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Antioxidant potential use of bioactive peptides derived from mung bean hydrolysates (*Vigna Radiata)*

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The antioxidant activity of bioactive peptides obtained from hydrolyzed mung bean (*Vidna radiata*) grown in Espinal Tolima was evaluated. Alkaline hydrolysis was performed with 1 N NaOH. Mung bean protein concentrate obtained was 82%. The enzymatic hydrolysis was performed using a randomized block design, with commercial enzymes: Alcalase®, trypsin® and Flavourzyme®. The kinetics of the hydrolysis degree (DH) was measured based on time. The factor evaluated was the reaction time in minutes and the response variable was the degree of hydrolysis (DH), which was 43.21% for Alcalase, 41.20% for Trypsin and 38.41% for Flavourzyme. Two experiments used for measuring the antiradical activity *in vitro* were positive for the three types of enzymes. The optimal antiradical capacity was obtained at 30 min for Alcalase and Trypsin, and 45 min, respectively for Flavourzyme. Antioxidant activity *in vitro* such as, ABTS, DPPH, ORAC and FRAC correlated with the in vivo assays. Mung bean hydrolysates could have antioxidant effects, a good alternative when incorporated into diet, as a dietary supplement or added to a food matrix.

Key words: Antioxidant, bioactive peptides, hydrolyses, mung bean.

INTRODUCTION

Enzymatic protein hydrolysates have been used to improve the functional properties of food products, formulate pharmaceuticals in specific clinical application, and obtain bioactive peptides (Torruco-Uco et al., 2008). Today, there is a great interest in protein hydrolysates and their various applications and uses. It is known that after hydrolysis, the biofunctional properties of proteins can be improved (Torruco-Uco et al., 2009).Generally, the resulting peptides have different biological activities in addition to being an energy source of essential amino acids (Betancur-Ancona et al., 2004; Tecson-Mendoza et al., 2009). Most bio-functional properties are based on amino acids sequences in the polypeptides, the type of enzyme used, and methods of recovery of the peptide. Bioactive peptides are often small peptide chains consisting of 2 to 15 amino acid residues (Segura-Campos et al., 2010; Megías et al., 2004). However, some studies mention that the bioactive peptides derived from food are only between 2 and 9 amino acid residues (El-adawy, 2000). Although, there may be exceptions

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because there are peptides with 20 amino acid residues, as lunasin peptide extracted from soybeans with anticancer activity tested in rats had 43 amino acid residues and a molecular weight of 5400 Da (Jeong et al., 2002). Bioactive peptides have been isolated from various sources, both animal and vegetable. Among the former are casein, cheese whey-based milk, fermented milk, muscle of chicken and fish, and the latter are those of plant origin which have been isolated from wheat gluten, soy, sunflower, spinach, etc (Das Neves et al., 2006; Megias et al., 2004). These peptides have various biological activities such as antihypertensives (Jenssen et al., 2004), opioids (Muñoz, 2011), antioxidants (Gómez et al., 2013) anticholesterolemic, (Torrugo.Uco et al., 2008), antimicrobial (Benitez et al., 2008), anticariogenic (Montano-Perez And Vargas-Albores, 2002), antithrombotic (Rojano et al., 2012) anti-cancer and immunomodulators (Martinez and Martinez, 2015), among others.

Mung bean is an important source of protein for human consumption in Asia. The bean is used for animal food in some countries. The main protein in mung bean seeds is vicilin (8S), which represents 89% globulin, followed by 7.6% of 11S, and 3.4% of the basic 7S globulin (Tecson-Mendoza et al., 2009). Only basic 7S and 11S have disulfide bonds. By isolating the main protein of mung bean, it is possible to use it to obtain many desirable features when added to processed foods, such as changes in foaming, emulsification and water absorption (El-adawy, 2000). The natural antioxidants based on peptides isolated from plant samples are effectively used for extending the storage period of food (Gómez et al., 2013; Benitez et al., 2008).The antioxidant activities are affected by the composition and sequences of amino acid present in those particular peptides and the molecular weight of the peptides (Montano-Perez And Vargas-Albores, 2002). In Colombia, mung bean is currently used for its high nutritional value as a flour in feeding pigs, fish and poultry. Similarly, it is used as green manure and sown and incorporated as organic matter at the beginning of growth to improve the physical, chemical and biological soil properties, and reduce weed populations and disease incidence. Mung bean hydrolysis products act as antioxidants in a food matrices. The values obtained are similar to reported values for some foods in the database of United States Department of Agriculture

MATERIALS AND METHODS

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), (±) -6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox) and potassium persulphate were purchased from Sigma-Aldrich.

Plant material

Mung bean was collected in the Municipality of Espinal Tolima. The seeds were cleaned by hand and grinded in an electric grinder brand Thomas Wiley. The flour obtained was passed through a mesh screen 200 µm. Afterwards it was defatted with hexane in a soxhlet apparatus by 8 h. Once evaporated, the hexane passed through a mesh screen of 100 µm to achieve a more homogeneous particle size. It was stored in airtight containers for later use.

Proximal composition of flour

The proximate composition of the mung bean flour was analyzed to determine crude protein, crude fat, ash, moisture, phosphorus, potassium, calcium and magnesium. The composition was determined according to the procedures described by AOAC (AOAC, 1990).

Mung bean protein concentrate (MBPC)

The protein concentrate was fractionated using an established method Betancur-Anacona et al., 2004), with some modifications. To raise the pH 1 N NaOH was added to the flour suspension to reach pH 9 and stirred for one hour at 450 rpm. The suspension was centrifuged at 9000 g × 15 min (centrifuge Hermle-Z32HK). The supernatant was adjusted to pH 4.5 with 1 N HCl, to isoelectric point of globulin, filtered and centrifuged at 15000 g for 20 min. The precipitate was washed with deionized water several times, and subsequently was lyophilized at -50°C.

Enzymatic hydrolysis

The method described by Pedroche et al 2002, was used for the hydrolysis of the protein concentrate. A randomized block design was used for it, where the blocks used were commercial enzymes (Alcalase® 2.4 L FG, Flavourzyme® 500 mg, and trypsin Novo Nordisk , Bagsvaerd, Denmark), reaction times (0, 5, 15, 30, 45 and 60 min) and the response variable was the degree of hydrolysis (DH). Hydrolysis was performed by individual treatments with the above enzymes, carrying out digestion with Alcalase®, Flavourzyme® Trypsin® up to 60 min. A hydrolysis curve was obtained from the pH-stat technique using the following parameters of hydrolysis: enzyme/substrate ratio (E/S) 3% for all enzymes; pH 8 at 50° C for Alcalase, pH 7 at 50°C for Flavourzyme and pH 8 at 37°C for trypsin. The hydrolysis was stopped by heating at 85°C for 15 min. The hydrolyzate was clarified through a 0.45 nm filter. The filtrate was centrifuged at 12,000 g × 10 min, lyophilized and stored in a freezer at -20°C.

The DH% for each enzyme was calculated by the pH-stat method, using the following equation:

$$\%DH = \frac{h}{h_{total}} x100 = \frac{V_{NaOH} * N_b}{MP * \alpha * h_{total}} x\ 100$$

$$\alpha = \frac{1}{1 + 10^{pK-pH}}$$

Where: %DH is the degree of hydrolysis; h is the number of broken peptide; h_{total} is total number of bonds available for hydrolysis proteolytic; V_{NaOH} is the total volume of NaOH consumed expressed in mL to keep the pH constant during the reaction. Nb is normality of the NaOH; MP is the mass of the protein; α is the degree of dissociation of the protein**.**

DPPH radical scavenging activity assay

The methodology described by Braca et al., 2002, was followed,

Table 1. Composition of the proximal mung bean flour cultivated in the Tolima Espinal.

% Humidity	% Ash	pH	E.E	% Protein	P	Ca	K	Mg
8.33	3.8	6.77	0.65	18.43	0.54	0.36	1.77	0.24

with slight modification. One milliliter of either hydrolysate or peptide solution at different concentrations (0-1 mg/ml) was mixed with 4 ml of 0.15 mM DPPH (in 95% ethanol). The mixture was then shaken vigorously using a mixer. The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Ethanol and Trolox was used as a control and standard sample, respectively. The radical scavenging capacity of the samples was measured as a decrease in the absorbance of DPPH radical and it was calculated using the following equation:

$$\%\ \mathrm{DPPH} = \left(\frac{\mathrm{A_C} - \mathrm{A_M}}{\mathrm{A_C}}\right) \times 100$$

Where: % DPPH, DPPH radical scavenging activity, expressed as a percentage. A_C: Absorbance control; A_M: Absorbance of the reaction mixture

From these values, the percent inhibition of 50% of radical (IC_{50}) of both samples, as pattern was determined using a linear regression model.

ABTS radical stabilizing activity

The methodology described by Kuskoski et al., 2004, was followed. The radical $ABTS^+$ was obtained by a mixture of ABTS (7 mM) and potassium persulfate (2.45 mM final concentration). This mixture was allowed to stand for 16 h at room temperature, time after which it was diluted with ethanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. The sample was prepared by mixing 3.43 mL $ABTS^+$ solution adjusted with 70 µL of the hydrolysates at different concentrations (0.2 to 200 µg/mL final concentration) and recording the absorbance values after 6 min of reaction. TROLOX was used as pattern (0.1-1 µg/mL).

$$\%\mathrm{ABTS} = \left(\frac{\mathrm{A_{ABTS}} - \mathrm{A_{6min}}}{\mathrm{A_{ABTS}}}\right) \times 100$$

Where: %ABTS is ABTS radical stabilizing activity, expressed as a percentage; A_{ABTS} is absorbance of $ABTS^+$ before adding the sample; A_6min is absorbance of the reaction mixture at 6 min.

Oxygen radical absorbance capacity (ORAC)

The methodology described by Zapata et al. (2014) was used. Trolox® was used as standard, under controlled conditions (temperature to 37 ° C and pH 7.4.) Absorbance was performed at 493 nm λ excitation and excitation slit 10 nm λ emission 515 and emission slit 15 with 1% attenuator. For the calibration of the technique 1×10^{-2} M of fluorescein solutions AAPH and phosphate buffer 0.6 M (75 mM, pH 7.4) were used. 30 µL aliquot of sample was mixed with 21 µL of fluorescein, 2.899 mL of phosphate buffer and 50 µL of AAPH. The protective effect of the antioxidant was calculated using the differences in areas under the decay curve of fluorescein between the target and the sample, and compared to the standard curve.

The results were expressed in µmol /micromoles Trolox equivalents per liter of sample (micromol Trolox / 100 g dry product), according to the following equation:

$$ORAC = \left(\frac{(AUC_{SAMPLE} - AUC_{CONTROL})}{(AUC_{TROLOX} - AUC_{CONTROL})}\right) f[TROLOX]$$

Where AUC_{SAMPLE} is the area under the curve of the sample, $AUC_{CONTROL}$ area under the curve for the control, AUC_{Trolox} area under the curve for the Trolox, f is the factor of dilution of the sample. The fluorescence was measured in a fluorescence spectrophotometer PerkinElmer® LS55.

Antioxidant activity reducing Fe^{+3}: FRAP Assay

This method evaluates the reducing power of a sample based on its ability to reduce ferric iron (Fe^{+3}) complexed with 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) to ferrous form (Fe^{+2}) which has an absorbance maximum at a wavelength between 590 and 595 nm. 50 50 µL of sample was added to 900 µL of a solution of FRAP (acetic acid –sodium acetate, pH 3.4 Buffer acid, TPTZ, $FeCl_3$, in 10:1: 1), after 30 min of reaction the absorbance was determined at a wavelength of 593 nm. This value was compared with the reference curve constructed with ascorbic acid as a primary standard, and the results were expressed as ascorbic acid equivalents (AEAC).

Anti-hemolytic activity

The anti-hemolytic activity was carried out following the methodology proposed by Nabavi et al., 2011 and Aguillon et al., 2013. Blood was centrifuged at 2500 rpm for 10 min; plasma and leucocytes were removed. Red cells were washed three times with isotonic saline phosphate buffer (PBS: 22.2 mM Na_2HPO_4, 5.6 mM KH_2PO_4, 123.3 mM NaCl and 10 mM glucose in distilled water, pH 7.4). The activity was determined using the following equation:

$$\%\ \mathrm{AAH} = \left(\frac{\mathrm{A_{b\ control}} - \mathrm{A_{b\ hydrolyzed}}}{\mathrm{A_{b\ control}}}\right) \times 100$$

Where: %AHH is anti-hemolytic activity, expressed as a percentage. Ab control is absorbance of control in this case ascorbic acid is used; hydrolyzed Ab is absorbance of the sample, in this case the hydrolysates.

RESULTS AND DISCUSSION

The results of the composition of mung bean seeds flour are shown in Table 1. Protein percentage agrees with that reported in the literature. Potasium concentration is comparatively high for plants (López Bellido, 1996). This suggests that mung bean can be a good source of Pottasium.

Enzymatic hydrolysis

The degree of hydrolysis for each enzyme is shown in Table 2. The highest degree of hydrolysis was obtained

Table 2. Percentage of hydrolysis for enzymes at different reaction times.

Hydrolysis time	Alcalase %DH	Trypsin %DH	Flavourzyme%DH
5’	28.81 ± 1.8	21.61 ± 1.1	19.21 ± 1.2
15’	33.61 ± 1.96	32.17 ± 1.4	24.01 ± 2.1
30’	43.21 ± 1.5	41.29 ± 1.9	33.61 ± 1.9
45’	38.41 ± 1.7	40.81 ± 1.2	38.41 ± 1.8
60’	40.81 ± 1.3	38.45 ± 1.8	37.45 ± 1.3

Data reported are the average of three different determinations in time ± standard deviation.

Table 3. Antioxidant activity of mung bean hydrolysates obtained with alcalase.

Hydrolysis time	Alcalase			
	IC_{50} ABTS (µg/mL)	IC_{50} DPPH (µg/mL)	TEAC (mM Trolox / 100 g sample)	mg. Ascorbic Acid/ 100 g sample)
5'	67.62 ± 1.69	163.22 ± 1.49	24.06 ± 0.51	226.34 ± 1.65
15'	54.25 ± 1.70	153.25 ± 1.50	32.09 ± 0.48	298.56 ± 1.97
30'	51.13 ± 1.91	141.13 ± 1.61	44.01 ± 0.65	327.31 ± 2.05
45'	52.52 ± 1.72	152.52 ± 1.42	52.16 ± 1.47	398.89 ± 2.13
60'	54.65 ± 1.83	169.65 ± 1.93	55.02 ± 3.20	427.12 ± 2.22

Values expressed as the average of three determinations ± standard deviation.

Table 4. Antioxidant activity of mung bean hydrolysates obtained with trypsin.

Hydrolysis time	Trypsin			
	IC_{50} ABTS (µg/mL)	IC_{50} DPPH (µg/mL)	TEAC (mM Trolox / 100 g sample)	mg. Ascorbic acid/ 100 g sample)
5'	110.31 ± 1.53	190.31 ± 1.53	12.23 ± 0.71	427.33 ± 2.63
15'	81.89 ± 0.71	171.89 ± 1.71	16.34 ± 0.52	478.65 ± 2.89
30'	82.39 ± 1.82	152.39 ± 1.82	19.32 ± 0.74	552.27 ± 2.96
45'	95.50 ± 1.93	195.50 ± 1.73	22.29 ± 1.25	580.36 ± 3.42
60'	116.29 ± 2.04	216.29 ± 1.89	29.59 ± 1.93	569.61 ± 3.02

Values expressed as the average of three determinations ± standard deviation.

after 30 min for both Alcalase **A**, trypsin **T**, and Flavourzyme **F.** The optimal hydrolysis time was 45 min. Similar result was observed in the degree of hydrolysis of Alcalase and trypsin enzymes, compared to Flavourzyme enzyme.

The degree of hydrolysis obtained is slightly higher than those reported in previous studies of legumes such as beans and soybeans, where the values of hydrolysis are between 20 and 31% for alcalase and 18 and 51% for flavourzyme (Martínez Ayala et al., 2015 and Viogue et al., 2008). The highest degree of enzymatic hydrolysis was obtained after 30 and 45 min in the three enzymes respectively, compared to 60 and 90 min reported in previous works for alcalase and flavourzyme (Betancur-Anacona et al., 2004; Megías et al., 2004).

Evaluation of antioxidant activity *in vitro*

The results of DPPH and ABTS antiradical activity, antioxidant activity as well as the ORAC and the FRAP, are presented for the three enzymes Alcalase, Trypsin and Flavourzyme in Tables 3 to 5 respectively.

The degree of hydrolysis for 30 min results in higher antiradical activity ABTS for alcalase and trypsin with IC_{50} values of 51.13 and 82.39 µg/mL, respectively. In the case of Flavourzyme enzyme the highest IC_{50} value was 93.44 µg/mL.

Table 5. Antioxidant activity of mung bean hydrolysates obtained with Flavourzyme.

Hydrolysis time	Flavourzyme			
	IC_{50} ABTS (µg/mL)	IC_{50} DPPH (µg/mL)	TEAC (mM Trolox / 100g sample)	mg. Ascorbic acid/ 100 g sample)
5'	223.32 ± 1.61	236.82 ± 1.71	10.21 ± 0.42	498.75 ± 2.98
15'	175.65 ± 1.52	195.95 ± 1.72	14.12 ± 0.37	507.62 ± 2.77
30'	143.13 ± 1.84	106.03 ± 1.74	17.04 ± 0.74	522.29 ± 3.12
45'	123.44 ± 1.65	93.44 ± 1.93	26.17 ± 1.01	518.93 ± 3.22
60'	174.92 ± 1.65	94.92 ± 1.85	43.41 ± 1.97	535.67 ± 3.31

Values expressed as the average of three determinations ± standard deviation.

Table 6. Oxygen radical absorbance capacity (ORAC) of selected foods, Release 2.0.

NDB No.*	Description	ORAC mmol TE/100 g
99459	Sumac, bran, raw	312.40
99465	Sorghum, bran, black	100.80
99464	Sorghum, bran, red	71.00
2030	Spices, pepper, black	34.05
16032	Beans, kidney, red,, mature seeds, raw	8.61
16014	Beans, black, mature, seeds, raw	8.49
16040	Beans, pink, mature, seeds, raw	8.32
16069	Lentils, raw	7.28
14097	Alcoholic beverage, wine, table, red, Cabernet Sauvignon	4.52
99586	Mangosteen, raw	2.51
99070	Tea, green, brewed	1.25
11052	Beans, snap, green, raw	0.80

*5-Digit Nutrient Databank number. Source: USDA data base of antioxidant activity, May, 2010.

According to the values obtained *in vitro* DPPH antiradical activity, an increased capacity of hydrolysis was observed at 30 min time with trypsin and Alcalase enzymes, with values of 141.13 g/mL and 152.39 µg/mL respectively; for flavourzyme enzyme hydrolysis timewhere most capacity antiradical activity in vitro is presented at 45 min with 123.44 µg/mL

ORAC assay

Antioxidant activities as measured by the ORAC method for mung bean hydrolysates with enzymes Alcalase, Flavourzyme and trypsin, at different times of hydrolysis are presented in Table 6. The ORAC values vary between 55.02 and 22.29 mmol Trolox / 100 g sample. ORAC values for some foods reported in the database of the United States Department of Agriculture (USDA) are shown in Table 6 (Haytowitz and Bhagwat, 2010).

Interestingly, only the protein hydrolysates bean exceeds the antioxidant potential of the seed, in which the secondary metabolites of phenolic origin are included. As can be seen, bean seeds have values between 0.80 and 8.61 mmol Trolox/100 g sample, ORAC values much lower than those obtained with mung bean hydrolysates. This means that mung bean hydrolysates obtained with Alcalase, Flavourzyme and trypsin Enzymes have a very high antioxidant potential. Some researchers suggested an intake between 3.0 to 5.0 ORAC/day, in order to promote oxidative balance in blood plasma and body tissues (Prior et al., 2003; Rojano et al., 2012).Therefore, mung bean hydrolysates could be a good alternative when incorporated into diet, either in the form of encapsulation or added to food matrix.

FRAP assay

In this test, the values of the redox potential of Fe^{+3} - TPTZ are comparable with that of ABTS; FRAP values for the hydrolysates are slightly higher than those reported in studies on foods like fruits and juices (Prior et al., 2003; Rojano et al., 2012).

Hydrolysates obtained in Tables 4 and 5 with trypsin and Flavourzyme had values of 535.67 and 580.36 (mg Ascorbic Acid/100 g sample) at 60 and 45 min respectively,

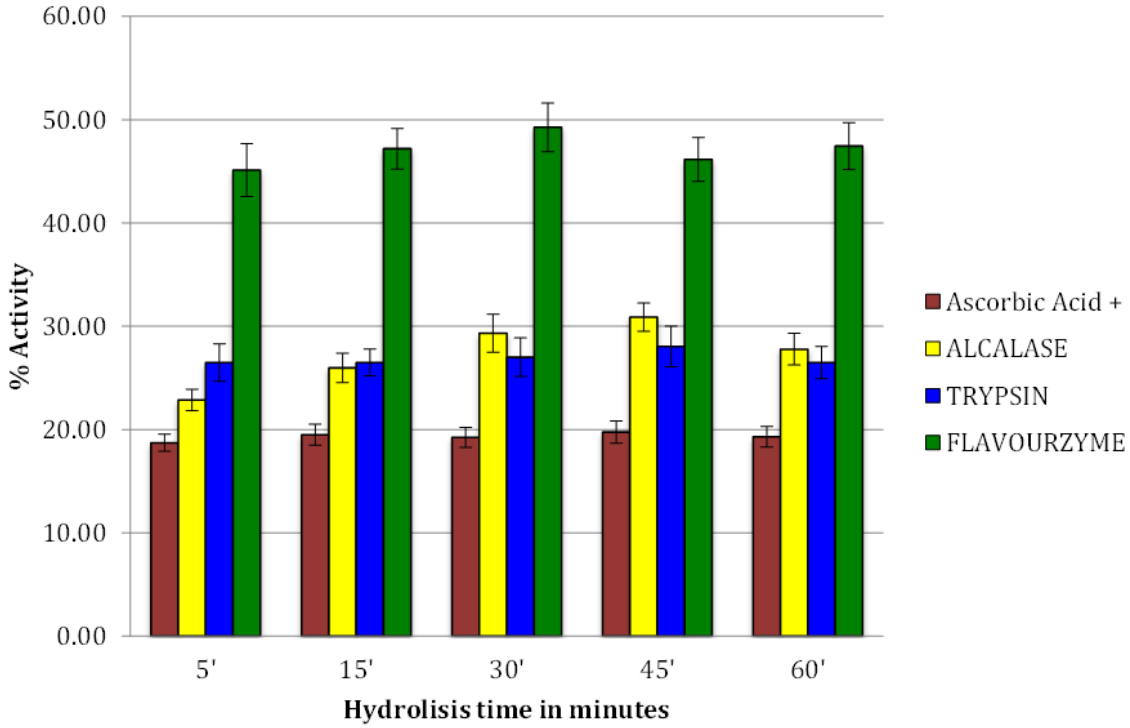

Figure 1. Anti-hemolytic activity of MBPC with alcalase, flavourzyme and trypsin. Values expressed as the mean of three determinations ± standard deviation.

whereas for Alcalase the highest value of 427.12 (mg Ascorbic Acid/100 g sample) was obtained at 60 min.

Anti-hemolytic activity

The results of the anti-hemolytic activity of mung bean hydrolysates at different times of hydrolysis are presented in Figure 1.

The results obtained inanti hemolytic activity of different hydrolysates mung bean against ascorbic acid used as a positive control are shown in Figure 1, being hydrolysates obtained with Flavourzyme the highest percentage of activity against those obtained with Alcalase and trypsin.

The hydrolysates show more ability to inhibit the hemolysis H_2O_2-induced than positive control. The antihemolytic activity of trypsin was the lowest compared to the other hydrolysates with Flavourzyme and Alcalase. All enzymatic hydrolysates were significantly different compared to ascorbic acid used as positive control.

Conclusion

The degree of enzymatic hydrolysis (%DH) of protein concentrates mung beans grown in the municipality of Espinal Tolima was more efficient with Alcalase and trypsin at 30 min with values of 43.21 and 41.29%, compared to 45 min used for Flavourzyme enzyme where a value of 38.45% was obtained. According to the values obtained for the two trials of antiradical activity *in vitro*, a higher capacity was observed in the hydrolysis time of 30 min with the enzyme Alcalase ABTS and DPPH respectively. Similar case with trypsin Enzyme, where at the same hydrolysis time (30'), and the highest valuesof antiradical activity *in vitro* was presented. For Flavourzyme enzyme, it is evident that the hydrolysis time where the increased capacity of antiradical activity *in vitro* occurs was at 45 min. A trend almost directly proportional was observed between the degree of hydrolysis (% HD) and antiradical activity, in the samples taken at different times, showing this behavior in each of the enzymes used. The hydrolysates with each one of the enzyme presented a higher value in the antihemolitic activity than those reported by the positive control in ascorbic acid.

Interestingly, only the protein hydrolysates bean exceeds the antioxidant potential of the seed, in which the secondary metabolites of phenolic origin are included. Evaluating antioxidant activity in cells has a very interesting report that correlates with the data obtained in the *in vitro* assays. Therefore, mung bean hydrolysates could be a good alternative when incorporated in diet, either in the form of encapsulation or added in a food matrix.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Aguillon J, Maldonado ME, Loango N, Arango SS, Landázuri P (2013). Antioxidant and antiproliferative activity of ethanolic and aqueous extracts from leaves and fruits juice Passiflora edulis. Perspect. Nutr. Hum. 15(1):13-25.

AOAC-Association of Official Agricultural Chemists (1990). Official Methods of Analysis, 14th ed. Washington DC.

Benitez R, Ibarz A, Pagan J (2008). Hidrolizados de proteína: procesos y aplicaciones. Acta Bioquím. Clín. Latinoam. 42(2):227-236.

Betancur-Ancona D, Gallegos-Tintoré S, Chel-Guerrero L (2004). Wet-fractionation of *Phaseolus lunatus* seeds: partial characterization of starch and protein. J. Sci. Food Agric. 84(10):1193-1201.

Braca A, Sortino C, Politi M, Morelli I, Mendez J (2002). Antioxidant activity of flavonoids from *Licania licaniaeflora*. J. Ethnopharmacol. 79(3):379-81.

Das Neves MRA, Campos T, Márquez LUM (2006). Modulação da Pressão Arterial por Hidrolisados Protéicos. Braz. J. Food Technol. III JIPCA, 81-86.

El-Adawy TA (2000). Functional properties and nutritional quality of acetylated and succinylated mung bean protein isolate. Food Chem. 70:83-91.

Gómez LJ, Figueroa OA, Zapata JE (2013). Actividad Antioxidante de Hidrolizados Enzimáticos de Plasma Bovino Obtenidos por Efecto de Alcalasa® 2.4 L. Inf. Tecnol. 24(1):33-42.

Haytowitz DB, Bhagwat S (2014). Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2. U.S. Department of Agriculture Agricultural Research Service. Pp. 10-41.

Jenssen H, Andersen JH, Uhlin-Hansen L, Gutteberg TJ, Rekdal Ø (2004). Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. Antiviral Res. 61(2):101-109.

Jeong JH, Lam Y, De Lumen OB (2002). Barley Lunasin Suppresses Ras-induced Colony Formation and Inhibits Core Histone Acetylation in Mammalian Cells. J. Agric. Food Chem. 50:5903-5908.

Kuskoski EM, Asuero AG, García-Parilla MC, Troncoso AM, Fett R (2004). Actividad antioxidante de pigmentos antociánicos. Ciênc. Tecnol. Aliment. 24(4):691-693.

Lopez Bellido L (1996). Nuevas técnica para determinar la calidad de las legumbres. Resultados de los trabajos realizados en la universidad de córdoba sobre la influencia de suelo y clima en la calidad de garbanzos y lentejas. 158: 77-83. http://www.magrama.gob.es/ministerio/pags/biblioteca/revistas/pdf_DYC/DYC_1995_25_77_83.pdf

Martínez AO, Martínez DV (2015). Proteínas y péptidos en nutrición enteral. Nutr. Hosp. 21(Supl.2):1-14.

Megías C, Yust MD, Pedroche J, Lquari H, Girón-Calle J, Alaiz M, Millán F, Vioque J (2004). Purification of an ACE Inhibitory Peptide alters Hydrolysis of Sunflower (*Helianthus annuus* L.) Protein Isolates. J. Agric. Food Chem. 52(7):1928-1932.

Megias C, Yust MM, Pedroche J, Lquari H, Girón-Calle J, Alaiz M, Millán F, Vioque J (2004). Purification of an ACE Inhibitory Peptide after Dydrolysis of Sunflower (*Helianthus annuus* L.) Proetin Isolates. J. Agric. Food Chem. 52:1928-1932.

Montano-Perez K, Vargas-Albores F (2002). Péptidos antimicrobianos: un mecanismo de defensa ancestral con mucho futuro. INCI. 27(1): 21-27.

Muñoz EJF (2011). Péptidos Opioides Endógenos, Dolor y Adicción. Synapsis 3(1):33-39.

Nabavi SF, Ebrahimzadeh M, Nabavi S, Eslami B (2011). Antioxidant activity of flower, stem and leaf extracts of Ferula gummosa Boiss. Grasas Aceites 61(3):244-250.

Pedroche J, Yust MM, Girón‐Calle J, Alaiz M, Millán F, Vioque J (2002). Utilisation of Chickpea Protein Isolates for Production of Peptides with Angiotensin I- Converting Enzyme (ACE)-Inhibitory Activity. J. Sci. Food Agric. 82(9):960-965.

Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, Hampsch-Woodill M, Huang D, Ou B, Jacob R (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL) of plasma and other biological and food samples. J. Agric. Food Chem. 51:3273-3279.

Rojano B, Zapata K, Cortés F (2012). Capacidad atrapadora de radicales libres de *Passiflora mollissima* (Kunth) L.H. Bailey (curuba). Rev. Cubana Plantas Med. 17(4):408-419.

Segura-Campos M, Chel-Guerrero L, Betancur-Ancona D (2010). Efecto de la digestión en la biodisponibilidad de péptidos con actividad biológica. Rev. Chil. Nutr. 37(3):386-391.

Torruco-Uco J, Chel-Guerrero L, Martínez-Ayala A, Dávila-Ortíz G, Betancur-Ancona D (2009). Angiotensin-I converting enzyme inhibitory and antioxidant activities of protein hydrolysates from Phaseolus lunatus and *Phaseolus vulgaris* seeds. Food Sci. Technol. 42:1597-1604.

Torruco-Uco JG, Domínguez-Magaña MA, Dávila-Ortíz G, Martínez-Ayala A, Chel-Guerrero LA, Betancur-Ancona DA (2008). Péptidos antihipertensivos, una alternativa de tratamiento de origen natural: una revisión. Cienc. Tecnol. Aliment. 6(2):158-168.

Zapata S, Piedrahita AM, Rojano B (2014). Capacidad atrapadora de radicales oxígeno (ORAC) y fenoles totales de frutas y hortalizas de Colombia. Perspect. Nutr. Hum.16:25-36.