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Evaluation of the antioxidant activity from bovine serum albumin protein fractions

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ABSTRACT

Objective: Evaluate the antioxidant activity of protein fractions obtained from (bovine serum albumin) BSA protein hydrolysates.

Design/methodology/approach: Bioinformatics tools, such as the NCBI database, were used to search for primary sequences of BSA proteins. The methodology included a prediction of peptides with antioxidant activity through various bioinformatics servers. The antioxidant activity was determined by different methods. Statistical analysis was performed to evaluate possible significant differences using the Student Newman Keulls test for group comparison.

Results: Through in silica hydrolysis the following peptides were found: valine-alanine-phenylalanine (VAF), lysine-tryptophan (KW), phenylalanine-tyrosine (FY), alanine-proline (AP), among others that may have antioxidant activity. The results showed that the fraction <1 kDa hydrolyzed with chymotrypsin, this fraction showed 84% copper chelation, 61% iron chelation, while 75% inhibition of the DPPH radical. In the case of the fraction <1 kDa hydrolyzed with pepsin, it only showed 16% iron chelation, while in the other methods no value was detected.

Study limitations/implications: The enzyme used for enzymatic hydrolysis generates low degrees of hydrolysis and generates oligopeptide dipeptides that may not be as like some of the tested methods, in addition to the protein concentration in the fraction <1 kDa with pepsin it had very low values that could not be detected by some antioxidant methods.

Findings/conclusions: The antioxidant activity of the <1 kDa fraction obtained with chymotrypsin showed greater antioxidant and chelating activity, compared to the <1 kDa fraction obtained with pepsin. However, at the concentration of 2% and 5% fluctuations are observed in both fractions, because probably the composition of amino acids that is present in both fractions determines the activity in each of the tested methods.

Keywords: BSA, fractions, antioxidants.

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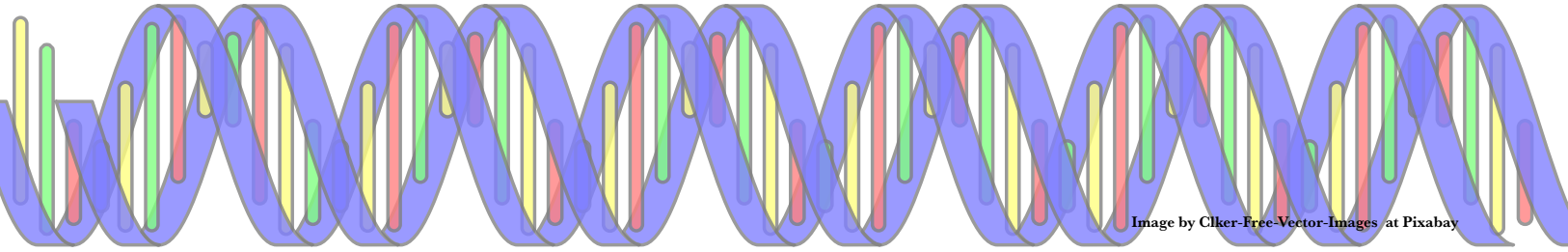
INTRODUCTION

The increasing rise in common lifestyle-related diseases, such as cardiovascular disorders, hypertension, atherosclerosis, and diabetes, among others, has become a serious global concern. Recent advances in the field of proteomics have promoted the use



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of protein hydrolysates or bioactive peptides as a therapeutic alternative to mitigate the risk factors related to these diseases. In recent years, numerous bioactive peptides from various food sources have been isolated and characterized, which have presented various biological activities, such as antihypertensive, antioxidant, opioid, antithrombotic, immunomodulatory, antidiabetic, hyploagglutinating, hypocholesterolemic and antimicrobial, mainly. Although all are important, antioxidant activity has been one of the most studied and widely related to chronic degenerative disorders, which is based on the ability to capture free radicals, inhibition of lipid peroxidation and properties of metal ion chelation (Bhat *et al.*, 2015; Choi *et al.*, 2017; Dhaval *et al.*, 2016; Lagrange & Clark, 2019).

Proteins are high molecular weight biomolecules that represent an important source of energy and essential amino acids necessary for growth and maintenance of physiological functions. Currently, there is great interest in the identification and characterization of bioactive peptides obtained from the hydrolysis of proteins of plant and/or animal origin. The bioactivity presented by these peptides depends mainly on the composition (structure, hydrophobicity, and charge) and amino acid sequence (Saadi *et al.*, 2015). An alternative to produce bioactive peptides is enzymatic hydrolysis that under controlled conditions *in vitro* or *in vivo* and by using specific proteolytic enzymes, the desired peptides can be isolated from a complex mixture made up of amino acids, oligopeptides and peptides of different lengths (Embiriekah *et al.*, 2018; Sarmadi & Ismail, 2010). It is known that the heat treatment involved in the inactivation of the enzymes can have an important effect on the bioactivity of the peptides (O'Loughlin *et al.*, 2014). Bovine serum represents a valuable source of high-quality protein. In addition to their balanced amino acid content, bovine serum proteins have been reported to be a rich source of bioactive peptides that exert important biological and physiological effects (Ballatore *et al.*, 2020; Hernández-Ledesma *et al.*, 2014; Morris & FitzGerald, 2008) show the antioxidant and cytoprotective activity of peptides (≤3 kDa) obtained from the hydrolysis of a serum protein concentrate (WPC 35). Pasiakos, (2015) demonstrated that whey proteins have advantageous digestive and absorbent properties that facilitate the rapid, but sustained delivery of essential amino acids to the body. Bovine serum proteins are considered proteins of high biological value according to the DIAAS (Digestible Indispensable Amino Acid Score) method developed by the Food and Agriculture Organization. The main proteins that make up bovine serum are α-lactalbumin (α-La), β-lactoglobulin (β-Lg), immunoglobulins (Ig), albumin (BSA), lactoferrin (LF) and lactoperoxidase (LP). The presence of leucine, isoleucine, tyrosine, methionine, proline and valine in the structure of these proteins contributes greatly to their biological properties (Tovar-Jiménez *et al.*, 2017).

One of the proteins present in bovine serum that has been widely used as a standard in various scientific studies is bovine serum albumin (BSA), due to its simple structure, good stability, low cost, easy availability, and versatile ligand-binding properties (Sengupta *et al.*, 2018). BSA is a water-soluble protein that occurs naturally in milk, has a molecular weight of 66.5 kDa, an isoelectric point of 4.7 in water at 25 °C, and is made up of a polypeptide chain of 583 amino acids. It has three intrinsic fluorophores: tyrosine, tryptophan (with two residues: Trp-134 and Trp-213) and phenylalanine. The Trp-134 residue is known to be

found on the outer surface of the protein, while Trp-213 is located within the hydrophobic structure. BSA, as well as human serum albumin (HSA, with 585 amino acid residues) are non-glycosylated globular transport proteins present in serum that play an important role in the circulatory system and in some specific functions of the organism. Both proteins have a structural homology of 75.6% in their three domains and a degree of similarity of 76% in their tertiary structures (Carter *et al.*, 1994; Majorek *et al.*, 2012; Simonelli & Arancibia, 2015).

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA, B4287, ≥98%), pepsin from porcine gastric mucosa (P7000, powder, ≥250 units/mg solid), and α-chymotrypsin from bovine pancreas (C4129 Type II, lyophilized powder, ≥40 units/mg protein) were obtained from Sigma Aldrich Co. LLC, St. Louis, MO, USA. All other reagents were of analytical grade.

Enzymatic hydrolysis of BSA

Hydrolysis was carried out according to José Goulart *et al.*, (2005), Adjonu *et al.*, (2014) and Fernández Alonso, (2015) with some modifications. Briefly, 2 g of BSA to be hydrolisated with chymotrypsin, was dissolved in 40 mL of a 5% (w/v) sodium phosphate buffer solution (pH 7.8, 0.01M), while 2 g the same protein to be treated with pepsin was mixed with 40 mL of a 0.001M citrate phosphate buffer solution (pH 2). In both cases, and before starting the hydrolysis reaction, the samples without enzymes were agitated at 125 rpm (MaxQ4000, Barnstead Lab-Line, Co. USA) during 30 min at 50 °C (chymotrypsin) and 37 °C (pepsin). Afterwards, the enzymes were added in a ratio of 1:10 enzyme/substrate. The hydrolysis time was 4 h and the pH was mantained constant by the addition of NaOH (0.1M) or HCl (0.1M) for chymotrypsin and pepsin respectively. At the end of the reaction, the samples were heated at 90 °C during 3 min, then were cooled at 25 °C and were stored at −18 °C until their analysis. The pH of the hydrolyzate of pepsine was modified from 2 to 6 before the ultrafiltration because of the membrane type used.

Ultrafiltration of the hydrolizates

Peptides were separated using a 10 mL cell with a filtration area of 4.1 cm^2 (Amicon 8010 Millipore, Bedford, MA, USA) and molecular cutting membranes (Ultracel®, Co. Millipore, U.S.A.) of 5, 3 and 1 kDa at a pressure of 25 psi (N_2) and at 25 °C following the manufacturer procedure. In all cases, the membranes were hydrated in distilled water during 2 h and were washed in the buffer solution, phosphate buffer pH 7 for chymotrypsine hydrolyzates and citrates buffer pH 2 for pepsine hydrolyzates, before their use. The filtration was done in three steps: first the hydrolizate was passed through 5 KDa membrane; this permeate was passed through 3kDa membrane and finally, the last permate was passed through 1KDa membrane. All the filtrated fractions were store at −18 °C until their analysis.

In silico analysis

Seed proteins sequences reported for BSA were obtained from the UniProt database (http://www.uniprot.org/) and NCBI database (https://www.ncbi.nlm.nih.gov/) with number P02769.4 for BSA.

While the development of proteolytic hydrolysis was carried out in silico, using the server for prediction of Biopep analysis. This tool was used to simulate hydrolysis to predict the possible enzymatic cleavage peptides that are released by the enzyme pepsin and chymotrypsin with which the BSA cleavages were obtained respectively. (http://www.uwm.edu.pl/biochemia/index.php/en/biopep) (Minkiewicz *et al*., 2019).

Antioxidant activity

Copper chelating activity

The ability of BSA fractions to chelate Cu^{2+} was determined according to Saiga *et al*., (2003). A volume of 200 μL of a 50mM sodium acetate buffer pH 6, 6 μL of a 4 mM pyrocatechol violet solution and 12 μL of $CuS0_4.5H_2O$ prepared a 1mg/ml, were added to 50 μL of fractions (3 mg protein /mL). Ethylenediaminetetraacetic acid (EDTA) was used as the reference molecule (1 mg/ml). Absorbance at 632 nm was measured using a microplate reader (Multiskan Spectrum, ThermoLab Systems, MA, USA). Copper chelating activity was calculated for iron, as described above.

$$\%\ Cooper\ chelating = \frac{\left(Abs_{blank} - Abs_{sample}\right)}{Abs_{blank}} \times 100$$

Where: Abs_{blank}=the absorbance observed for the blank, and Abs_{sample}=the absorbance observed for the sample.

Iron Chelating Activity

The ability of BSA fractions to chelate Fe^{2+} was measured by the method of Saiga, Tanabe, & Nishimura, (2003). A volume of 30 μL of a 2 mM ferrous chloride solution and 150 μL of 100 mM acetate buffer at pH 4.9 were added to 50 μL of BSA fractions (3 mg protein/mL). After 30 min of incubation at room temperature, the reaction was inhibited by the addition of 12.5 μL of a 40 mM ferrozine solution. Ethylenediaminetetraacetic acid (EDTA) was used as the reference molecule (1 mg/ml). After 10 min, the absorbance was measured at 560 nm by using a microplate reader (Multiskan Spectrum, Thermo Lab Systems, MA, USA). A decrease in absorbance corresponds to an increase in iron chelating capacity. The ability of the samples to chelate ferrous ion Fe^{2+} is defined as follows:

$$\%\ Iron\ chelating = \frac{\left(Abs_{blank} - Abs_{sample}\right)}{Abs_{blank}} \times 100$$

Where: Abs_{blank}=the absorbance observed for the blank, and Abs_{sample}=the absorbance observed for the sample.

Evaluation of radical scavenging activity of the hydroxyl $(-OH)$

The hydroxyl radical scavenging capacity was measured using modified method as described previously de Avellar *et al.*, (2004). 50 μL of a 0.75 mM 1, 10-phenanthroline and 25 μL of a 0.75 mM $FeSO_4$, 150 μL of phosphate buffer pH 7.4 and 50 μL of H_2O_2 0.1% (v/v %) were added to 50 μL of fractions (3 mg protein/mL). Ascorbic acid was used as a positive control (1 mg/mL). The mixture was incubated at 37 °C for 60 min, and the absorbance was measured at 536 nm in a microplate reader (Multiskan Spectrum, ThermoLab Systems). The % hydroxyl radical scavenging activity were determined using the following equation:

$$\%\ OH\ radical\ scavenging\ activity = \frac{\left(A_s - A_1\right)}{\left(A_0 - A_1\right)} \times 100$$

where: A_s it is the absorbance of the sample; A_1, is the absorbance of the control (distilled water instead of the sample); and A_0, is the absorbance of the blank solution containing 1, 10- phenantroline and $FeSO_4$.

Evaluation of the scavenging activity of the superoxide radical (O_2^-)

Scavenging activity of the superoxide radical (O_2^-) for the BSA fractions was generated from the pyrogallol autooxidation reaction according to the method of Udenigwe and Aluko, (2012). 50 μL of BSA fractions (3 mg/mL) were mixed with 80 μL of 50 mM Tris-HCl-EDTA buffer pH 8.3 in a 96-well microplate followed by the addition of 40 μL of 1.5 mM pyrogallol dissolved in 10 mM HCL. The scavenging activity of the superoxide radical (O_2^-) induced by pyrogallol was measured as the absorbance at 420 nm at room temperature. Scavenging activity of the superoxide radical (O_2^-) was calculated using the following equation:

$$\%\ Scavenging\ activity\ of\ O_2^- = \frac{\left(Abs_{blank} - Abs_{sample}\right)}{Abs_{blank}} \times 100$$

The Tris-HCl buffer solution was used as blank (Abs_{blank}) and the absorbance of the samples with pyrogallol and Tris-HCl-EDTA buffer (Abs_{sample}).

RESULTS AND DISCUSSION

The peptide fractions obtained from the protein hydrolysates obtained with pepsin and chymotrypsin, presented a protein content that ranged between 14.39-6.34 mg/mL and 106-18.86 mg/mL respectively. The protein content decreased proportionally to the

molecular weight cutoff of the membranes used in the separation of the protein hydrolyzed fractions, observing the highest amount of protein in the 5-10kDa fraction and the lowest in the <1kDa fraction.

To determine the percentage of amino acids according to their polarity, the number of dipeptides, tripeptides, and free amino acids, as well as the fragments that probably may have some biological activity, the in-silico hydrolysis of bovine serum albumin (BSA) in the BIOPEP database, using pepsin and chymotrypsin as proteolytic enzymes individually. In Figure 1 the analysis showed that in the case of hydrolysis with pepsin, a higher proportion of peptides with more than 5 amino acids is obtained, while only 11 free amino acids and 12 tripeptides are observed, of which only one VAF is the showing angiotensin converting enzyme inhibitory activity. In the case of hydrolysis with chymotrypsin, a greater number of free amino acids and dipeptides are obtained that show antihypertensive and antioxidant activity. It should be mentioned that the number of peptides and their length will depend on the activity and specificity of the enzyme. In the case of pepsin, it acts on peptide bonds of amino acids with hydrophobic side chains, while chymotrypsin acts on residues of aromatic amino acids and Leu (Damodaran & Parkin, 2017).

Antioxidant activity

Copper quelating activity

The hydrolysate obtained from hydrolysis with chymotrypsin was subjected to ultrafiltration to obtain peptides of different molecular weight ranging from fractions

***In silico* hydrolysis with pepsin**

MKWVTF - ISL - L - L - L - F - SSAYSRGVF - RRDTHKSEIAHRF - KDL - GEEHF - KGL - VL - IAF - SQYL - QQCPF - DEHVKL - VNEL - TEF - AKTCVADESHAGCEKSL - HTL - F - GDEL - CKVASL - RETYGDMADCCEKQEPERNECF - L - SHKDDSPDL - PKKPDPNTL - CDEF - KADEKKF - WGKYL - YEIARRHPYF - YAPEL - L - YYANKYNGVF - QECCQAEDKGACL - L - PKIETMREKVL - TSSARQRL - RCASIQKF - GERAL - KAWSVARL - SQKF - PKAEF - VEVTKL - VTDL - TKVHKECCHGDL - L - ECADDRADL - AKYICDNQDTISSKL - KECCDKPL - L - EKSHCIAEVEKDAIPENL - PPL - TADF - AEDKDVCKNYQEAKDAF - L - GSF - L - YEYSRRHPEYAVSVL - L - RL - AKEYEATL - EECCAKDDPHACYSTVF - DKL - KHL - VDEPQNL - IKQNCDQF - EKL - GEYGF - QNAL - IVRYTRKVPQVSTPTL - VEVSRSL - GKVGTRCCTKPESERMPCTEDYL - SL - **IL** - NRL - CVL - HEKTPVSEKVTKCCTESL - VNRRPCF - SAL - TPDETYVPKAF - DEKL - F - **TF** - HADICTL - PDTEKQIKKQTAL - VEL - L - KHKPKATEEQTVMENF - **VAF** - VDKCCAADDKEACF - AVEGPKL - VVSTQTAL - A

***In silico* hydrolysis with chymotrypsin**

M - **KW** - VTFISL - L - L - L - FSSAY - SRGVFRRDTHKSE - IAHRFKDL - **GE** - E - HFKGL - VL - IAFSQ - Y - L - Q - Q - CP - FDE - HVKL - VN - E - L - TE - FAKTCVADE - SHAGCE - KSL - HTL - FGDE - L - CKVASL - RE - TY - GDM - ADCCE - KQ - E - P - E - RN - E - CFL - SHKDDSP - DL - P - KKP - DP - N - TL - CDE - FKADE - KKFW - GKY - L - Y - E - IARRHP - Y - **FY** - **AP** - E - L - L - Y - Y - AN - **KY** - N - GVFQ - E - CCQ - AE - DKGACL - L - P - KIE - TM - RE - KVL - TSSARQ - RL - RCASIQ - KFGE - RAL - KAW - SVARL - SQ - KFP - KAE - FVE - VTKL - VTDL - TKVHKE - CCHGDL - L - E - CADDRADL - AKY - ICDN - Q - DTISSKL - **KE** - CCDKP - L - L - E - KSHCIAE - **VE** - KDAIP - E - N - L - P - P - L - TADFAE - DKDVCKN - Y - Q - E - AKDAFL - GSFL - Y - E - Y - SRRHP - E - Y - AVSVL - L - RL - AKE - Y - E - ATL - E - E - CCAKDDP - HACY - STVFDKL - KHL - VDE - P - Q - N - L - IKQ - N - CDQ - FE - KL - **GE** - Y - GFQ - N - AL - IVRY - TRKVP - Q - VSTP - TL - **VE** - VSRSL - GKVGTRCCTKP - E - SE - RM - P - CTE - **DY** - L - SL - **IL** - N - RL - CVL - HE - KTP - VSE - KVTKCCTE - SL - VN - RRP - CFSAL - **TP** - DE - TY - **VP** - KAFDE - KL - FTFHADICTL - P - DTE - KQ - IKKQ - TAL - **VE** - L - L - KHKP - KATE - E - Q - TVM - E - N - FVAFVDKCCAADDKE - ACFAVE - GP - KL - VVSTQ - TAL - A

Figure 1. In silico hydrolysis of BSA with pepsin and chymotrypsin individually. The fragments in bold are those that show antioxidant activity in each of the hydrolysis.

>10 kDa, <10 kDa, <5 kDa, <3 kDa and <1 kDa. Once the fractions were obtained, the copper chelation test was carried out (Figure 2a), where it was observed that the 5, 3 and 1 kDa fractions showed the highest chelation of this metal, which allows to elucidate that these peptides can trap said metal and with this stop oxidation reactions in the body. The percentage of chelation of these last three fractions is greater than 80%, so it gives a relationship with the amount of short chain peptides obtained in enzymatic hydrolysis. However, the fractions greater than 5kDa showed less chelating activity, this inhibition being around 20%. Also, the fraction <10 KDa showed the lowest activity with only 7% inhibition. On the other hand, the fractions obtained from the pepsin hydrolysate (Figure 2b), yielded values below 30% of metal chelation, being below the values obtained with chymotrypsin. Metal chelation measures the degree of protection against oxidation reactions, which are catalyzed by transition metals such as Cu^{2+} and Fe^{2+} (Saiga *et al.*, 2003) and which can catalyze the generation of reactive oxygen species such as the hydroxyl radical (•OH) and the superoxide anion O_2^- (Stohs, 1995). These *in vivo* oxidation reactions are apparently involved in the pathogenesis of neurodegenerative diseases (Mandel *et al.*, 2006). Copper chelating peptides can prevent the oxidative activity of this metal by chelating it. Therefore, they can be useful, not only preventing the oxidative activity of this metal that can damage the cells of the luminal space of the stomach, but also, they can prevent the oxidation of LDL induced by copper, if they reach the bloodstream (Burkitt, 2001). Copper chelating peptides, being rich in His, have been shown to prevent the oxidative activity of this metal. The imidazole ring of this residue is directly involved in bonding with copper. On the other hand, it has also been observed that these peptides are rich in Arg and although this amino acid lacks chelating properties, it can favor the union of the peptide with the metal ion (Megías *et al.*, 2008).

The fractions obtained from the chymotrypsin hydrolysate (Figure 3a), showed activity to chelate iron, ranging between 40 and 70% activity. In this way, the fraction <10 kDa and the fraction <5 kDa, showed the highest values, 67.91±0.16 and 68.83±0.02 respectively. On the other hand, in the fractions obtained from the pepsin hydrolysate

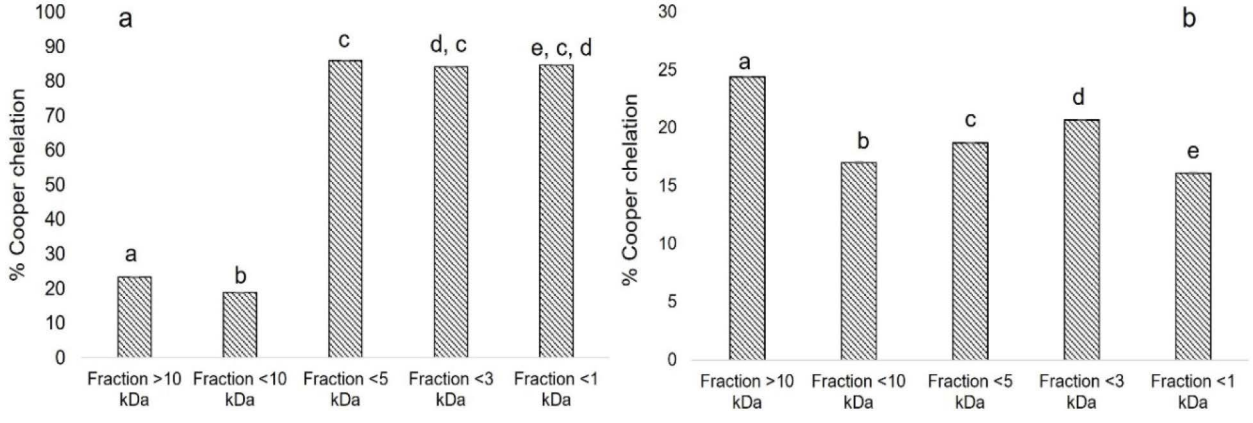

Figure 2. Cooper chelating activity in BSA protein fractions, obtained by hydrolysis with chymotrypsin a) and pepsin b). The results represent the mean of three independent determinations. Different letters indicate significant difference ($p<0.05$). Unifactorial ANOVA, group comparison by Student Newman Keuls (SNK).

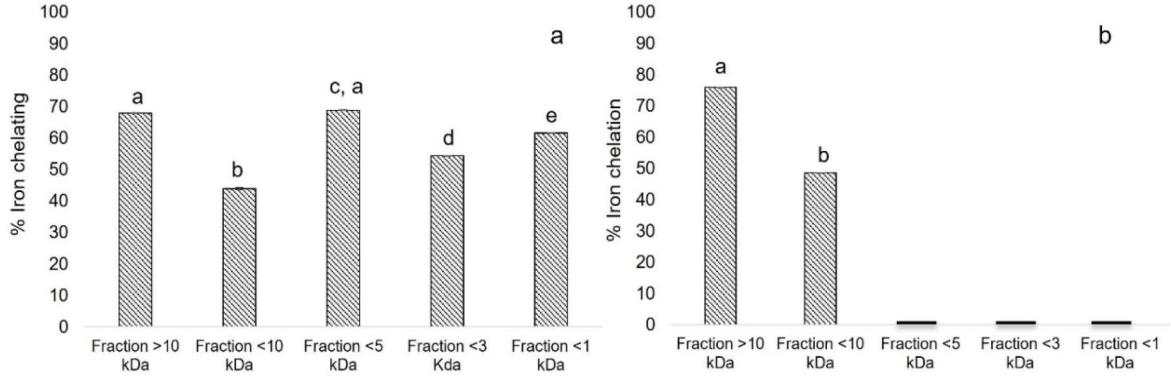

Figure 3. Iron chelating activity in BSA protein fractions, obtained by hydrolysis with chymotrypsin a) and pepsin b). The results represent the mean of three independent determinations. Different letters indicate significant difference ($p<0.05$). Unifactorial ANOVA, group comparison by Student Newman Keuls (SNK).

(Figure 3b), only the fraction >10 kDa and <10 kDa, showed activity to chelate, in the case of the remaining fractions no activity was detected, this probably because the protein concentration was very low, and therefore the amount of amino acids present did not manage to trap the metal.

Chelation of this metal appears to be at least partially responsible for the antioxidant activity that has been found in several amino acids, including Tyr, Met, His, Lys, Arg, and Trp (Huang *et al.*, 2010). Furthermore, iron chelation by His, Glu, Asp, and Cys shows results in iron absorption and can also lead to reduction of ferric to ferrous ion (Storcksdieck Genannt Bonsmann *et al.*, 2007).

The scavenging activity of the SO^{-2} radical of the fractions obtained from the hydrolysate with chymotrypsin and pepsin is observed in Figure 4a and 4b respectively. The protein fractions obtained from the chymotrypsin hydrolysate showed a better ability to inhibit the SO^{-2} radical except for the <1kDa fraction that showed no activity. In the case of the fractions obtained from the pepsin hydrolysate, all the fractions except for the fraction <5 kDa and <1 kDa showed no activity.

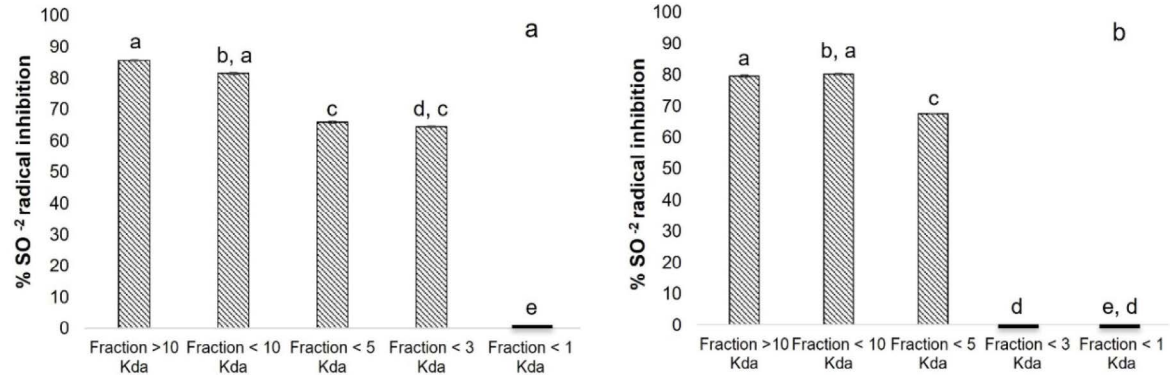

Figure 4. % SO^{-2} radical inhibition in BSA protein fractions, obtained by hydrolysis with chymotrypsin a) and pepsin b). The results represent the mean of three independent determinations. Different letters indicate significant difference ($p<0.05$). Unifactorial ANOVA, group comparison by Student Newman Keuls (SNK).

The trapping activity of the −OH radical of the fractions obtained from the hydrolysate with chymotrypsin and pepsin is observed in Figure 5a and 5b respectively. The protein fractions obtained from the chymotrypsin hydrolysate showed a better ability to inhibit the −OH radical except for the <1kDa fraction that showed no activity. On the other hand, the fractions obtained with pepsin also showed radical trapping activity, however the percentage of inhibition in all the fractions was around 50%, this being half of what was obtained with the fractions obtained with chymotrypsin. In addition, it was also observed that the fraction <1 kDa did not show activity.

On the other hand, Ballatore *et al.*, (2020), demonstrated that the protein fractions (<3 kDa) of whey obtained by hydrolysis with trypsin show SO^{-2} and −OH scavenging activity, which compared with our results the fraction <3 kDa showed the same activity but only with the fraction obtained with chymotrypsin. Peng *et al.*, (2010), determined that the fractions between 0.1-2.8 kDa fractionation with a Sephadex G-10 gel filtration column obtained from whey protein isolate hydrolyzed with alcalase for 5 h, showed strongest free radical scavenging effects, which was evidenced by the electron spin resonance (ESR) of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) and of scavenging hydroxyl (OH) and superoxide radicals. This activity is important to determine since the superoxide anion is normally formed in cellular oxidation reactions. Although it cannot directly initiate lipid oxidation, the superoxide radical can produce hydrogen peroxide and hydroxyl radical, through dismutation and other types of reactions (Dorman *et al.*, 2004).

As it has been shown above, superoxide can participate in many important epigenetic processes including DNA methylation/demethylation, histone methylation/demethylation, and histone acetylation/deacetylation. Therefore, the disruption of superoxide balance might stimulate dangerous changes in these processes. As it has been discussed above, the effects of superoxide on epigenetic processes might be more prominent ones in pathologic states characterized by the enhanced levels of ROS such as cancer, aging, cardiovascular diseases, and diabetes mellitus (Afanas'ev, 2015).

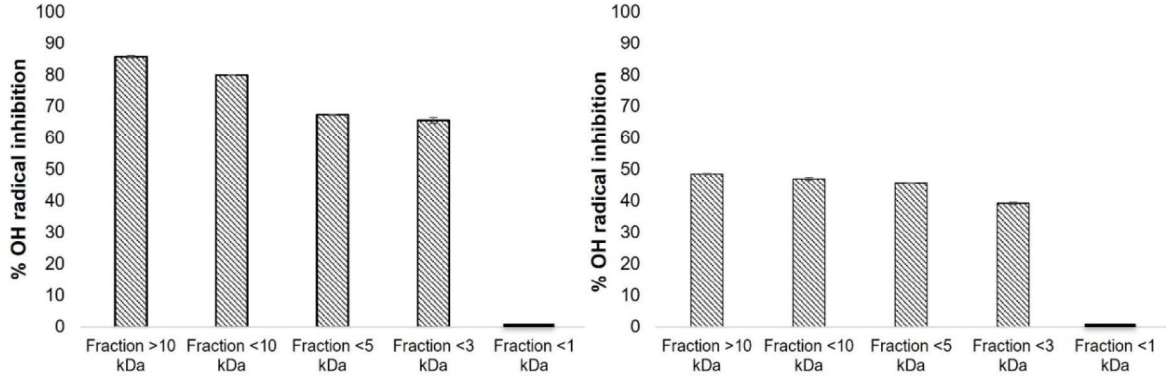

Figure 4. % OH radical inhibition in BSA protein fractions, obtained by hydrolysis with chymotrypsin a) and pepsin b). The results represent the mean of three independent determinations. Different letters indicate significant difference ($p<0.05$). Unifactorial ANOVA, group comparison by Student Newman Keuls (SNK).

CONCLUSIONS

The antioxidant activity present in the protein fractions of BSA hydrolysed with chymotrypsin and pepsin can be attributed at least in part to their ability to trap radicals, this due to the presence of various amino acids or short peptides in the range of <10 kDa to <3 kDa that showed the extinction of radicals, playing an important role in the general antioxidant effect of the protein fractions. Much more research is needed to isolate the individual peptides responsible for the antioxidant activity of BSA, as well as to identify the amino acid sequence, which will allow a better structure-functionality relationship of the peptides.

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