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Original Research Article

Antioxidant activity of peptides obtained from reserve proteins of *Salvia hispanica L*

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Abstract

The objective of the present study was to evaluate the antioxidant activity of peptides obtained by enzymatic hydrolysis from the reserve proteins of chia seeds. The contents of the protein fractions of albumins, globulins, prolamins and glutelinsin the seeds were 3.1, 3.2, 2.6 and 3.8 mg/g of chia flour respectively. Treatment with the Eap1 protease made it possible to obtain peptides from 1.4 mg/g of chia flour. The fractions with the highest amounts of released peptides were globulin, prolamin and glutelin. Allthe fractions of protein and peptideswere inhibitedby two radicals:1,1-diphenyl-2-picrylhidazil (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). The glutelin and prolamin peptides inhibited the DPPH and ABTS radicals at 59 and 4%. Chia seeds contain reserve proteins that were inhibited by the DPPH and ABTS radicals, as were the peptides released from them by enzymatic hydrolysis. Therefore, they may have potential applications in the food, pharmaceutical and cosmetics industries.

Keywords: Chia, peptides, inhibition, ABTS radical, DPPH radical

INTRODUCTION

Salvia hispanica L., commonly as chia, is classified as a pseudo-cereal belonging to the family *Lamiaceae*. Its cultivation and utilization were considered essential elements of Mesoamerican culture, where it was used to produce flours and oils, as well as having medicinal, alimentary, artistic and even religious applications (Cahil, 2004). By the year 3500 B.C., chia had become a staple cultigen in central Mexico that later spread to Central America in the period 1500-900 B.C. However, with the arrival of the Spaniards in the Americas, their cereals soon displaced this crop, though it did survive in mountainous areas of Mexico and Guatemala (Ayerza and Coates, 2006). Its increasing importance in recent years is due to its contents of protein (15-25%), fats (30-33%), carbohydrates (26-41%), dietaryfiber (18-30%), and ash (4-5%). In addition, it is a source of several vitamins, minerals and antioxidants (Ixtaina *et al.,* 2008; Vázquez-Ovando *et al.,* 2010; Ixtaina *et al.,* 2011). Because of these properties, it has been used as a study model to determine its potential benefits for treating such health problems as cardiovascular diseases and obesity, for regulating intestinal transit and for controlling cholesterol and triglyceride levels. It may also have the capacity to prevent diseases like type II diabetes and some cancers (Poudyal *et al.,* 2012).

The proteins of chia seeds perform several functions: structural, biological and as reserves or storage. The latter is proportionally the most important as reserve proteins are deposited in protein bodies during endosperm development, where they supply carbonated and nitrogenated skeletons in the germination phase. Isolating proteins entails taking into account their physicochemical properties (Argos *et al.,* 1985; Shewry and Halford, 2002; Nehete *et al,* 2013), since different proteins are soluble in distinct solvents, as follows: albumins in water, globulins in saline solutions, prolamins in alcohols, and glutelins in acids and bases (Dangaran *et al.,* 2009). The hydrolysis of alimentary proteins to

obtain bioactive peptides is a key focus of current research which has found that they may present antioxidant activity by eliminating radicals, inhibiting lipid peroxidation and chelating metallic ions. The precise structure and sequence of different amino acids, however, can affect these activities. Antioxidant peptides can be obtained by digesting proteins of both animal and vegetable origin using techniques based on enzymes, microbial fermentation orgastrointestinal digestion. Enzymatic hydrolysis is an approach that has been widely used to produce antioxidant proteins from alimentary proteins. Proteases like papain (of vegetable origin) and pepsin-trypsin (of animal origin) have been utilized to obtain these peptides (Sarmadi and Ismail, 2010).

Current knowledge holds that chia seeds contain large amounts of proteins; however, no reports on the hydrolysis of their reserve proteins or evaluations of the antioxidant capacity of their peptides appear in the literature. Thus, the objective of this study was to fraction the reserve proteins, hydrolyze them using the Eap1 aspartyl-protease produced by the *Sporisorium reilianum* fungus, and then evaluate the antioxidant activity of their peptides by reducing two radicals: 1,1-diphenyl-2-picrilhidazil (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). These peptides could have health benefits and applications in the food, pharmaceutical and cosmetics industries.

MATERIALS AND METHODS

Chia seeds gathered in the state of Puebla, Mexico, were utilized. They were conserved in plastic recipients at a temperature of 25°C until use.

Chemical analysis

Bromatological characterization was performed–following the protocols outlined in Mexican Norms– to determine Humidity (NMX-F-083-1986), ash (NMX-F-066-S-1978) and protein (NMX-F-068-S-1980) content, as well as ethereal extract (NMX-F-089-S-1978) and Dietary fiber (NMX-F-089-S-1978). The carbohydrate content was obtained by difference between 100 and the sum of ashes, ethereal extract, proteins and dietary fiber.All analyses were carried out in quintuplicate.

Mucilage-free seeds

The chia seeds were hydrated to extract mucilage at a proportion of 1:10for 24 h. Once they were covered with mucilage, the seeds were frozen for 24 h and then lyophilized for 96 h. The mucilage was removed by friction and the mucilage-free seeds recovered (Olivos-Lugo *et al.,* 2010)

Obtaining flour

The mucilage-free seeds were ground up to obtain a fine powder, which was later defatted and left to dry at ambient temperature for 24 h(Vázquez-Ovando *et al.,* 2010).

Sequential fractioning of the proteins

Extraction of the protein fractions (albumins, globulins, glutelins,prolamins) from the defatted flour was performed following the protocol modified by De la Rosa *et al.* (1992).

Protein quantification

The method proposed by Bradford (1976) was used toquantify the protein fractions. A protein pattern curve was elaborated withBovine serum albumin at a concentration of 1 mg/mL.

Obtaining aspartyl protease Eap1 from the *S.reilianum* fungus

The aspartyl protease Eap1 was obtained from the *S.reilianum* fungus following the procedure in Mandujano e*t al.* (2013).

Enzymatic hydrolysis of the protein fractions ofchia seeds

This procedure required taking 100 µL of eachprotein fraction plus 50 µL of Eap1, equivalent to 11.57 mU of activity. This reaction was incubated at 37°C for 30 min, then 500 µL of TCA at 10% were added. This mixture was agitated for 1 min and then centrifuged at 13,000 rpm for 10 minutes. The soluble peptides in the supernatant were quantified using the technique reported by Lowry *et al.* (1951). Absorbance was measured at a wavelength of 590 nm.

Antioxidant activity of the fractions and peptides

The antioxidant test with the DPPH radical (1,1-diphenyl-2-picrilhidazil) was determined in accordance with Moraes et al., (2008). The percentage of inhibition was calculated using the following equation:

Table 1. Chemical composition of chia seeds.

Component	%/100 g dried basis
Humidity	7.4±0.233
Ashes	3.42±0.309
Ethereal extract	33.4±2.75
Dietary fiber	22.7±1.45
Other carbohydrates	12.78±0.32
Proteins	20.3±0.309

±standard deviation

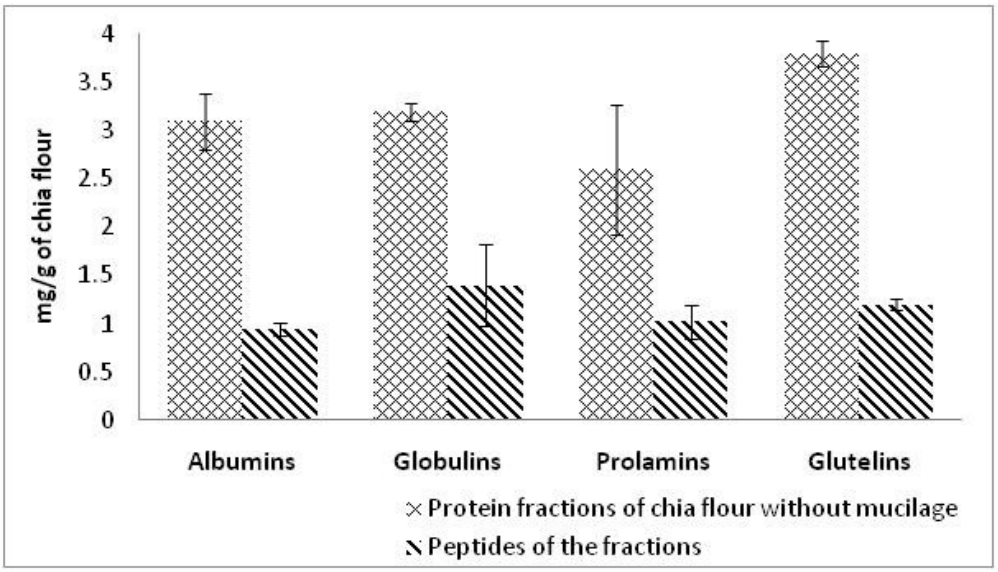

Figure 1. Reserve protein fractions and peptides released by enzymatic hydrolysis ofchia flour.

$$\%\ of\ inhibition = \frac{Abs\ (t=0) - Abs\ (t=45)}{Abs\ (t=0)} \times 100$$

where Abs (t=0) is the absorbance of the DPPH radical at time zero, and Abs (t=45) is the absorbance of the reduced DPPH radical at 45 min.

The antioxidant test with ABTS was performed in accordance with Re et al. (1999). The percentage of inhibition was calculated using the following equation:

$$\%\ of\ inhibition = \frac{Abs\ (t=0) - Abs\ (t=6)}{Abs\ (t=0)} \times 100$$

where Abs (t=0) is the absorbance of the ABTS radical at time zero, and Abs (t=6) is the absorbance of the reducedABTS radical at 6 min.

RESULTS AND DISCUSSION

The table 1 shows the results of the bromatological analysis as percentages of the dry base of the chia seeds, *S.hispánica.* The protein value determined was 20.3% ± 0.309. Raw fiber and fat were also found to have high values. Ash and moisture content, in contrast, were low, but reached values similar to those reported by Reyes *et al.* (2008) and Sandoval and Paredes-López (2012). Although the nutritional value of chia seeds changes from one variety to another, their protein content tends to be similar to that of lentils (23%) and chick peas (*garbanzo*) (21%) and, therefore, higher than cereals like wheat (14%), corn (14%), rice (8.5%), oats (15.3%) and barley (9.2%) (Ayerza and Coates, 2011). Reports on other varieties of chia have found levels as high as 28.4%, so it is clear that these seeds are a rich source of proteins, in addition to having high values for fats and dietary fiber, which improve their nutritional properties. These findings suggest that chia seeds are an excellent option for use by industries that transform products for human and animal consumption (Monroy-Torres *et al.,* 2008).

According to their solubility, the reserve proteins extracted from mucilage-free chia flour, *S.hispánica.,*

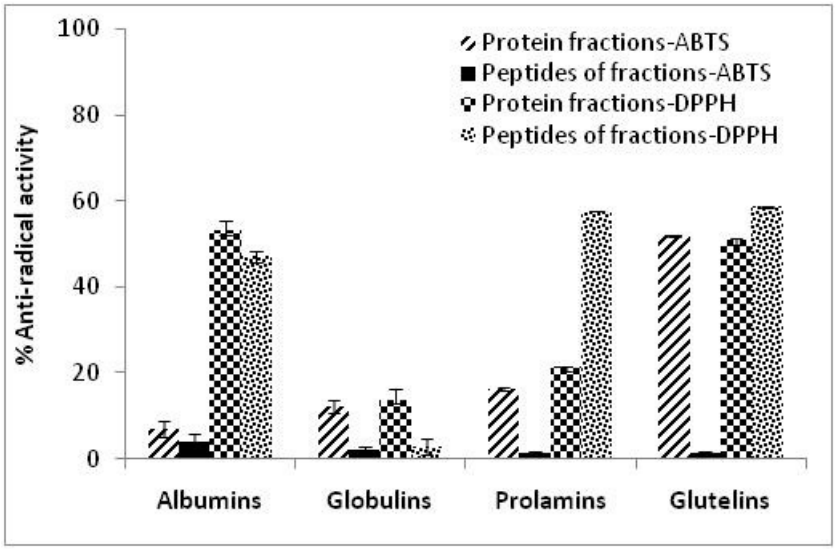

Figure 2. Percentage of antioxidant activity of the reserve protein fractions andpeptides released, evaluated using ABTS and DPPH.

presented values of 2.6-3.8 mg of protein/gofflour. Albumins, globulins and glutelins appeared in the highest quantities (Figure 1). These results are similar to those reported by Sandoval-Oliveros and Paredes-López (2012)] and Orona-Tamayo *et al.,* (2015) as well as in studies of the following cereals: cotton, peas and broad beans (*habas*) (Nikokyris and Kandylis, 1997). In addition to their reserve function, these types of proteins can aid metabolism by providing amino acids during the germination and growth of seedlings, though percentages will depend on the type of vegetable source from which they are derived and the extraction method used (Nehete *et al.,* 2013; Shewry et al., 1995).

Hydrolysis of the fractions using the aspartyl protease Eap1 made it possible to obtain peptides at values below 1.4 mg/g of chia flour. The peptides released in the greatest quantities were globulins, prolamins and glutelins (Figure 1). The enzyme Eap1 has the capacity to degrade proteins from plants like corn and to coagúlate milk, thanks to its stability over broad pH and temperatura ranges (Mandujano-González *et al.,* 2013). Also, it has the capacity to hydrolyze proteins from dairy serum and so release peptides that have greater antimicrobial activity than those generated using proteases like trypsin and chymotrypsin (Tovar-Jiménez *et al.,* 2017). The type of protease employed in hydrolysis plays a crucial role in obtaining peptides, since amino acids of different sizes, amounts and composition can be obtained, and these factors will exert a direct influence on the antioxidant activity of the hydrolyzed proteins (Korhonen *et al.,* 1998; Wu *et al.,* 2003).

All the protein fractions extracted from *S.hispánica* presented antioxidant activity against ABTS and DPPH (Figure 2). To estímate the antioxidant activity of biological components, studies often utilize techniques that involve the radicals 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and 1,1-diphenyl-2-picrylhidazil (DPPH) (Miller and Rice-Evans, 1997; Brand-Williams *et al.,* 1995), together with reducing ferric antioxidantpower (FRAP) (Benzie and Strain, 1999). Various biological sources are currently being analyzed in the search for peptides with antioxidant capacity due to their capacity to reduce the reactive oxygen species that participate in damaging biological structures during metabolism. This isespecially important because their use causes no secondary effects (Tovar-Pérez *et al.,* 2009; Hernández-Ledesma *et al.,* 2014).

In the present study, glutelins presented the highest percentage of inhibition in both radicals, at 55%, while the albumins inhibited the DPPH radical at 52% and ABTS at 8%. The globulins and prolamins, meanwhile, presented inhibitions below 20% with both radicals. Our study also found that while all the peptides released during enzymatic hydrolysis inhibited the ABTS and DPPH radicals, the glutelins and prolamins showed the greatest inhibition at 59% for the DPPH radical and 4% for ABTS. Thealbumin peptides showed 48% inhibition of the DPPH radical and 5% for ABTS, while the globulins had the lowest percentages of inhibition for both radicals, at just 5% (Figure 2). Orona-Tamayo *et al.,* (2015) reported that during enzymatic hydrolysis of proteins from chia using simulated gastrointestinal digestion, the albumin and globulin peptides had the highest antiradical activity against ABTS and DPPH. Previous studies have determined that the presence of the amino acids Tyr, Trp, Met, Lys, Cys and His are responsible for the antioxidant activity of peptides. Those with aromatic residues can donate protons to radicals that are deficientin electrons, while those containing His exist in relation to the hydrogen donor, the lipid proxy radical, entrapment

and/or the capacity to chelate metallic ions of theimidazole group. Finally, the SH group in cysteine performs acrucial antioxidant action due to its direct interaction with radicals (Saito *et al.,* 2003; Wang *et al.,* 2005; Rajapakse *et al.,* 2004, Qian *et al.,* 2008).

Peptides of vegetable origin mayhavebetter characteristics, since plants present diverseactive compounds to which antioxidant activity has been attributed. These peptides aid by donating electrons that can stop radical chain reactions. The United Nations Food and Agriculture Organization (FAO) considers that proteins from foliage plants are of high quality for consumption by humans because they abound in nature, have great nutritive value and are cholesterol-free. Moreover, they can satisfy the nutritional needs of vegetarians who avoid consuming proteins of animal origin due to health or religious considerations (Sarmadi and Ismall, 2010; Nagai *et al.,* 2007).

CONCLUSIONS

The seeds of the chia plant, *S.hispanica* are a rich vegetable source ofproteins. The study of their peptides, therefore, is potentially very important because they can present biological activity that may have applications in the food, pharmaceutical and cosmetics industries. In the present study, the Eap1 protease hydrolyzed the reserve proteins of chia seeds to release peptides that showed antioxidant activity on the DPPH and ABTS radicals. However, several factors play crucial roles in producing peptides. These include the type of protease used, time, and the conditions of hydrolysis. This is because amino acids of different sizes, amounts and compositions may be generated, and these characteristics will directly influence the antioxidant activity of the hydrolyzed peptides. These results should encourage the conduction of additional research designed to determine if other proteases will produce larger amounts of peptides with better antioxidant activity. Another significant effect is that it will be possible to purify and characterize peptides in order to ascertain their properties and perhaps establish biotechnological applications.

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Conflict of Interests

The authors of the article, report no relationships that could be construed as a conflict of interest.

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