerably the EAI and ESI, mainly at the lowest DH [6, 40]. This phenomenon was related to the increase of solubility, molecular flexibility of peptides and the enhancement of the potential of hydrophobic amino acid residues to interact with the oil, while the hydrophilic residues interact with water thanks to protein hydrolysis. However, higher DH decreased both EAI and ESI due to the excessive decrease in molecular weight of the peptides generated by proteolysis. In fact, the obtained smaller peptides were unable to unfold and re-orient rapidly at the interface and the viscoelastic film, formed at the water–oil interface, was insufficient to resist to the coalescence of droplets.Previous studies have reported that the EAI and ESI are improved by limited proteolysis [4, 38].

The EAI of BCSPI and BCSPH decreased when the concentration increased, while ESI increased with the increase of the concentration of BCSPI and their hydrolysates (Table 2). Indeed, the low concentration enhances peptides adsorption to the interfacial oil- water area which improves the emulsifying capacities. However, the high concentration facilitates the molecules rearrangement as well as the formation of a strong water–oil interface.

Foaming properties

The FC and the FS values of BCSPI and BCSPH as a function of concentration are depicted in Table 2. Partial hydrolysis of BCSPI enhanced significantly ($p < 0.05$) FC and FS values, however, foaming proprieties decreased slightly with excessive protein hydrolysis. The highest foaming properties were observed at the lowest DH

Table 2 Techno-functional properties of BCSPI and its hydrolysates BCSPH with different DH as function of concentration

	Concentrations (g/100 mL)	BCSPI	DH = 12.58%	DH = 14.74%	DH = 16.90%	DH = 19.63%
Emulsifying activity index (m^2/g)	1	$155.5 \pm 0.6^{a,\square}$	$189.5 \pm 0.5^{b,\square}$	$150.5 \pm 0.3^{c,\square}$	$126.9 \pm 0.0^{d,\square}$	$110.6 \pm 0.6^{e,\square}$
	2	$122.2 \pm 0.3^{a,\#}$	$168.6 \pm 0.5^{b,\#}$	$119.0 \pm 0.5^{c,\#}$	$100.5 \pm 0.1^{d,\#}$	$81.6 \pm 0.5^{e,\#}$
	3	$97.6 \pm 0.5^{a,\$}$	$122.8 \pm 0.3^{b,\$}$	$90.4 \pm 0.2^{c,\$}$	$76.1 \pm 0.2^{d,\$}$	$55.1 \pm 0.2^{e,\$}$
	4	$78.7 \pm 0.4^{a,\blacklozenge}$	$108.0 \pm 0.2^{b,\blacklozenge}$	$74.1 \pm 0.2^{c,\blacklozenge}$	$50.5 \pm 0.4^{d,\blacklozenge}$	$34.1 \pm 0.2^{e,\blacklozenge}$
	5	$55.3 \pm 0.1^{a,\bullet}$	$88.0 \pm 0.1^{b,\bullet}$	$52.5 \pm 0.5^{c,\bullet}$	$38.4 \pm 0.3^{d,\bullet}$	$23.5 \pm 0.5^{e,\bullet}$
Emulsifying stability index (min)	1	$33.3 \pm 1.8^{a,\square}$	$34.0 \pm 0.1^{a,\square}$	$30.3 \pm 0.4^{b,\square}$	$28.5 \pm 0.2^{c,\square}$	$21.4 \pm 0.4^{d,\square}$
	2	$43.3 \pm 0.6^{a,\#}$	$48.1 \pm 0.2^{b,\#}$	$40.4 \pm 0.3^{c,\#}$	$37.7 \pm 0.4^{d,\#}$	$29.2 \pm 0.2^{e,\#}$
	3	$46.9 \pm 0.9^{a,\$}$	$50.5 \pm 0.2^{b,\$}$	$44.7 \pm 0.1^{c,\$}$	$41.1 \pm 0.3^{d,\$}$	$37.8 \pm 0.3^{e,\$}$
	4	$50.3 \pm 0.9^{a,\blacklozenge}$	$54.6 \pm 0.5^{b,\blacklozenge}$	$48.3 \pm 0.4^{c,\blacklozenge}$	$44.9 \pm 0.6^{d,\blacklozenge}$	$42.2 \pm 0.1^{e,\blacklozenge}$
	5	$53.5 \pm 0.6^{a,\bullet}$	$58.1 \pm 0.1^{b,\bullet}$	$49.8 \pm 0.7^{c,\bullet}$	$47.1 \pm 0.2^{d,\bullet}$	$43.6 \pm 0.6^{e,\bullet}$
Foam capacity (%)	1	$81.9 \pm 2.8^{a,\square}$	$90.5 \pm 0.2^{b,\square}$	$81.9 \pm 0.1^{c,\square}$	$66.0 \pm 0.9^{d,\square}$	$50.6 \pm 0.9^{e,\square}$
	2	$70.9 \pm 1.3^{a,\#}$	$81.0 \pm 1.4^{b,\#}$	$70.7 \pm 0.5^{c,\#}$	$58.2 \pm 0.2^{d,\#}$	$46.1 \pm 0.3^{e,\#}$
	3	$58.1 \pm 1.9^{a,\$}$	$71.5 \pm 2.1^{b,\$}$	$54.8 \pm 1.2^{c,\$}$	$50.6 \pm 0.9^{d,\$}$	$37.8 \pm 0.5^{e,\$}$
	4	$43.3 \pm 2.1^{a,\blacklozenge}$	$60.3 \pm 0.4^{b,\blacklozenge}$	$42.7 \pm 1.0^{a,\blacklozenge}$	$41.9 \pm 0.4^{c,e,\blacklozenge}$	$33.9 \pm 0.8^{d,e,\blacklozenge}$
	5	$38.6 \pm 1.3^{a,\bullet}$	$51.0 \pm 1.4^{b,\bullet}$	$36.8 \pm 0.6^{c,\bullet}$	$33.5 \pm 0.2^{d,\bullet}$	$25.6 \pm 0.9^{e,\bullet}$
Foam stability (min)	1	$54.1 \pm 0.1^{a,\square}$	$60.1 \pm 0.2^{b,\square}$	$33.5 \pm 2.2^{c,\square}$	$31.1 \pm 0.6^{d,\square}$	$21.9 \pm 0.8^{e,\square}$
	2	$67.5 \pm 0.7^{a,\#}$	$70.4 \pm 0.1^{b,\#}$	$60.6 \pm 0.8^{c,f,\#}$	$58.9 \pm 0.9^{d,f,\#}$	$45.7 \pm 0.6^{e,\#}$
	3	$84.6 \pm 0.5^{a,\$}$	$121.5 \pm 2.2^{b,\$}$	$76.5 \pm 0.8^{c,\$}$	$70.8 \pm 1.1^{d,\$}$	$63.1 \pm 0.5^{e,\$}$
	4	$104.8 \pm 0.2^{a,\blacklozenge}$	$156.6 \pm 0.6^{b,\blacklozenge}$	$99.3 \pm 0.9^{c,\blacklozenge}$	$91.5 \pm 1.1^{d,\blacklozenge}$	$84.9 \pm 0.4^{e,\blacklozenge}$
	5	$126.0 \pm 1.4^{a,\bullet}$	$184.7 \pm 0.4^{b,\bullet}$	$123.1 \pm 1.3^{c,\bullet}$	$121.6 \pm 0.9^{d,\bullet}$	$102.8 \pm 0.8^{e,\bullet}$

All values given are means of three determinations $(\bar{x} \pm SD)$

Means in row with different small letters are significantly different ($p < 0.05$)

Means in column with different symbol are significantly different ($p < 0.05$)

BCSPI black cumin seeds protein isolate, *BCSPH* black cumin seeds protein hydrolysates, *DH* degree of hydrolysis, *SD* standard deviation

(DH = 12.58%) for both FC and FS. This could be probably explained by the production of amphiphilic peptides with a smaller molecular weight during hydrolysis. In fact, the obtained peptides are more flexible and they are able to absorb rapidly to the air–water interface during bubbling leading to the formation of a stable viscoelastic cohesive film via intermolecular interactions. Moreover, the lowest surface tension was found at DH = 12.58% (Fig. 2) which may explain again the highest FC and FS at the lowest DH. Our finding was in agreement with that reported by Arteaga et al. [9] for pea protein hydrolysates in which enzymatic hydrolysis ameliorated the foam ability properties.

On the other hand, FC of both BCSPI and BCSPH decreased with increasing of concentration, while FS increased with increasing of concentration. In fact, the low concentration allows proteins to be more flexible to migrate rapidly to the air–water interface, however, the high concentration of proteins improves the foam stability.

Antioxidant activities

DPPH radical scavenging capacities of samples are shown in Fig. 4a. The BCSPI and BCSPH possessed a DPPH free radical scavenging activity in the range of the concentrations tested. However, BCSPH showed higher scavenging activity than BCSPI. In fact, hydrolysis leads to generate smaller peptides able to cross cellular membranes and act as an antioxidative compound [8]. All samples exhibited a concentration dependent scavenging activity against DPPH radicals and they were significantly ($p < 0.05$) lower than that of BHA at the same concentration. These results are in agreement with those found for protein hydrolysates from canola [41] and Rice bran [42]. For BCSPH, the protein hydrolysate with DH = 19.63%, exhibited the highest DPPH radical-scavenging activity ($p < 0.05$) ($IC_{50} = 0.23$ mg/mL). Our finding was similar to that reported for chickpea protein and camel milk casein hydrolysates [4, 43]. Thus, the results show that BCSPH, at relatively high DH, potentially contained substances which are hydrogen donors.

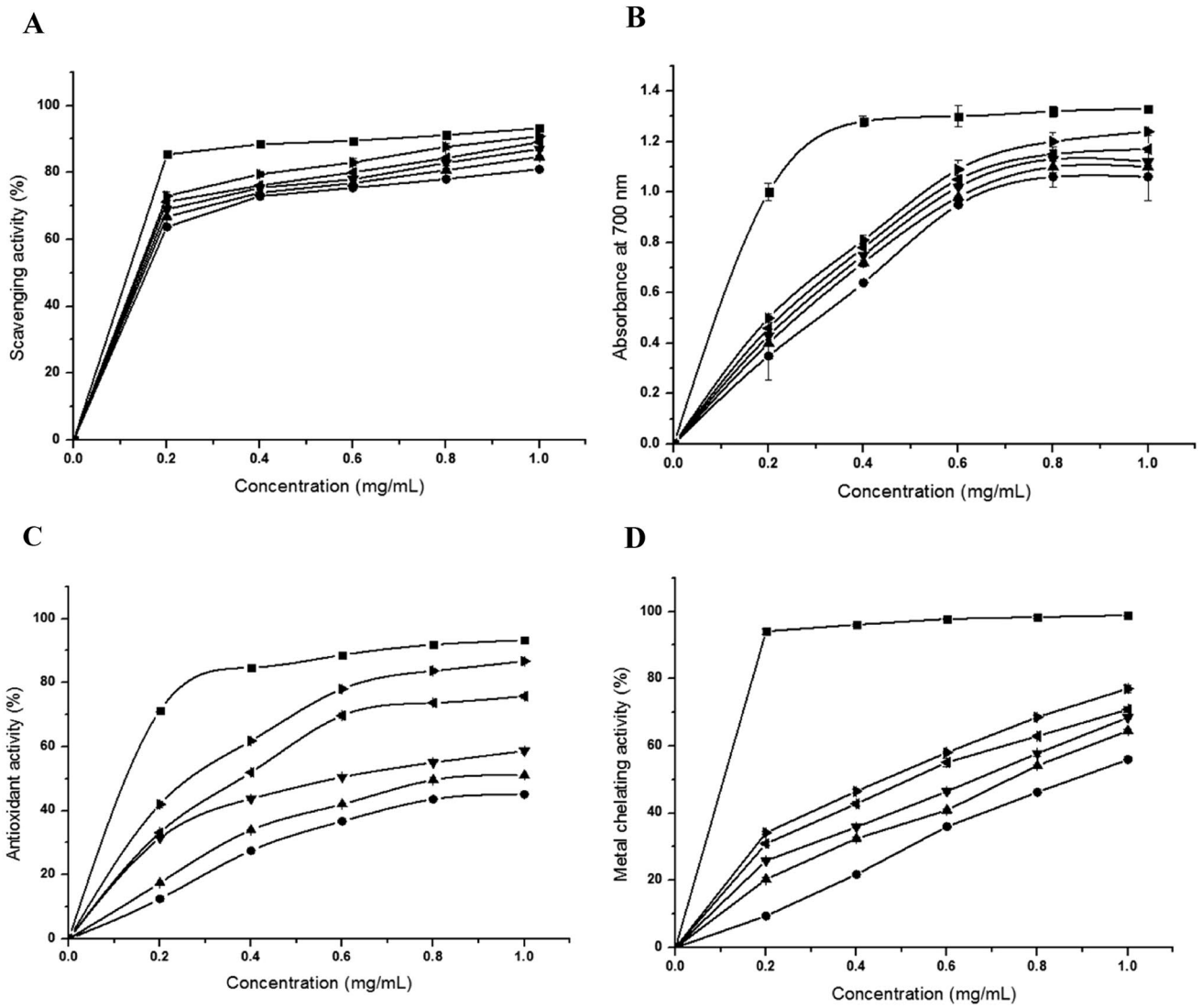

Fig. 4 Antioxidant activities of BCSPI and BCSPH at different concentrations. **a** DPPH radical-scavenging assay. **b** Reducing power assay. **c** Antioxidant assay using the ß-carotene bleaching method. **d** Metal chelating assay. BHA and EDTA were used as positive controls. (■) BHA or EDTA; (●) BCSPI; (▲) BCSPH 12.58%; (▼) BCSPH 14.74%; (◄) BCSPH 16.90%; and (►) BCSPH 19.63%

The reducing power assay is generally used to evaluate the ability of an antioxidant to donate electrons to free radicals. As shown in Fig. 4b, reducing power of samples appeared to be concentration-dependent and increased with increasing concentration. In addition, the highest reducing power was significantly ($p < 0.05$) exhibit by BHA, followed by BCSPH and BCSPI, respectively. The reducing power in BCSPH was dependent on the DH. Some researchers [7, 26, 44] have reported that the reducing power ability increased with increasing of DH.

Antioxidant activities of BCSPI and BCSPH were analyzed using β-carotene bleaching inhibition assay and the results are represented in Fig. 4c. In this assay, the antioxidant activity of all samples increased with increasing samples concentration. BCSPH showed higher antioxidant capacity than BCSPI in the range of concentrations tested. However, BHA displayed a significantly ($p < 0.05$) higher antioxidant activity than that of BCSPH at all concentrations tested. The IC_{50} values were determined. A lower IC_{50} indicates a strong antioxidant activity. The hydrolysate at DH = 19.63% had the most antioxidant capacity ($IC_{50} = 0.39$ mg/mL) followed by the protein hydrolysate at DH = 16.90% ($IC_{50} = 0.49$ mg/mL). In fact, these results were similar to those previously reported by Jamdar et al. [7] for peanut protein hydrolysates.

Ferrous ion (Fe^{2+}) is the most powerful pro-oxidant among metal ions. The BCSPI and BCSPH showed very strong metal (Fe^{2+}) chelating activity (Fig. 4d). This capacity of samples and EDTA increased with increasing concentration. BCSPH had higher antioxidant activity than

BCSPI at all concentrations tested. As the DH increased, metal chelating activity of the BCSPH increased ($p < 0.05$). The protein hydrolysate with DH = 19.63%, exhibited the highest metal chelating activity ($p < 0.05$) (77.07% at 1 mg/mL). Therefore, it suggests that high degree of cleavage of peptide bonds renders a hydrolysate with high metal chelating activity [6, 42].

Conclusion

In the present study, the techno-functional properties and the antioxidant activity of black cumin seeds protein isolate (BCSPI) and hydrolysates (BCSPH) were evaluated. All BCSPH had a significant ($p < 0.05$) protein content higher than that obtained for BCSPI. BCSPH presented better solubility and antioxidant activity compared to that of BCSPI. BCSPH, with their excellent solubility over a wide pH range, can be used in different food formulations, especially in acidic foods. BCSPH were found to be effective natural antioxidants that can be used in food systems. Moreover, BCSPH with the lowest DH (DH = 12.58%) exhibited higher emulsifying and foaming properties than that of BCSPI. The results revealed that BCSPH could create new opportunities for the development of promising techno-functional ingredients, which presented interest antioxidative properties, for use in a wide range of food and pharmaceutical products. Accordingly, this study may be of interest for example for the meat industry, which requires several ingredients with high antioxidant potential and effective emulsifying properties.

Further researches should be done to isolate and identify some specific peptides from BCSPH which are responsible of the antioxidant activities. Moreover, a study of amino acid composition and structural characteristics of these hydrolysates is required.

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Declarations

Conflict of interest The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Ethical approval This article does not contain any studies with human or animal subjects.

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