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Evaluation of the Oxidative Activity of Protein Isolate Extracted from Wheat Bran and Basil Seeds

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Abstract: The study was carried out at University of Basra with the aim of extracting a protein from wheat bran and basil seeds and testing it as an antioxidant and compared with butylated hydroxy toluene (BHT) and ascorbic acid outside the body of the organism from November 14, 2019 to February 1, 2020. The data was collected on antioxidant effectiveness, reducing power, scavenging of hydrogen peroxide, chelating ability of ferrous ion, 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The protein isolate extracted from wheat bran and basil seeds had an antioxidant activity of 88.15 and 85.32%, respectively and reducing power of 87.64 and 90.27%, respectively. The ability to scavenge hydrogen peroxide was 50.31 and 46.77%, respectively. The ability to remove free radicals by DPPH was 82.33 and 78.86%, respectively. The results showed that the protein isolate extracted from wheat bran and basil seeds had 58.22 and 46.72% ferrous ion chelating, receptively.

Keywords: Protein isolates, Antioxidant, Wheat bran, Basil seeds

Many synthetic antioxidants are currently commonly used which largely include butylated hydroxy toluene (BHT) and butylated toluene anisole (BHA), and many concerns have arisen about the efficacy of these antibiotics in recent years. Because of its negative effects on public wellbeing, synthetic antioxidants require accurate use (U.S. Food and Drug Administration -FAD). This is attributed to questions about its toxicity outside such thresholds (Manhiani et al 2013). Proteins serve play roles within the body of living organisms: catalyzing biochemical processes, replicating DNA, reacting to stimuli, supplying cohesion to cells and organisms, and moving molecules from one location to another (Fleurence et al 2018). In addition, proteins have the advantage of being used as antioxidants based on the type of amino acid (Zhang et al 2012). Wheat bran is a dietary fiber that has essential health functions, such as accelerating the excretion of intestinal waste, reducing the risk of intestinal and rectal cancer, decreasing blood cholesterol levels, and helping the development of beneficial bacteria in the intestine (Brennan 2005). Basil seed (*Ocimum bacilicum* L.) belongs to the Labiate family and is native to Asia (Iran and India), Africa and America. Using various parts of basil in herbal medicine for the treatment of a wide variety of diseases, experimental experiments have shown that basil contains oil compounds that act as potent antioxidants anticancer, antiviral, antimicrobial properties and prevention of cardiovascular disease (Bozin et al 2006, Abi Beaulah et al 2014). Proteins are commercially classified into animal proteins and plant proteins and, owing to the high price of animal proteins, require protein processing from unusual (untapped) sources (Arshad et al 2007). The aim of the current study is to extract proteins isolated from wheat bran and basil seeds and to determine their potential as natural antioxidants.

MATERIAL AND METHODS

Extraction of protein isolates: The protein was extracted according to the method mentioned by AL-Sadoon and Naji (2016) with some modifications. Extracted with extraction solution (1N) from sodium hydroxide (1:20) and protein was deposited using hydrochloric acid (HCL) (1N). Protein was dried using a rowing device and stored in plastic bottles in the refrigerator.

Antioxidant effectiveness: Estimated by method of Al-Moussawi and Al-Halfi (2012). The antioxidant capacity was determined using the linoleic acid system. The reaction mixture consists of 4.1 ml of linoleic acid at a concentration of 2.5 percent in ethanol, 4 ml of alcohol extract, 8 ml of 0.05 molar phosphate buffer solution 7 and 3.9 ml distilled water. One ml of 80 Tween at a concentration of 0.05 percent ethanol. The mixture was incubated at 40°C for 24 hours. The degree of oxidation was determined by the thiocyanate method, mixing 0.1 ml of the mixture, adding 9.7 ml of 75 percent ethanol and 0.1 ml of ammonium thiocyanate at a concentration of 30 percent. The 0.1 ml of ferric chloride at a concentration of 0.02 M in 3.5 percent hydrochloric acid three minutes later was added. Absorption was measured at a wavelength of 500 nm, butylated hydroxy toluene (BHT) and ascorbic acid were used for comparison. The control sample was prepared in the same way above except for 4 ml of ethanol instead of plant extract. The percentage inhibition of

linoleic fatty acid peroxides was calculated using the following formula:

$$\%\text{Antioxidant Effectiveness} = 100 - \frac{\text{Sample absorbance reading}}{\text{The absorbance reading of the control}} \times 100$$

Reducing Power: The method of Al-Moussawi and Al-Halfi (2012) was followed, which included mixing 2.5 ml of alcoholic proteins isolate extract with 2.5 phosphate buffer solution 200 mM with pH 6.6 and 2.5 ml of potassium ferricyanide solution (1 %) bosom. The mixture at a temperature of 50C° was kept for 20 minutes after the reaction was stopped by adding 2.5 ml of trichloro acetic acid (10 %). The central centrifugation of the mixture was carried out at a speed of 2000 rpm for 10 minutes. Separate the top layer of the solution and add 5 ml of distilled water and 1 ml of ferric chloride (0.1 %). The measurement of absorption was at a wavelength of 700 nm. The control sample was prepared by adding all the previous materials except the addition of 2.5 ml of ethanol instead of the alcohol extract of the protein isolate.

$$\%\text{Reducing Power} = 100 - \frac{\text{Sample absorbance reading}}{\text{The absorbance reading of the control}} \times 100$$

Scavenging of hydrogen peroxide: Determined according to Türkoğlu et al 2010) by taking 1-5 mg ml^{-1} of protein isolate and 0.6 ml of 0.002 M H_2O_2 prepared in 0.1 M phosphate buffer at pH 7.4. Kept at laboratory temperature for 10 min, measured absorbance along 230 nm, the equation was used below:

$$\%\text{Capability of capture} = \frac{\text{Absorbance of the control sample}}{\text{Absorbance of the control}} \times 100$$

Free radical scavenging (DPPH): The method of DPPH tests for protein isolate relative to industrial and natural antioxidant was followed (AOAC 2008). The 3 g of isolated protein, synthetic antioxidant (BHT) and natural antioxidant (ascorbic acid) added to 30 ml of methanol The 60 microliters of the previous extract sample 3 was added 3 ml of DPPH solution and put in a bath of water for 20 minutes at a temperature of 25 m. The controlled sample contained 60 microliters of distilled water and DPPH solution was applied. The absorption of the spectrophotometer was estimated at wavelength of 517 nm and the behavior of free radical sweeping was determined.

$$\%SA = 100\left(\frac{1 - AC}{AD}\right)$$

Chelating ability of ferrous ion: The method of Gülçın et al (2003) was used. Mixed 0.4 ml of alcoholic extract with 0.4 ml of ferric chloride 2 ml molar with 0.4 ml of 8-Hydroxyquinoline at a concentration of 5 molars (prepared ethanol) incubate the mixture at room temperature in a dark place for 10 minutes. Absorption was measured along 562 nm wavelength and the results were compared with (EDTA 2Na) the control sample.

$$\%\text{Chelating ability of Ferrous ion} = \frac{\text{Sample absorbance reading}}{\text{The absorbance reading of the control sample}} \times 100$$

Statistical analysis: Data were statistically analyzed using the SPSS statistical program (SPSS 2018) .

RESULTS AND DISCUSSIONS

Antioxidant effectiveness: The antioxidant effectiveness of the industrial compound (BHT) and ascorbic acid was 94.18 and 92.12 percent respectively as compared to the basil seed isolate. Both did not differ significantly from wheat bran protein isolate (Table 1). This may be because of hydrophobic amino acids, as shown that hydrophobic amino acids have antioxidant efficacy. Peptides that are high in amino acids hydrophobic properties such as cysteine, histidine, phenylalanine, tryptophan, tyrosine, and methionine possess strong as antioxidant efficacy (Ren et al 2008).

Reducing Power: The reducing power of protein isolates extracted from wheat bran and basil seeds, showed non-significant difference. The wheat bran protein isolate had less antioxidant effect (87.64%) than basil seed isolate (90.27%), while the antioxidant effectiveness of the industrial compound (BHT) and ascorbic acid was 93.45, 89.91 respectively. The reductive activity is used as a significant indicator of the antioxidant capacity. The activity of the reducing force is determined by reducing the Ferricyanide / Fe^{+3} complex to Fe^{+2} relying on strength samples that have a higher reducing power are more likely to give an electron and form a stable substrate and thus impede the chain reaction of radicals. Samples that have a higher reducing power are more likely to give an electron and form a stable substrate and thus impede the chain reaction of radicals . The presence of amino acids such as leucine, tryptophan, histidine, isoleucine, tyrosine, methionine and lysine in protein contributes to the strong reducing energy (Qian et al 2008).

Scavenging hydrogen peroxide: The antioxidant effectiveness of protein isolates extracted from wheat bran and basil seeds, indicated significant difference (Table 1) . The wheat bran protein isolate had an antioxidant effect of approximately 50.31%, while the basil seed isolate had an antioxidant function of approximately 46. 77%, while the antioxidant effectiveness of the industrial compound (BHT) and ascorbic acid was approximately 87.41, 82.19 respectively. This method was very important for the safety of cellular structures, as hydrogen peroxide was a very important compound due to its ability to infiltrate cell membranes within cells. It is also a harmful material for cells because it increases the root of hydroxyl, which has toxic

Table 1. Antioxidant activity of the protein isolate (Mean ± standard deviation)

Treatments	Antioxidant effectiveness (%)	Reducing power (%)	Scavenging hydrogen peroxide (%)	DPPH (%)
BHT	94.18 ± 0.22[a]	93.45 ± 0.11[a]	87.41 ± 0.06[a]	83.76 ± 0.13[a]
Ascorbic acid	92.12 ± 0.16[a]	89.91 ± 0.24[a]	82.19 ± 0.10[a]	75.52 ± 0.12[a]
Isolated wheat bran	88.15 ± 0.31[ab]	87.64 ± 0.10[a]	50.31 ± 0.37[b]	82.33 ± 0.46[a]
Isolated basil seeds	85.32 ± 0.08[b]	90.27 ± 0.33[a]	46.77 ± 0.21[b]	78.86 ± 0.63[a]

Means in the same column with different letters show significant differences ($p<0.05$).

Table 2. Ferrous ion chelating for protein isolate (Mean ± standard deviation)

Treatments	Ferrous ion chelating (%)
EDTA 2Na	75.84 ± 0.07[a]
Isolated wheat bran	58.22 ± 0.60[b]
Isolated basil seeds	46.72 ± 0.20[b]

Means in the same letters show non-significant differences ($p<0.05$)

effects. In addition, the inhibited amount of enzymes can be oxidized by the primary groups (SH-) They provide a biological benefit for cells to regulate the level of hydrogen peroxide within the cell The low molecular weight 1.5 K Da short-chain peptides containing the amino acid sequence Phe-Tyr-Tyr-Asp-Trp have the ability to reduce the oxidative effect of the peroxide Hydrogen (Chai et al 2013).

Free radical scavenging (DPPH): The free radical scavenging of protein isolates extracted from wheat bran and basil seeds, appearance non-significant difference. The wheat bran protein isolate has an anti-free radical effect of approximately ٨٢.٣٣%, while the basil seed isolate has an anti-free radical effect of approximately ٧٨.٨٦%, while the industrial compound (BHT) and ascorbic acid has an anti-free radical effect of was approximately ٨٣.٧٦, ٧٥.٥٢ respectively. The explanation for the ability of the protein isolate to scavenge the free root is correlated with the reductive force of the protein isolate, since the reductive intensity is increased by the peripheral amino acids that generate the electron and interact with the free radical (Zeng et al 2014).

Ferrous ion chelating: The ferrous ion chelating of protein isolates extracted from wheat bran and basil seeds showed a significant difference (Table 2). The wheat bran protein isolate had the ability to bind ferrous ion (58.22%) while the basil seed isolate had the ability to bind ferrous ion function of approximately to 46. 72%, The effectiveness of the industrial compound (EDTA 2Na) was approximately 75.84. The reason the protein isolate has the capacity to bind to the ferrous ion can be traced back to its containment of cyclic amino acids and hydrophobic amino acids (Ajibola et al 2011).

CONCLUSIONS

Some plant wastes, such as wheat bran and basil seeds, have effective compounds that have biological properties as natural antioxidants that can be used as healthy food supplements for humans or animals. It is also possible to use protein isolates as natural preservatives to support manufactured food products.

REFERENCES

AOAC 2000. Official Method of Analysis. Horwitz WL, George W and Latimer Jr. (Eds.). 18th ed. Washington, D.C.*, Association of official Agricultural Chemists*: 2200pp.

Abi Beaulah G, Mohamed Sadiq A, Sivakumar V and Jaya Santhi R 2014. Cardioprotective activity of methanolic extract of Croton sparciflorus on isoproterenol induced myocardial infarcted wistar albino rats. *Journal of Medicinal Plants* **2**(6): 01-08.

Ajibola CF, Fashakin JB, Fagbemi TN and Aluko RE 2011. Effect of peptide size on antioxidant properties of African yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. *International journal of molecular sciences* **12**(10): 6685-6702.

Al-Moussawi AHJ and Al-Halfi SAH 2012. Isolation identification and determination the antioxidant activity of phenolic compound to some plants extracts. *Journal of University of Karbala*. 2nd Sci. Conference Faculty Agriculture: 1218-1228. (In Arabic). https://www.iasj.net/iasj?func=fulltext&aId=92740

AL-sadoon AA and Naji EZ 2016. Evaluation of chemical composition and study some of the functional properties of the flour and isolated protein from the Iraqi wheat industry residue. *Tikrit Journal for Agricultural* **16**(4): 160-175.

Arshad MU, Anjum FM and Zahoor T 2007. Nutritional assessment of cookies supplemented with defatted wheat germ. *Food Chemistry* **102**(1): 123-128.

Bozin B, Mimica-Dukic N, Simin N and Anackov G 2006. Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of Agricultural and Food Chemistry* **54**(5): 1822-1828.

Brennan CS 2005. Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition and Food Research* **49**(6): 560-570.

Chai HJ, Chan YL, Li TL, Shiau CY and Wu CJ 2013. Evaluation of lanternfish (*Benthosema pterotum*) hydrolysates as antioxidants against hydrogen peroxide induced oxidative injury. *Food Research International* **54**(2): 1409-1418.

Fleurence J, Morançais M and Dumay J 2018. Seaweed proteins. In Proteins in food processing (pp. 245-262). Woodhead publishing. DOI: 10.1533/9781855738379.1.197

Gülçın İ, Oktay M, Kıreçcı E and Küfrevıoğlu Öİ 2003. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chemistry* **83**: 371-382.

Manhiani PS, Northcutt JK, Han I, Bridges WC and Dawson PL 2013. Antioxidant activity of carnosine extracted from various poultry tissues. *Poultry Science* **92**(2): 444-453.

Qian ZJ, Jung WK and Kim SK 2008. Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, *Rana catesbeiana* Shaw. *Bioresource Technology* **99**(6): 1690-1698.

Ren J, Zhao M, Shi J, Wang J, Jiang Y, Cui C and Xue SJ 2008. Purification and identification of antioxidant peptides from grass carp muscle hydrolysates by consecutive chromatography and electrospray ionization-mass spectrometry. *Food chemistry* **108**(2): 727-736

SPSS 2018. *Statistical Packages of Social Sciences*. Version 24 for Windows. SPSS. Inc.

Türkoğlu S, Çelik S, Türkoğlu I, Çakılcıoğlu U and Bahsi M 2010. Determination of the antioxidant properties of ethanol and water extracts from different parts of (*Teucrium parviflorum*) Schreber. *African Journal of Biotechnology* **9**: 6797-6805.

Zeng Y, Guan Y, Han W and Sun Y 2014. Antioxidant peptides from freshwater clam extract using enzymatic hydrolysis. *African Journal of Food Science* **8**(3): 148-154.

Zhang HJ, Zhang H, Wang L and Guo XN 2012. Preparation and functional properties of rice bran proteins from heat-stabilized defatted rice bran. *Food Research International* **47**(2): 359-363.

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