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Effects of gamma irradiation on antioxidant activity of Ergosan

M. Heidarieh[1*], A.Borzouei[1], S. Rajabifar[1], F. Ziaie[1], Sh. Shafiei[2]

[1]Agricultural, Medical and Industrial Research School, Karaj, Iran
[2]Departments of Clinical Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Background: The approaches chosen for control of Outbreaks of infectious diseases in Aquatic farming industry include improvement of environmental conditions, stocking of specific pathogen free (SPF) brood stockings, and application of vaccines and immunostimulants. Despite numerous studies on the effects of Ergosan on immune system of aquatic animals, there is no data available on antioxidant activities of Ergosan. The aim of the present study was to investigate and evaluate the radical scavenging activities of Ergosan extract by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, and the possible effects of gamma irradiation on its assumed radical scavenging activities. ***Materials and Methods:*** Ergosan was irradiated with gamma rays (10, 20, 30, 40 and 50 kGy), and their structural changes and antioxidant activities were investigated by UV absorbanceand DPPH (1,1-diphenyl-2-picrylhydrazyl) assays, respectively. ***Results:*** The gamma irradiation decreased the average pH of irradiated Ergosan, and UV spectra of irradiated product showed increase in the number of carboxyl groups and double bonds. Our results showed that 30 kGy irradiated Ergosan suspension had significant higher level of antioxidant activity in comparison with non-irradiated Ergosan ($P<0.05$). Also, the reducing power values of 30 and 50 kGy irradiated Ergosan were higher than that of non-irradiated ($P<0.05$) and the other doses of irradiation couldn't make any significant difference in reducing power of Ergosan. ***Conclusion:*** Results indicate that the 30 kGy irradiated Ergosan might be an appropriate candidate for the use in aquatic animal diets as a natural antioxidant agent besides its immunostimulant role. **Iran. J. Radiat. Res., 2012; 9(4): 245-249**

Keywords: *Ergosan, gamma irradiation, antioxidant activity, reducing power.*

INTRODUCTION

Outbreaks of infectious diseases cause significant economic loss in the Aquatic farming industry [(1)]. The approaches chosen for control of pathogenic agents include improvement of environmental conditions, stocking of specific pathogen free (SPF) brood stockings, and application of vaccines and immunostimulants [(2, 3)]. Many studies have evaluated the effects of different immunostimulants on aquatic animals. Ergosan, an algine based immunostimlant (Schering Plough Aquaculture, UK), has been shown to improve the function of phagocytic cells located in the head kidney [(4, 5)]. In a study by Peddie *et al.* (2002) [(6)] intraperitoneal injection of 2.5 mg /kg Ergosan in the rainbow trout increased the number of neutrophils, degree of respiratory burst of phagocytes and production of interleukins and chemokines while no effect was observed on lysozyme and protease activity 7 days post-administration. In addition, it has been shown that the mechanism of alginates on the fish immune system may improve the oxygen transfer through the cell membrane of lymphocytes and macrophages, raising metabolic activity, which results in improved disease resistance and increased capacity for renovation of injured tissues [(6,7)]. In another research by Heidarieh *et al.* (2010) Ergosan significantly enhanced the body weight and also increased survival rate of juvenile shrimp which were experimentally infected with Vibrio harveyii and WSSV [(8)]. Despite numerous studies on the effects of Ergosan on immune system of aquatic animals, there is no data available on antioxidant activities of Ergosan. A variety of free radical scavenging antioxidants has been found in

Corresponding author:
Dr. M. Heidarieh,
Agricultural, Medical and Industrial (AMIRS-NSTRI), Research School, Karaj, Iran.
Fax: *+98 261 4411107*
E-mail: *mheidarieh@nrcam.org*

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marine algae [9, 10]. The aim of the present study was to investigate and evaluate the radical scavenging activities of Ergosan extract by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, and the possible effects of gamma irradiation on its assumed radical scavenging activities.

MATERIALS AND METHODS

Preparation of Ergosan suspension

Preparation of Ergosan suspension was performed as previously described by Scott Peddie *et al.* Ergosan (Schering Plough Aquaculture, UK) is composed of 0.002% unspecified plant extract, 1% alginic acid from Laminaria digitata, and 98.998% algal based carrier. Ergosan (Supplied in powdered form) was suspended in sterile 0.15 M phosphate buffered saline (pH 7.2) to reach the appropriate concentration and then sonicated on ice for 30 min [6].

Gamma irradiation

The Ergosan solution was irradiated with a gamma cell instrument model PX-30 – IssIedovapel (Russia) at a dose rate of 0.22 Gy/sec in, Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, Iran.

The applied dose levels were 10, 20, 30, 40 and 50 kGy. Dosimetry was performed with Fricke reference standard dosimetry system and after irradiation process the samples were stored at 4 ℃ for further experiments.

Irradiated Ergosan solutions were measured at 25°C by using a spectrophotometer (Cecil CE- 2021). The UV spectra were recorded between 200–500 nm [9].

DPPH radical scavenging activity

The free radical scavenging activity of Ergosan samples was measured by DPPH method previously described by Brand- Williams *et al.* with minor modifications [11]. Briefly, 100 µl of each sample was added to 2 ml DPPH ethanol solution (100 mM) in a test tube. After incubation at 37°C for 30 min, 1 ml chloroform was added and centrifugation at 3000 g for 5 min was performed. The absorbance of clear solution was determined at 517 nm using spectrophotometer. 100 mM DPPH ethanol solution was used as control. DPPH radical scavenging activity was calculated according to the following equation:

$$\textit{Scavenging activity}\ (\%) = [(\textit{absorbance of the control} - \textit{absorbance of the sample}) / \textit{absorbance of the control}] \times 100.$$

Reducing power

According to the method previously described by Oyaizu (1986) [12] the reducing power of polysaccharides was determined. 1mL of irradiated Ergosan suspensions were mixed with 2.5mL of 0.2M sodium phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide ($K_3Fe(CN_6)$) and incubated in a water bath at 50°C for 20min. then 2.5mL of 10% trichloroacetic acid added to each sample. The mixtures were then centrifuged at 750×g using a centrifuge (Eppendorf, 5417 R) for 5min at 25 ℃. 5mL of the supernatant was mixed with 5mL distilled water and 1mL of 1% ferric chloride. The absorbance of the final mixtures was measured at 700 nm. Increase in absorbance was considered as a measure of the reducing power of irradiated Ergosan suspensions [9, 12].

Statistical analysis

All the data were statistically analyzed by one-way analysis of Variance (ANOVA) and Tuky's HSD test multiple comparisons of the means using EXCEL software. The level of significances were $P<0.05$.

RESULTS AND DISCUSSION

Effect gamma radiation on DPPH radical scavenging activity

We measured the radical scavenging

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activity of irradiated and non-irradiated Ergosan using DPPH assay. The total antioxidant activity of each group is presented in figure 1. According to the Tuky's HSD test, there was a significant higher level of total antioxidant capacity in irradiated samples of Ergosan in comparison to non-irradiated ($P<0.05$). It was also observed that the 30 kGy irradiated Ergosan is more potent to reduce the stable radical DPPH in comparison with other treatments ($P<0.05$). We also measured reducing power of irradiate and non-irradiated Ergosan (figure 2). Only the reducing power values of 30 and 50 kGy irradiated Ergosan were higher than that of non-irradiated ($P<0.05$) and the other doses of irradiation couldn't make any significant difference in reducing power of Ergosan.

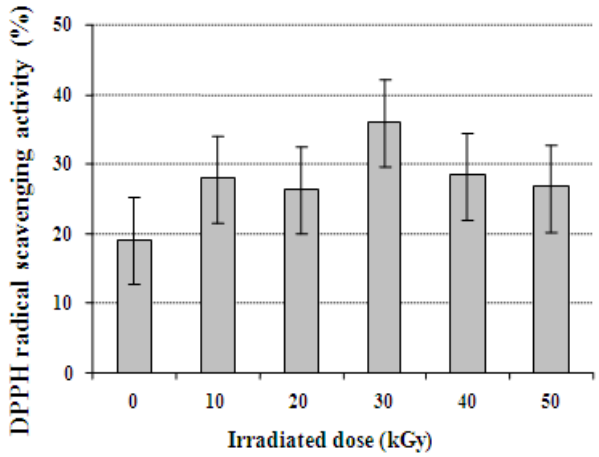

Figure 1. Effects of gamma doses on DPPH radical scavenging activity.

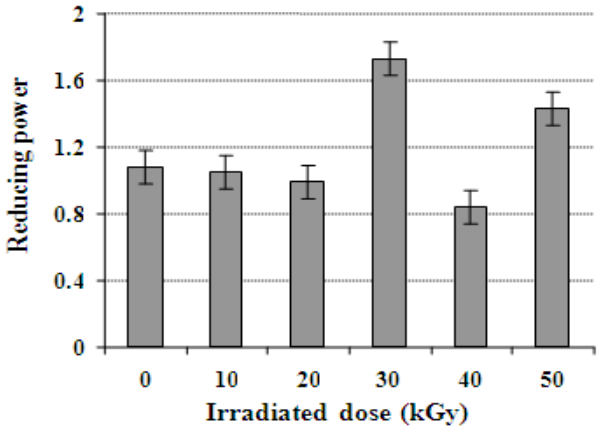

Figure 2. Effects of different gamma doses on reducing power.

Antioxidants are particularly important at sites of infection or in immunologically active tissues where activated phagocytic cells (as part of the innate immune response) release reactive oxygen species (ROS) into the surroundings in the process termed the respiratory burst [13, 14]. Innate immune system, Enzymatic and non-enzymatic antioxidant are the most important biological defences against environmental stress [15]. In the past decade, major changes made in aquatic animals feed composition (especially salmon diets) towards high lipid feed demand an increase of antioxidant supplements.

DPPH radical scavenging activity was already reported in the crude fucoidan from Scytosiphon lomentaria [10, 16]. There are documentations about the use of brown algae (Ergosan) as an immunostimulant agent in aquatics diet [7, 8, 14 and 17] but so far there is no report about the antioxidant activity of it.

Our research indicates that Ergosan has the potential in scavenging free radicals and can be a vital source of antioxidant. Our results showed that 30 kGy irradiated Ergosan suspension have significant higher level of antioxidant activity in comparison to non-irradiated Ergosan (figure 1).

UV absorbance assay

The UV spectra of the irradiated and non-irradiated Ergosan are shown in figure 3. The peak UV absorption of all samples was at ~300 nm except for 30 kGy irradiated one. The data showed gamma irradiation decreased pH of Ergosan suspensions shown in figure 4. The decreasing pH of the irradiated Ergosan has significant difference in comparison with non-irradiated Ergosan ($P<0.05$).

The structural changes of Ergosan corresponding at UV peak at ~300 nm could be due to formation of carboxyl group in irradiated form. Gamma irradiation also decreased pH of Ergosan significantly, that it confirms the formation of carboxylic group. These results are agreement with

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Nagasawa *et al.* (2000) who suggested the increase in the absorbance at 300 nm could be assigned to double bonds of polysaccharides formed after main chain scission and/or hydrogen abstraction reaction by irradiation [18]. Similarly, Ulanski and Rosiak (1992) reported that the absorbance at ~250 attributed to carbonyl group was increased with increasing radiation dose [19].

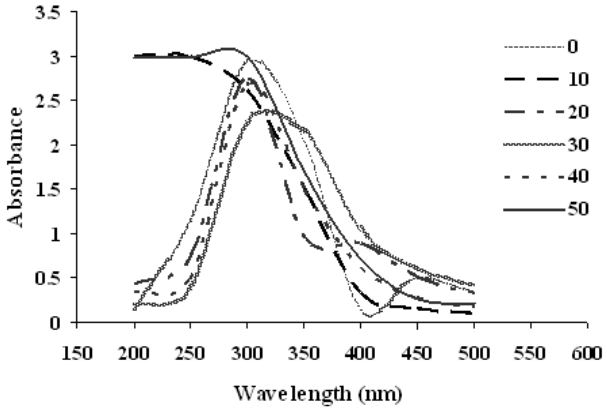

Figure 3. UV spectra of Ergosan with different doses of gamma irradiation.

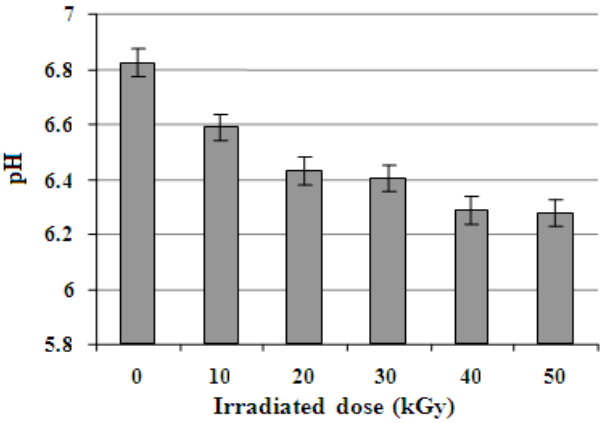

Figure 4. Effect of different gamma doses on pH of Ergosan solution.

In conclusion results indicate antioxidant properties of Ergosan and that gamma irradiation could be an effective method for increasing antioxidant activity of this product. Also from the obtained results it can be deduced that the 30 kGy irradiated Ergosan might be an appropriate candidate for use in aquatic animal diets as a natural antioxidant agent besides its immunostimulant role.

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