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The Effect of Time and Dose from Gamma Irradiation (Cesium-137) on Protein Levels and Meat Color Index of Cork Fish (Channa Striata)

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ABSTRACT

The study aimed to determine the effect of time and dose of gamma-ray irradiation on protein levels and storability based on the red color index of cork fish (channa striata). The results showed that the longer the irradiation time and the larger the dose given, the higher the protein level and absorbance value produced. The method used was UV-Vis spectrophotometry to determine protein levels and Image-J software to see the shelf life based on the red color index. The sample consisted of control, irradiated with irradiation time of 35 minutes (6 mGy), 40 minutes (7 mGy) and 45 minutes (8 mGy) and an irradiation distance of 150 cm. The protein levels at control time, which were recorded at 35, 40, and 45 minutes, were 0.138%, 0.187%, 0.276%, and 0.347%, respectively. These results suggest that there may be a correlation between time and protein levels. Further analysis may be needed to fully understand the implications of these findings. The percentage close to stable on the first day with percentages of 1.54%, 1.66%, 1.76%, 2.50%. Additionally, it seems that a higher dose of irradiation may also result in a higher protein content and absorbance value.

Keywords: *Cesium-137, Cork Fish, Irradiation, Protein, UV-Vis Spectrophotometer*

Introduction

Gamma irradiation is an induced mutation therapy using gamma rays. Mutations are the main source of all genetic variation and provide the raw materials for evolution. The evolution of living things cannot occur without mutations. One or more new special traits can be added or removed by mutation without affecting the existing traits as a whole [1]. Ionizing radiation energy is a specific type of electromagnetic energy used in food irradiation. Ionizing radiation is the energy of radiation that bounces electrons off atoms. This type of radiation transforms neutral atoms into positive ions, atoms that have lost electrons. The World Health Organization (WHO) states that irradiation is a safe method of preserving food throughout the world. WHO also states that food which is irradiated in certain doses does not harm nutrition and does not pose a risk of poisoning [2]. Indonesian Food and Drug Authority (BPOM RI) in 2008 recommended the total dose of radiation absorbed by fish products should not be more than 10 kGy. The choice of radiation dose is very important so that the radiation cycle becomes efficient and productive.

A dose of 5 kGy is the maximum dose that can be used on fish and shellfish to reduce the number of certain pathogenic microorganisms. The maximum absorbable dose of fish and shellfish products for shelf-life extension is 3 kGy. Meanwhile, according to several studies that moderate doses of 2-10 kGy can be used to kill insects, parasites, pathogenic microbes, fungi, and yeast [3]. In addition, irradiation time is also defined as the time needed to reach the desired absorbed dose. The dose level of the radioactive substance has a significant impact on the irradiation duration. In addition to the dose rate, additional elements that determine the length of radiation exposure, such as attenuation of radiation intensity [4]. Basically, the color of the meat is influenced by the amount of muscle myoglobin, which is the color pigment present in the animal's muscles. The color intensity increases from purple to dark red as the myoglobin level increases. Color is an indicator of a physico-chemical change. Color change during storage time is an important quality measure. Thus, color analysis has been widely used to identify color variations in products during storage [5].

One of the methods that can be used to analyze protein levels is to use the UV-Vis spectrophotometry method [6]. A spectrophotometer is used together with a method for analyzing near ultraviolet (190-380) nm and visible ultraviolet (380-780) nm electromagnetic radiation known as Ultraviolet-Visible (UV-Vis) spectrophotometer [7]. Research related to gamma irradiation of foodstuffs has been carried out by several researchers such as the effect of gamma irradiation and long storage at cold temperatures on the properties of Jenaha fish fillets (*Lutjanus Sp*) [8]. Based on the results of the analysis, the irradiation technique did not change the water content, ash content, or pH of the fish fillets, but it did decrease the protein content and slightly increase the fat content compared to fillets without irradiation. A different study is the effect of gamma irradiation and cold storage at 4˚C on the number of bacterial pathogens of cork fish fillets [9].

Based on the description above, a study was conducted entitled the effect of time and dose of gamma irradiation (Cesium-137) on protein content and flesh color index of cork fish (*Channa striata*). The method used is the UV-Vis spectrophotometry method [10] to determine protein levels and uses Image-J software [11] to see the color change that occurs after storage of the cork fish (*Channa striata*) sample. The objective of the study was to determine the effect of time and dose of gamma irradiation (Cesium-137) on protein content and storability based on the red color index of cork fish (*Channa striata*). The time used for irradiation of gamma irradiation (Cesium-137) was 35 minutes, 40 minutes and 45 minutes. The value of the irradiation dose is determined based on the rate of kerma and irradiation time.

Research Methods

The equipment used is a source of Cesium-137 radiation (*Indonesia-BATAN*), Phantom solid water, CCTV Monitor, UV-Vis Spectrophotometer (*Spectrophotometer Visible Faithful Type 722-Wavelength range: 320-1050 nm*), Ionizing Chamber Detector (*800 cc Model/Serial No.: A6 / XQ 202020*), Analytical Balance, Centrifuge, Hot place, Magnetic stirrer, Centrifuge tube, Chemical glass, Vacuum plastic container, Test tube, Test tube rack, Mortar and Pestle, Knife, Measuring flask, Measuring cup, Micropipette, Irradiator *(Model: IBT 121)*, Camera Cellphone. The materials used are cork fish meat (*Channa striata*), Aquades, Bovine Serum Albumin solution (*22% for BSA Serological application*), Na_2CO_3 (*Merck Repack-Sodium Carbonate Anhydrous 99.9%*), NaOH (*Brand: Merck-Sodium hydroxide 80%*), $CuSO_4$ (*High Purity Copper Sulfate 98%*), Folin Ciocalteau (*Nitra Kimia-Follin Denish Phenol Reagen*).

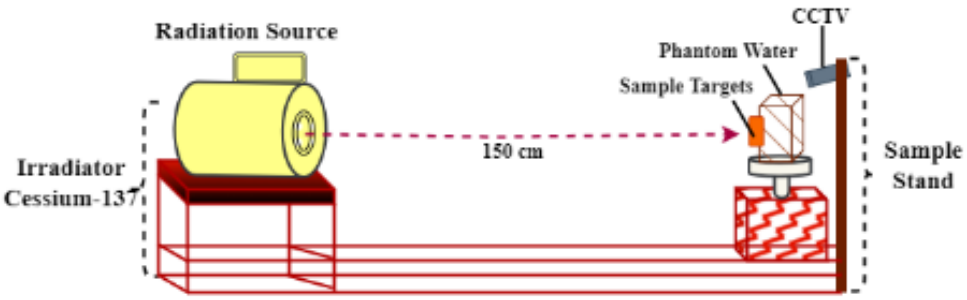

Figure 1. Scheme of irradiation on Cesium-137 equipment for cork fish samples

The irradiation method uses an irradiator and Cesium-137 based on a predetermined time. Prepare an irradiator with a calibrated Cesium-137 source as shown in Figure 1. Place the fish meat sample in front of the gamma radiation source for 35, 40 and 45 minutes with an irradiation distance of 150 cm. The radiation dose produced by a Cesium-137 irradiator uses equation (1). D is the radiation dose (mGy), k is the rate of kerma water or Hp (10) with a value of 10.276 mGy/hour. t is irradiation time (hours).

$$D = k.t \quad (1)$$

As much as 20 mg of BSA was dissolved in 100 mL of distilled water, then pipetted 25 mL and redissolved in 50 mL of distilled water. 1 gram of NaOH dissolved in 250 mL of distilled water. 0.5 grams of Sodium Potassium Tatrate is dissolved in 50 mL of distilled water. 2 grams of Na_2CO_3 is dissolved using 100 mL of the prepared NaOH solution. 0.25 $CuSO_4$ is dissolved using 50 mL of the Sodium Potassium Tatrate solution that has been prepared. Mix 50 mL of reagent (Na_2CO_3 and NaOH) with 1 mL of reagent ($CuSO_4$ and Sodium Potassium Tatrate) only at the time of use. Folin ciocalteau reagent is dissolved in distilled water in a ratio of 1:1 before use.

The protein standard curve was prepared by inserting 0 (blank) into the test tube, 0.1; 0.2; 0.4; 0.6; 0.8 and 1 mL BSA to obtain concentrations of 6.25 µg/mL, 12 µg/mL, 25 µg/mL, 37.5 µg/mL, 50 µg/mL and 62.5 µg/mL, respectively. Then add water until the total volume of each is 4 mL. Add 5.5 mL of mixed reagents (Na_2CO_3 and NaOH) and CuSO4 and Sodium Potassium Tatrate each into a test tube, then homogenize and leave for 10-15 minutes at room temperature. Adding 0.5 mL of reagent (folin ciocalteau with a ratio of 1:1) into each test tube and then homogenizing rapidly after adding the reagent. The sample was allowed to stand for approximately 30 minutes until a blue color formed. Then put the solution into the cuvette and read on the UV-Vis spectrophotometer to find the maximum wavelength.

Figure 2 is a sample of cork fish meat whose protein content value will be determined. Samples of cork fish that had been given gamma irradiation treatment (Cesium-137) weighed as much as 5 grams each. Then it was dissolved in 4 mL of distilled water and extracted. The extract was filtered and then separated into a test tube. The precipitated protein was separated by a centrifuge at 9,000 rpm for 10 minutes. The precipitate, which is a protein, was redissolved as much as 0.02 mL into 100 mL of distilled water. Then take 1 mL of the protein sample and add 3 mL of distilled water then carry out the procedure as in the standard curve preparation treatment starting from adding reagents and testing using a UV-Vis spectrophotometer. Then the protein content is determined from the absorbance value which is obtained from the sample solution using the standard curve.

Figure 2. Samples of cork fish meat after filleting before being extracted

Determination of protein levels in the sample is analyzed using equation (2). Testing the storage capacity of cork fish meat samples based on changes in the red color index of the fish meat. Samples that have been irradiated at room temperature (without irradiation) will be left in a (plastic) container by observing the length of storage based on the red color index. Preparing tools (smartphone camera and image-J software) and materials (cork fish). The operation of the Image-J Software is (1) Open the Image-J software on the computer, then select the file menu (Ctrl+ O) then enter the image to be analyzed. (2) click on the line drawing in the application then draw a line on the sample then select the Analyze menu, then click set scale. (3) Click on the oval image in the application, then draw a line on the sample following the pattern of the image. (4) Then click on the edit menu, choose clear outside. (5) Then click on the image menu, then select Adjust, then select Color Threshold and click color space, then select RGB and Thresholding method, then select triangle. (6) After that, click the Analyze menu, then select measure (Ctrl+M) and the area of the sample area to be analyzed will appear. (7) Then click again on the Analyze menu, then select color histogram, the data values from the analyzed images will appear as shown, then save the data as a result of Image-J analysis. The determination of the red color index in the sample can be calculated by using equation (3). PM is the percentage of red [12].

$$\text{Protein Levels} = \frac{\text{Protein Weight}}{\text{Sample Weight}} .100\% \quad (2)$$

$$PM = \frac{\text{Number of Red Pixels}}{\text{Number of Sampled Pixels}} .100\% \quad (3)$$

Results and Discussion

The results of irradiation of gamma-ray radiation (Cesium-137) can be seen in table 1. From the table it can be seen that for 35 minutes of irradiation the dose released is 6 mGy, for 40 minutes of irradiation the dose is released is 7 mGy and for 45 minutes of irradiation The released dose was 8 mGy. It can be seen from these data that the longer the time given, the greater the dose issued.

Table 1. Results of gamma irradiation (Cesium-137)

Sample	Exposure time to Cesium-137 irradiation (minutes)	Exposure time			Dose (mGy)	Abs
		Seconds	Minutes	Hours		
A	35	2100	35	0.58	6	Without Abs
B	40	2400	40	0.68	7	Without Abs
C	45	2700	45	0.77	8	Without Abs

The dose received by a tissue is determined by the intensity of the absorbed photons, the duration of exposure or irradiation to the tissue and the distance between the tissue and the radiation source. Absorbed dose is the amount of energy absorbed by a substance per mass unit. The absorbed dose is a quantity that is limited by the amount of energy absorbed by biological tissues in radiation [13]. Determination of the standard protein curve aims to determine whether the concentration of the substance to be analyzed by the UV-Vis Spectrophotometer method has a linear relationship or not significantly. Figure 4 is the absorbance values for each concentration. The table shows that the higher the concentration of the analyte, the greater the absorbance value produced. The relationship between concentration and absorbance is expressed by a calibration curve.

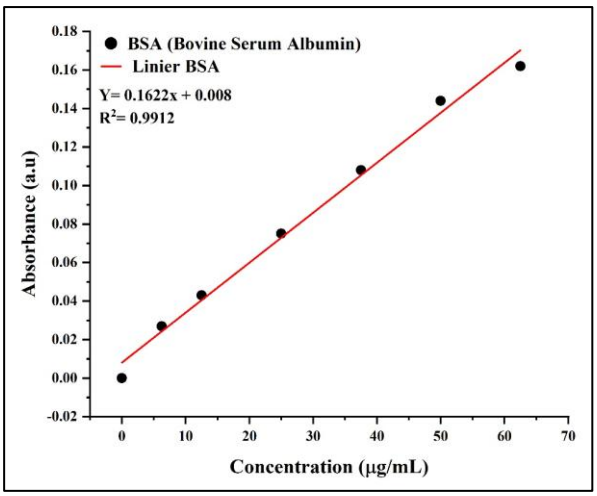

Figure 3. Graph of the relationship between BSA solution concentration and absorbance

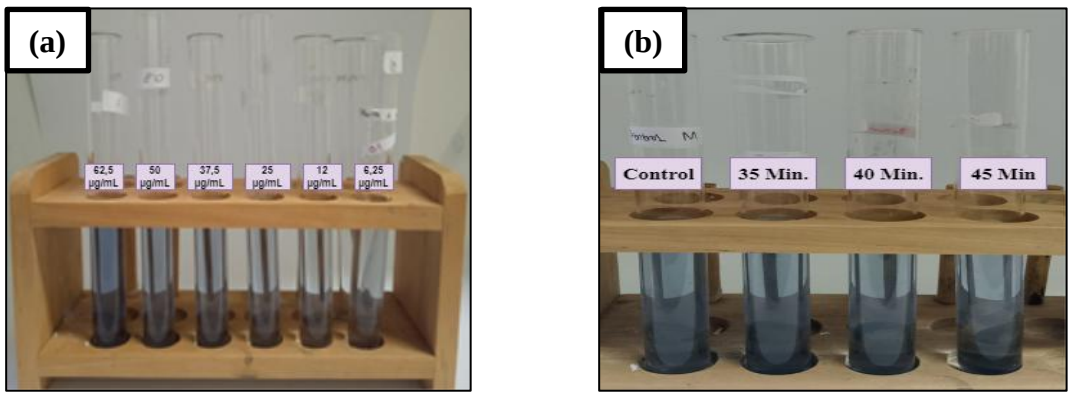

Figure 4. (a) Solution standard curve of protein with each concentration and (b) Cork fish protein sample solution

Based on the results of the calibration curve in Figure 3 above, we obtain a linear regression equation y = 0.1622 x + 0.008 with a correlation coefficient (R) = 0.9912. The calibration curve above shows the linearity relationship between concentration and absorbance as evidenced by an increase in the linear line. Linearity can be accepted if the value (R^2) is close to 1 (Indonesian National Standard). The color formed at the beginning of the addition of the reagent is clear in color. Then after standing for 30 minutes at room temperature the sample solution turns blue (figure 4.a). This blue color is formed due to the reaction of Cu2+ with peptide bonds and reduction of phosphomolybdic acid and phosphotungstic acid by tyrosine and tryptophan which are protein residues). The color that is formed is mainly from the reduction of phosphomolybdic acid and phosphotungstic acid. Therefore, the color formed depends on the levels of tyrosine and tryptophan in the protein [14].

Determination of protein levels was carried out on cork fish samples that had undergone radiation treatment with different variations of irradiation time (figure 4.b). Determination of protein levels was carried out using the spectrophotometric method (Lowry), using a UV-Vis spectrophotometer at a wavelength of 650 nm. The Lowry method is basically used for determining protein content using a UV-Vis spectrophotometer. Standard curve is a calibration curve of a set of standard solutions. The ideal solution should have the same composition as the sample. Single solutions are extremely rare in the molar absorbance literature. Proteins containing phosphotungsten salts show a blue color in an alkaline state, the intensity varies according to protein content (figure 4). To estimate the amount of protein in solution, optical density (OD) or absorbance at certain wavelengths is very necessary [15]. The absorbance values of cork fish samples with variations in irradiation time are shown in table 2. The relationship between time, dose and absorbance.

Table 2. Results of the analysis of protein content of 5 grams of cork fish samples

Irradiation time (minutes)	Dose (mGy)	Absorbance (a.u)	Sample protein levels (%)
0	0	0,064	0,138
35	6	0,084	0,187
40	7	0,12	0,276
45	8	0,149	0,347

Based on table 2, it can be seen the relationship between dose, time and absorbance, that is, during the control period, the resulting dose was 0 mGy with an absorbance of 0.064 a.u, in 35 minutes the resulting dose was 6 mGy with an absorbance of 0.084 a.u, 40 minutes of the resulting dose 7 mGy with an absorbance of 0.12 a.u and within 45 minutes the resulting dose was 8 mGy with an absorbance of 0.149 a.u. It can be seen from the graph that the longer the irradiation time given, the higher the dose and absorbance value produced. The absorbance value is linear with increasing time and radiation dose. The absorbance value describes the number of particles that are formed over time. The quantity of particles created increases as the absorbance value increases [16]. As a result, an increase in absorption during the irradiation process indicates that the number of particles increases with increasing exposure to gamma rays [17].

Based on table 2, it can be seen the relationship between time, dose and protein levels, that is, during the control period, the dose produced was 0 mGy and the protein level was 0.138%, at 35 minutes the resulting dose was 6 mGy with a protein level of 0.187%, at 40 minutes the resulting dose was 7 mGy with a protein level of 0.276% and in 45 minutes the resulting dose was 8 mGy with a protein level of 0.347%. The histogram shows a linear line, the longer the irradiation time is given, the higher the dose issued and the protein level produced will be higher.

Irradiation only causes structural changes in proteins. The structure in question is the basic structure of the protein, namely the sequence of amino acids making up the peptide backbone. The mechanism of protein structure changes due to gamma irradiation is divided into two parts, namely the direct mechanism and the indirect mechanism. The protein structure changes in the process directly due to gamma radiation impacting the protein molecule. Gamma irradiation decomposes water molecules through an indirect method culminating in the radiolysis process of water. The water molecule is broken down into simpler molecules during radiolysis and results in the formation of free radicals. Hydrogen bonds in proteins can be broken and protein molecules rearranged by free radicals [18].

Based on the three effects of gamma radiation, one effect is on protein. This is in accordance with research conducted by Mamluatul Hasanah concerning the Effect of Gamma Ray Irradiation on Protein, Fat and Free Radicals of Beef (Bos Taurus) with a given radiation dose of 0 kGy, 1 kGy, 2 kGy, 5 kGy and 7 kGy [19]. The results of the study showed that at the highest dose (7 kGy) the protein content was also higher. This happens because the radiation energy given is large enough so that it can inhibit the growth of pathogenic bacteria. The effect of gamma rays on protein is capable of disrupting chemical bonds and depolymerizing polysaccharides. Termination of hydrogen bonds can change the shape of biomolecules and affect biological

activity, possibly triggering apoptosis or inhibiting cell proliferation. Apoptosis is initiated from a state of stress, such as DNA damage caused by ionizing radiation (gamma rays). Apoptosis is defined as the signal of death. These signals can be produced intracellularly or extracellularly by ionizing radiation [20]. The color index observed in this research is the red index. The results of observing the color in the sample can be seen in table 3.

Table 3. The results of observing the percentage of red color

No.	Irradiation time (minutes)	Percentage of Red Per Day (%)						
		1	2	3	4	5	6	7
1	Control (0)	1.54	1.05	0.80	2.49	3.13	2.09	0.92
2	35	1.66	1.52	0.70	3.03	2.74	3.09	1.80
3	40	1.76	2.41	1.24	3.19	2.96	3.42	1.74
4	45	2.50	1.81	2.78	4.36	3.87	6.15	1.31

The table above shows the change in the percentage of red color in the sample against irradiation time. In the control sample (0), the highest percentage of red color index was found on day 5 with a percentage of 3.13%, at 35 minutes the highest percentage of red was on day 6 with a percentage of 3.09%, at 40 minutes the percentage of red the highest percentage was found on day 6 with a percentage of 3.42% and at 45 minutes the highest percentage of red was on day 6 with a percentage of 6.15%. The relationship between irradiation time and the red index in the sample can be seen in Figure 5.

Based on the data presented in Figure 5, it appears that there is a relationship between Cesium-137 irradiation time and the red color index in cork fish samples. On the first day, the red color index in the control sample increased linearly with the irradiation time variation. The percentage value was 1.54%, with the highest percentage found at 45 minutes of 2.50%. On the second day, the red color index in the control sample experienced a non-constant percentage. The percentage value was 1.05%, with the highest percentage found at the 40 minute time variation and then decreased again at the 45 minute time variation.

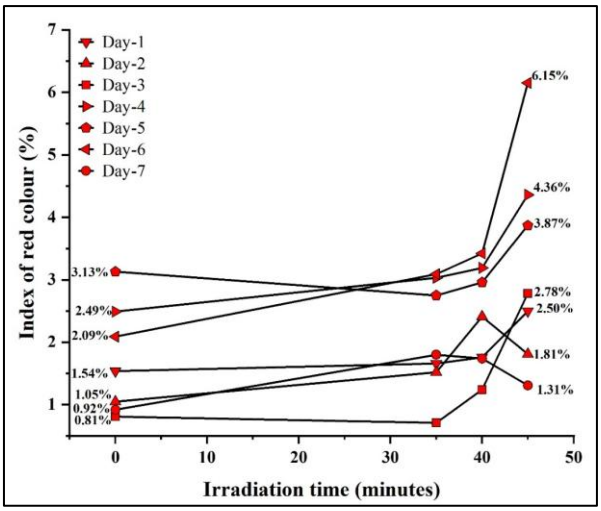

Figure 5. Graph of the relationship between the percentage of red color and irradiation time

On the third day, the red color index in the control sample experienced a non-constant percentage. The percentage value was 0.81%, then decreased at 35 minutes of time variation and increased again at 45 minutes of variation with a percentage of 2.78%. On the fourth day, there was a linear increase in the red index as shown in the graph above. In the control variation, the percentage value was 2.49%, with the highest percentage found in the 45 minute time variation with a percentage of 4.36%. On the fifth day, the percentage of the red color index in the sample was not constant. In the control variation, the percentage value was 3.13%, then decreased at 35 minutes and increased again at 45 minutes with a percentage of 3.87%.

Based on the data provided, it appears that the red color index in the sample experienced a linear increase on the sixth day. Specifically, the control variation had a percentage value of 2.09%, with the highest percentage occurring in the 45 minute time variation at 6.15%. On the seventh day, the percentage of the red color index in the control variation was 0.92% and increased at 35 minutes with a percentage of 1.80%, then decreased until 45 minutes with a percentage of 1.31%. Overall, it seems that the percentage value remained relatively stable from the first day to the seventh day, with the percentage increase not varying too greatly

between each variation of irradiation time. It is worth noting, however, that the percentage value of the red color index that is not constant or unstable may be influenced by a lack of accuracy in taking pictures of the sample.

Conclusion

The effect of time and dose of gamma irradiation (Cesium-137) on protein level is that the longer the irradiation time and the greater the dose of irradiation given, the higher the protein content in the sample, while the effect of gamma irradiation time (Cesium-137) on shelf life is based on index the red color in the cork fish sample, namely from the first day to the seventh day, the percentage value is close to a stable percentage, namely on the first day. The relationship between absorbance and protein content is that the higher the absorbance value of a sample, the greater the protein content in the sample and there is no relationship between absorbance and shelf life of the sample based on the red color index.

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