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ANTIOXIDANT PROPERTIES OF SEER FISH MEAT

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ABSTRACT

Antioxidant compounds found in our diet act as a protective shield to our body against diseases. The antioxidant and biochemical properties of enzymatically hydrolyzed seer fish (*Scomberomorus commerson)* protein were studied. The muscle of seer fish was hydrolyzed with three different commercially available enzmymes pepsin, trypsin and papain. The moisture, ash and protein content were quantified. The antioxidant properties of the hydrolysates were determined by using LPO inhibition assay, DPPH radical scavenging assay and ferric reducing power activity. The free radical scavenging potential is further confirmed by ESR spectral analysis through DPPH quenching assay and hydroxyl radical scavenging potential. All the three hydrolysates exhibits antioxidant activities however the pepsin fraction yielded more quantity of hydrolysate and readily quenches the free radicals efficiently than that of the fraction of trypsin and papain.

KEYWORDS : Lipid Peroxidation (LPO), 1, 1- diphenyl-2 picrylhydrazyl (DPPH), Electron spin resonance (ESR), Antioxidants, Enzymes, Hydrolysates, Scavenging potential, Free radical.

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I. INTRODUCTION

People must breathe in **oxygen** to live. Continuously on the move in the blood stream and transported to every cell, oxygen is necessary for all essential bodily functions. Oxidation is an essential reaction in all living organisms. However, a small amount of this oxygen gets loose and produces unstable by-products called free radicals. Body processes, such as metabolism, as well as environmental factors, like excess exposure to the sun, cigarette smoke and air pollution, excess alcohol, and even x-rays can produce free radicals. The formation of free radicals and other reactive oxygen species is unavoidable during the oxidative metabolic process.

A free radical is an atom or molecule with an unpaired electron. Unpaired electrons make for very unstable, highly reactive atoms and/or molecules. Researcher and writer Michael Dye explains it this way: "This is a very hazardous, unnatural and unstable state, because electrons normally come in pairs. This odd, unpaired electron in a free radical causes it to collide with other molecules so it can steal an electron from them, which changes the structure of these other molecules and causes them to also become free radicals. This can create a self-perpetuating chain reaction in which the structures of millions of molecules are altered in a matter of nanoseconds (a nanosecond is a billionth of a second).It is now recognized that free radicals are contributing causes to more than 60 diseases.

Effects of Excess of Free Radicals

Free radicals damage DNA, RNA, Proteins, and enzymes, Lead to the formation of tumors and cause cancers, Cardiovascular diseases, Nervous disorders, Premature ageing, Parkinson's and Alzheimer's Diseases, Rheumatic and pulmonary disorders.

Antioxidants test

Antioxidant means "against oxidation." Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken (Dekker *et al.,* 2001). After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats, and oil.

Functions of Antioxidants:

i. Antioxidants such as Vitamin C and Vitamin E boost our immune system (Wintergerst *et al.,* 2006).
ii. Certain phytochemicals have beneficial effect on heart diseases.
iii. Antioxidants lower the level of low-density lipoprotein cholesterol, thus preventing plaque deposition in the blood vessels.
iv. It is beneficial in cancer prevention (Bartlett and Eperjsei, 2003).
v. Antioxidants neutralize substances that can damage the genetic material by oxidation.

Compounds that function as antioxidants

Vitamin C, Vitamin E, Phytochemicals, Carotenoids, Flavonoids, Phenols, Minerals.

II. MATERIALS AND MEHODS

Fish Protein Hydrolysate

Dissected fish parts, enzymes, mortar and pestle, beaker, Water bath, magnetic stirrer, centrifuge tubes, sodium di-hydrogen phosphate and Di-sodium hydrogen phosphate, glycine

The muscle of seer fish was taken. The sample was thawed and 30g of the sample was exactly weighed. The weighed sample was ground into a paste using mortar and pestle. The paste was then taken into a 1000ml beaker and 0.3g of the enzyme was added and 100ml of corresponding buffer was added. The pH was checked and the contents were stirred with a magnetic stirrer. This set up was incubated at 37^{o}C for 6 hours. After 6 hours, the beaker was placed in a water bath at 100 ^{o}C for 5 minutes to stop the enzyme activity. The contents were then transferred into a centrifuge tube and were centrifuged at 10000 rpm for 15 minutes. The debris was discarded and the supernatant obtained is the fish protein hydrolysate. The fish protein hydrolysates were lyophilized to obtain powdered sample.

Antioxidant Assays

(a) Lipid Peroxidation Inhibition Assay*:*

Lyophillized samples, Potassium di-hydrogen phosphate, di-potassium hydrogen phosphate, ethanol, distilled water, ferrous chloride, Ammonium thiocyanate, Linoleic acid.

In vitro lipid peroxidation inhibition activities of the samples were determined by usin the methods of Osawa and Namiki (1985). 1mg of the fish protein hydrolysate was dissolved in 5ml of 50mM phosphate buffer (pH-7.0). 65µl of linoleic acid was added to 5 ml of 99.5% ethanol and this linoleic acid solution was mixed with the above solution and up to 12.5ml using distilled water. This solution was incubated in dark at 45 C for 7 days.

Spectrophotometer Readings

100 µl of the incubated sample was taken to which 4.7ml of 75% ethanol is added. 100 µl of 30% ammonium thiocyanate was added and mixed well. 100 µl of 20mM ferrous chloride was added. As soon as ferrous chloride is added, color formation occurs and the intensity of the color is measured at 500nm.

(b) DPPH Radical Scavenging Assay*:*

Fish protein hydrolysate, 1, 1- diphenyl-2.DPPH radical scavenging activity was measured by the method given by Wu (2003). Different concentrations of the sample such as 0.5mg, 1.0mg, 1.5mg, 2.0mg, 2.5mg and 3.0mg were taken in different test tubes and were dissolved in 1ml of ethanol. 4ml of 0.004% DPPH solution in methanol solution was added to each test tube. The test tubes were incubated at room temperature for 30 minutes. The absorbance was read at 517nm. DPPH solution was considered as control. The percentage inhibition was calculated as follows.

$$I\% = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

ESR Measurement

(a) Scavenging Effect on DPPH Radical

The DPPH scavenging activity was determined using ESR spectrometer using the methods of Nanjo (1996). A 500µl peptide solution was added to 500µl DPPH in ethanol solution. After mixing vigorously for 10 seconds, the solution was transferred into a capillary tube, and the scavenging activity of peptide on DPPH radical was measured using an ESR- Spectrometer. The spin adduct was measured on an ESR spectrometer exactly 2 minutes later.

(b) Hydroxyl Radical Scavenging Activity

Hydroxyl radicals were generated by iron-catalyzed Haber-Weiss reaction and the generated radicals rapidly reacted with nitrone spin trap DMPO. The hydroxyl radical scavenging activity of the sample was determined by the methods of Rosen (1984). Peptide sample was mixed with 0.3M DMPO, 10mM ferrous sulphate and 10mM Hydrogen peroxide in a phosphate buffer solution of pH 7.2. This mixture was then transferred into a 100µl capillary tube. After 2.5minutes the ESR spectrum was recorded using ESR Spectrometer.

Proximate Analysis

(a) Moisture Content:

The moisture content of the fishes was determined according to the methods of AOAC

(1995). 5g of the raw sample was weighed and incubated in an incubator at 100º C for 48 hours. After 48 hours the samples were removed and weighed. The difference in weight gives the moisture content of the sample.

(b)Ash Content:

The ash content of the fishes was determined according to the methods of AOAC (1995). The dried samples were weighed and placed in crucibles. These crucibles were then placed in a muffle furnace at 420º C for 3 hours. The crucibles were removed and the ash obtained was weighed.

(c)Protein Content:

Bovine serum albumin (BSA), alkaline mixture, Folin's reagent. The protein content of the seer fish was determined by the method of Lowry (1951). The stock solution was prepared by dissolving 1mg of Bovine serum albumin in 1ml of distilled water. Different concentrations of the standard such as 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml were taken in different test tubes and made up to 1ml using distilled water. 4.5ml of alkaline copper sulphate reagent (analytical reagent) was added and the solutions were mixed well. The solutions were incubated at room temperature for 10 minutes. 0.5 ml of reagent Folin Ciocalteau solution (reagent solutions) was added to each tube and incubated for 30 min. the absorbance was measured at 540nm. The absorbance was plotted against protein concentration to get a standard calibration curve. The protein content of the fish sample was determined by using this standard curve.

III RESULTS AND DISCUSSION

The muscle seer fish was hydrolyzed using pepsin, papain and trypsin enzymes. The hydrolysates obtained were examined for their antioxidant activities using assays such as DPPH radical scavenging assay, lipid peroxidation assay, reducing power hydroxyl radical and DPPH radical scavenging assays using ESR spectrometer. All the hydrolysates exhibiting antioxidant activity however the pepsin hydrolysate of the seer has higher antioxidant *activity.*

The moisture, ash and protein content of the fish were determined. The antioxidant peptides thus obtained can be further purified and the activity of these antioxidant peptides can be examined against cell lines and their in vivo antioxidant properties can be explored using animal models for future drug discovery efforts. Hence the future research must be focused in developing novel methods to apply these antioxidants and other bioactive compounds for the human health promotion.

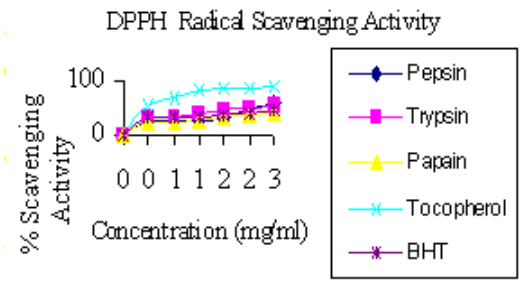

Figure.1

ESR spectrum showing the DPPH radical scavengingactivity of pepsin hydrolysate of seer fish muscle

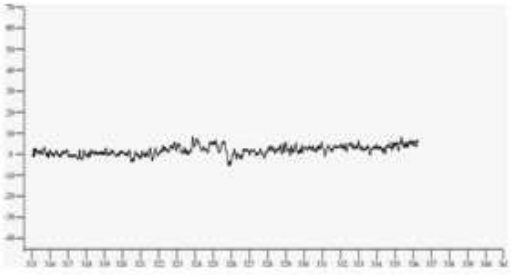

Figure.2
ESR spectrum showing the DPPH radical scavengingactivity of Trypsin hydrolysate of seer fish muscle

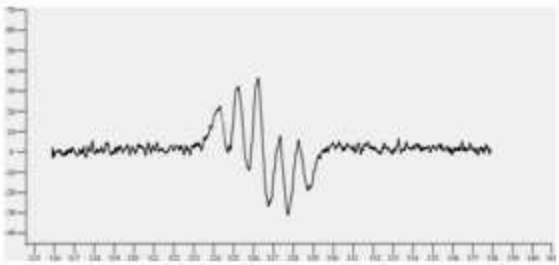

Figure.3
ESR spectrum showing the DPPH radical scavenging activity of papain hydrolysate of seer fish muscle

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