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Isolation and characterisation of enzymatic hydrolysed peptides with antioxidant activities from green tender sorghum

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ABSTRACT

The antioxidant activities of bioactive peptides isolated from green tender sorghum (GTS) protein hydrolysate were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonate) (ABTS), Fe^{2+} chelating activity and reducing power assay. Molecular mass and amino acids sequences of the purified peptides were identified using matrix assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS/MS). Molecular weight distribution of the peptides from fractions F2B (875.5 Da) and F3A (858.5 Da) revealed higher antioxidant activity with 74.19% and 77.64% of inhibition, respectively. High performance liquid chromatography (HPLC) demonstrated hydrophobic amino acids content in green tender sorghum protein isolate (GTSPI), green tender sorghum protein hydrolysate (GTSPH) (>10 kDa), GTSPH (3–10 kDa) and GTSPH (<3 kDa) was found to be higher especially, 13.8, 74.5, 100.7 and 117.7 mg/g respectively, which contributed to higher degree of free radical inhibition. Oil holding capacity of GTSPI was 3.32 mL/g, while GTSPH exhibited only 1.13 mL/g. Purified peptides were identified in sub-fractions F2A (VPPSKLSP), F2B (VAITLTMK), F2C (GLLGKNFTSK), F2D (LDSCKDYVME), F2E (HQTSEFK), F3A (VSKSVLVK) and F3B (TSVEIITSSK) with ion table for identified peptide sequences. GTS Protein produced peptides with strong free radical scavenging capacity upon enzymatic hydrolysis and can be utilized to develop health related nutraceutical ingredients.

1. Introduction

Oxidation is the key origin for the pathogenic disorders and various chronic diseases. High level of oxidative stress lead to cellular damage due to significant imbalance between free radicals and the antioxidant defence system (Rodrigues et al., 2016). Free radicals attack on bio-molecules such as proteins, nucleic acids and lipids which lead to lipid peroxidation and weakening of the antioxidant enzymes (Kepekci, Polat, Çelik, Bayat, & Saygideger, 2013). From last two decades there are number of scientific papers have been published which deals on correlation of diet and extraordinary antioxidant supplementation to maintain, or even to improve our well being (De Menezes et al., 2013). Human diet contains some nutritional and non-nutritional components, which can be carried out to mutate the threat of initial or various infuriating human ailments (McDonald, 1998, pp. 323–340). Current research investigations have drawn attention to the function and bioactivities of protein and its hydrolysates from food sources because peptides generated by hydrolysis may be used as an alternative source in the prevention and management of cardiovascular diseases. Furthermore, food proteins have been served as a substantial role in human health improvement with their well known nutritional values (Sila & Bougatef, 2016). Peptides derived from food proteins carry aromatic rings, excessive donor electrons and appropriate hydrophobic character which can be a great source of physiological relevant antioxidants. Amino acids (2–20 residues) formed antioxidant peptides and immobile in parent protein which can be released during trypsin digestion (Shahidi & Zhong, 2008). Enzymatic hydrolysis is the most reliable and an effective method to produce peptides with functional properties (Iwaniak, Minkiewicz, Darewicz, Protasiewicz, & Mogut, 2015). The optimistic properties of these functional components may not be observed in acute health conditions because the lesser amount of these active ingredients than therapeutic amounts and biologically inactive due to the interaction with other components of food matrix (Parada &

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Aguilera, 2012). Hence, to increase the amount of these molecules and their absorption directly into the blood circulation, transportation to the targeted cellular site, these bioactive components can be isolated and administered in the form of nutraceuticals (McClements et al., 2015). Numerous research papers have been published on free radical scavenging activity of hydrolysates or bioactive peptides from plant sources. Food crops like pearl millets (Agrawal, Joshi, & Gupta, 2016), oat bran (Gao, Smith, & Tsopmo, 2014), foxtail millets (Amadou et al., 2013), soybean (Abu-Salem, Mahmoud, El-Kalyoub, Gibriel, & Abou-Arab, 2013), and buckwheat protein (Tang, Peng, Zhen, & Chen, 2009) have been shown to possess bioactive peptides. In most developing countries, plant-based foods are the significant parts of our day-to-day diet.

Sorghum is one of important cereal crop for the people in different parts of Africa who live in desert and semi-arid areas. Sorghum has a well adaptation in these types of areas due to its temperature and drought tolerant capability (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). Sorghum is gluten-free hence there is an increasing interest in its consumption and utilization (Pontieri et al., 2013). It also possesses some health benefiting properties, such as anti-inflammatory, cholesterol-lowering, inhibition of human esophageal, slow digestibility and colon cancer cell growth (Moraes et al., 2012). Recently most of the studies on sorghum are related to the area of its utilization for ethanol production (Wang et al., 2012). The main component of sorghum is protein after starch. However, sorghum variety, soil condition and environmental factors affect the essential amino acids profile. Protein digestibility has been varied from 30 to 70% in different verities of sorghum. As the other major cereals, sorghum has the similar amount of protein content, but the quality of protein is inferior (Massoud & Abd El-Razek, 2011). A number studies has been conducted regarding the application of sorghum and their constituent but the identification of peptides with antioxidant activity and their amino acids composition has not been reported earlier.

Therefore, the objective of present work was to investigate the peptides with free radical scavenging potential from green tender sorghum (GTS) (*S. bicolour*) using different chromatographic techniques. Extracted protein was first hydrolysed with alcalase enzymes and then purified with ultra filtration, gel filtration and Reverse phase ultra-flow liquid chromatography **(**RP-UFLC). Finally, the sequence of antioxidant active peptide fraction was identified using matrix-assisted laser desorption/ionization- Time-of-flight-mass spectrometer (MALDI-TOF-MS/MS).

2. Materials and methods

2.1. Chemicals and reagents

Sodium hydroxide, sephadex G-25, bovine serum albumin, β-mercaptoethanol, Bradford reagent, trypsin from porcine pancreas, 1,1-diphenyl-2-picrylhydrazyl (DPPH),o-phthaldialdehyde (OPA), ferrous sulphate, sodium tetrahydroborate, sodium dodecyl sulphate, casein tryptone, 2, 2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonate) (ABTS), potassium persulphate, 1–10, phenanthroline, 2,4,6-trinitrobenzenesulfonic acid (TNBS), potassium ferricyanide, trichloroacetic acid, trifluroacetic acid, trolox, butylated hydroxytoluene and α-cyano-4-hydroxycinnamic acid were purchased from Sigma-Aldrich, India. Ethanol, methanol, hexane, acetonitrile, hydrochloric acid and formic acid were analytical grade and purchased from Merck India Pvt. Ltd. Ferrous chloride, ferric chloride, ferrozine and hydrogen peroxide were procured from Himedia. Alcalase® 2.4L, an endopeptidase from *Bacillus lichenformis* (EC number 3.4.21.14) (≥0.75 Anson units/ml) was also bought from Sigma-Aldrich, India.

2.2. Material processing and protein extraction

Green tender sorghum (GTS) was obtained from CSK Himachal Pradesh Agricultural University, Palampur, India. GTS was processed using previously described method of Agrawal et al. (2016). The extraction method of protein from GTS flour was adopted from Najafian & Babji, 2014 with some changes. Defatted GTS flour was soaked in 0.02 mol/L phosphate buffer, pH 6.5 (1:100, g:mL) and homogenized at room temperature for one hour. The solution was then centrifuged at 13,000 × g for 20 min at 4 °C. The supernatant was collected and passed through a filter (0.22 μm) from Merck Millipore, India. This isolated protein was lyophilised and put in −20 °C for further investigation.

2.3. Determination of functional properties of GTS protein

2.3.1. Solubility of protein (PS)

PS was evaluated by following the previously described method (de Andrade Ferreira, Freire, de Souza, Cortez-Vega, & Prentice, 2013). Un-hydrolyzed protein and its hydrolysate sample (0.5 g) were mixed separately with 0.1 mol/L NaCl (2 mL) and de-ionised water (48 mL) in a 100 mL beaker. The pH was adjusted with solution of 1 mol equi/L HCl and 1 mol equi/L NaOH. The solution was kept on a magnetic stirrer for 30 min followed by centrifugation at 9000 × g for 20 min at ambient temperature. Bradford assay was used to determine the total soluble protein content in collected supernatant. Following equation was used to calculate the protein solubility:

$$\text{PS}(\%) = \text{protein content in supernatant/total protein content in sample} \times 100 \quad (1)$$

2.3.2. Oil holding capacity (OHC)

OHC was determined using previously described method (de Andrade Ferreira et al., 2013). Unhydrolyzed protein and its hydrolysate sample (0.5 g) were weighed separately and mixed with soybean oil (10 mL) in centrifuge tubes and shaken for 10 min. After that, the centrifugation was performed at 8000 × g for 25 min on above mixture. The amount of oil retained by the isolates is the difference between the added and un-retained oil. The OHC was determined from following equation:

$$\text{OHC (mL/g)} = \text{oil retained (mL)/protein mass (g)} \quad (2)$$

2.3.3. Water holding capacity (WHC)

WHC was measured from previous reported method (de Andrade Ferreira et al., 2013). Dispersion of protein and its hydrolysate (1:10 g:mL) was prepared at variable pH (3–9 pH). A smooth paste was obtained by adding 0.1 mol/L sodium chloride to above dispersion. Corresponding buffer solution was mixed according to corresponding pH up to final volume of 40 mL. Stirring was performed for 15 min followed by centrifugation at 9000 × g for 25 min at ambient temperature. Bradford method was used to determine to calculate soluble proteins content in supernatant and deduced from total proteins. The WHC was calculated following the equation:

$$\text{WHC (mL/g)} = \text{amount of water retained (mL)/original mass of protein} \quad (3)$$