THE INFLUENCE OF *Ficus carica* AND *Averhoa blimbi L.* WATER BATH ON FREE RADICAL AND PROTEIN LEVEL OF GAMMA RADIATION EXPOSED-BEEF

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Article Info

Article history:

Received Jul 12th, 2017
Revised Aug 20th, 2017
Accepted Oct 26th, 2017

Keyword:

Gamma radiation,
Antioxidant,
ESR,
Ficus carica,
Averhoa blimbi,
Free Radical,
Protein

ABSTRACT

Ionizing radiation has been used in various technologies. One example of its use in industry is food preservation process.Ionizing radiation is able to kill microorganism. However, it also causes free radicals. Beef exposed with gamma radiation from Cobalt 60 will be ionized and its tissue will be damaged due to free radicals. The damage also can occur on its protein. It needs antioxidant to prevent the free radicals. The study aims to find out the influence of gamma radiation on beef before and after adding the antioxidant. Antioxidant used is water bath of *Ficus carica* and *Averhoa blimbi*. Then the beef sample is tested its free radicals using ESR. The result shows that when added with antioxidant the amount of free radical decreases for 77.46%. It is due to the fact that *Ficus carica* and *Averhoa blimbi* contain flavonoid compound, vitamin C, tannin, saponin, and beta-carotenewhich act as antioxidant. Then the protein content is tested using kjehdahl method. The result shows that the protein level in the beef is 21.03% and when the beef is exposed to gamma ray it decreases to 20.53%. When antioxidant is added, the protein level is 19.81% and 19.06%.

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I. INTRODUCTION

Beef is one of farm products in Indonesia. It contains protein, vitamin B12, niacin, vitamin B6, iron, zinc and phosphor needed by human body. Today the need for beef is increasing. The supply of local beef is not able to fulfill the demand. Imported beef needs a longer time to reach Indonesia. To preserve them, the importer uses irradiation technique. However, the side effect of the technique is the free radicals.

Gamma radiation is uncharged particle which can cause ionization. Therefore, it is called ionizing radiation. The interaction between ionizing radiation and compound is ionisasi.[1] Gamma radiation can cause damage on human cells due to the interaction between cells and ionizing radiation. Exposed to radiation, human body, particularly cells, will absorb the radiation energy. The process occurs very fast, approximately in only 10^{-16} second. Since most part of a cell is water, the first process occurs on the cell is ionization with H_2O compound.[2]

Usually, radiation damages big molecules (bio macromolecules) such as lipid, protein and carbohydrate. The damage leads to severe effect. Cell damage will bring negative impact on structure and its function. Biologically, bio macromolecule compound plays an important role. Therefore, the damage of the structure and its function inhibits the organ work system in general.[3] Free radical is very reactive. It can form chain reaction which leads to chemical change and destroys various living cell components such as protein, thiol non protein cluster, lipid, carbohydrate, nucleotide.[4]

Antioxidant is an electron donor reductant. It weighs a small molecule but it affords to inactivate oxidation reaction by cutting chain reaction. Therefore, it prevents the formation of new radicals.[5]

The existence of free radicals can be controlled using antioxidant compounds. The study uses antioxidant compound in *Ficus carica* and *Averhoa blimbi*. Their antioxidants mostly consist of phenolic, vitamin C, B, tannin, saponin and beta-carotene.

Previous studies use extracted antioxidant materials. Therefore, it needs a new technique which brings a better result. The study aims to analyze the influence of *Ficus carica* and *Averhoa blimbi* water bath before and after gamma radiation on beef free radical and protein level. In addition, it also analyzes the influence of soaking time of the antioxidants.

2. RESEARCH METHOD

In the study, the researchers use 2 cm^3 beef sample. The source of radiation is Cobalt-60 with activity 74 kBq in 1993. The water bath of both fruits is 250 ml with soaking time variation.

First step is cutting the beef into 2 cm^3 slices. Then, it is exposed to gamma radiation for 10, 15, and 20 minutes. After that, it is soaked with the fruit water bath for ten minutes. The water bath is from soaking the fruits for 1, 12 and 24 hours.

The last step, after the treatment the beef is ground and tested for its free radicals and protein level.

3. RESULTS AND ANALYSIS

Most of nutrient content in beef is water. It contains 73.1 gram. The high level of water will potentially form free radicals if it is exposed to gamma ray.Free radicals can be captured during the test using ESR and it can be seen using factor g.

The formed free radical is hydroperoxide(H_2O_2) andperoxy(*OOH). They occurs due to the damage of physical chemical process The primary and secondary ionization process lead to the damage of water molecules in the cell. The ionization process occurs due to the interaction between ionizing radiation and water molecules of beef and creates photoelectrical effect. It releases the electron from the atom since the energy of electron bond on the nucleus is lower than the approaching radiation energy. It causes the electron interacts with water compound in the beef. The reaction is as follow:

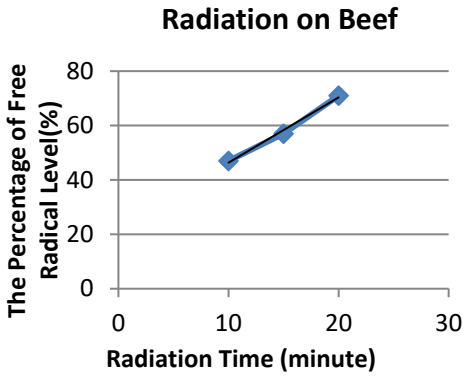
$$H_2O + e^- \rightarrow H^+ + OH^*$$
$$OH^* + OH^* \rightarrow H_2O_2 \text{ (Hydroperoxide)}$$
$$O_2 + e^- + H^+ \rightarrow {}^*OOH \text{ (peroxy)}$$

The process also captures other free radicals as shown in the Table 1.

Table 1. The Type of Captured Free Radicals

Radiation Time	Code	Factor g	Free Radical Type
10 min	1	1.9201157	-
	2	1.8631782	FeS
	3	1.9879504	Hidroperoxide
	4	2.0040832	Ethyl Hidroperoxide
15 min	1	1.9850795	-
	2	1.9521511	Peroxy
	3	2.015601	Hidroperoxide
	4	1.98231	FeS
20 min	1	1.8679273	C
	2	2.0052872	-
	3	1.9555221	FeS
	4	1.8612043	

In table 1, captured free radicals are Hydroperoxide 49.63%, FeS 46.70%, Peroxy 16.80%, Ethyl 16.70% and C 16.71%. Then, a graph of radiation time and free radical level can be drawn. From the data, it can be seen that the highest percentage is that of Hydroperoxide.



Graph 1. The Relation between Radiation Time and Free Radical Level

From the graph, it can be seen that the radiation time affects the free radical level. The longer the radiation the higher the free radical level.

The Mechanism of Antioxidants and Free Radicals

The antioxidant acts as a protector against free radicals. It is able to terminate chain reaction of free radicals. It can be obtained from fruits, vegetables and seeds. The compound in *Ficus carica* and *Averhoa blimbi* contains flavonoid, vitamin C, tannin, saponin dan beta-carotene.

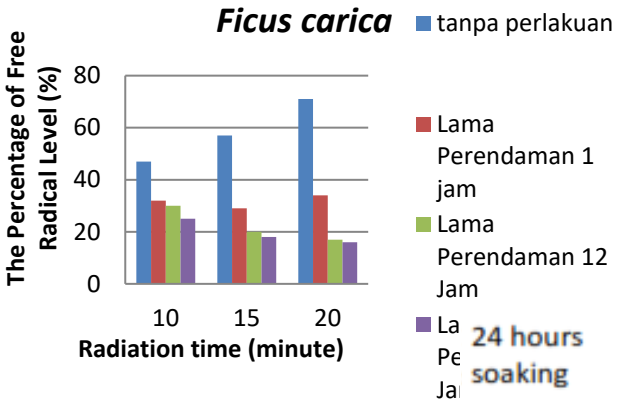
When the beef is soaked in the water bath of *Ficus carica* and *Averhoa blimbi*, no free radical is captured compared to that of without antioxidant. It shows the existence of antioxidant performance to reduce free radicals. The mechanism of antioxidant in protecting against free radicals is by donating one atom. The data shows no existence of free radicals on 24 hours soaking time.

Table 2. Types of Free Radical Captured by adding Antioxidant

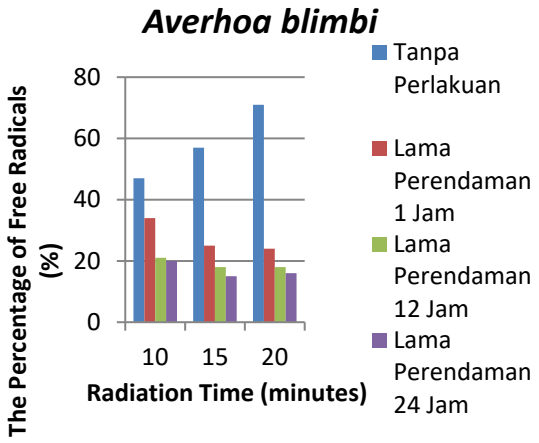
Radiation Time	Code	Factor g	Types of Free Radical
10 min	1	1.881403	-
	2	1.97148	-
15 min	1	1.896277	-
	2	1.935108	-
20 min	1	1.938712	-
	2	1.905687	-

Table 2 identified the absence of types of free radical captured. It showed the performance of antioxidant compound within both fruits in preventing free radical. Afterward, 24 hours soaking time was very effective with 77.46 % effectiveness.

Interaction between antioxidant compound and free radical creates a new radical and more stable antioxidant radical. This stable antioxidant radical cannot bond with other molecules and produce new radical. The difference of soaking time affects the percentage of free radical level, as shown in the following graph.



Graph 2.The Relation between Percentage of Free Radical Level and Radiation Time in *Ficus carica* water bath.



Graph 3. The Relation between the Percentage of Free Radical Level and the Radiation Time in *Averhoa blimbi* water bath .

Graph 2 and 3 shows the decreasing percentages of free radical level before and after adding antioxidant.

Analysis of Protein Level

Free radical is able to damage the structure and function of biomacromolecule such as protein which can reduce the amount of protein. It is due to the relation between radiation doses of gamma rays to living tissue. The interaction between free radical and protein is as follow:

$$H^* + P \rightarrow H_2 + P^*$$

$$OH^* + P \rightarrow H_2O + P^*$$

The effect of gamma rayradiation on the protein can terminate the chemical bond or polysaccharide depolymerization. The termination of hydrogen bond can change biomolecule conformation and affect its biological activities. Therefore, it may lead to apoptosis or inhibit cell division. While cell division is inhibited, mitochondria changes due to oxidative phosphorylation disorder and electron transport so that protein synthesis is inhibited as shown by data of protein level test.

In table 3, the percentage of protein level decreases when antioxidant is added. It is due to the vitamin C content in *Ficus carica* and *Averhoa blimbi*which are 1.2 mg and 18 mg, respectively. Organic acid is able to penetrate lipid membrane within cell. While the cell retains neutral pH, it transports an excess of proton. The excessive transportation makes

the cell unable to reproduce energy. It will run out of energy to live. In addition, the duration of soaking will affect percentage of protein level because protein will have denaturation when its pH changes. It will dissolve when the pH is low, that is during soaking process.

Table 3. The Result of Protein Level Test

No	Code	Protein (%)
1.	Fresh beef	21.03
2.	Beef in 10 minutes radiation	20.53
3.	Beef in 1 hour *Ficus carica* water bath	19.81
4.	Beef in 1 hour *Averhoa blimbi* water bath	19.06

4. CONCLUSION

The study shows a relation between gamma radiation and the level of free radical and protein in the beef. The free radical level will increase when the antioxidant is added. It shows the performance of antioxidant in preventing free radicals. Referring from the results, it is suggested to do test on the durability of beef when an antioxidant is added and test on other biomacromolecules such as fat, vitamin, and carbohydrate.

ACKNOWLEDGEMENTS

We extend our sincere gratitude to the head of Universitas Islam Negeri Maulana Malik Ibrahim Malang Laboratory, the head of Advanced Physics Laboratory of Faculty of Mathematics and Science, Brawijaya University, and the head of Food Laboratory Faculty of Agriculture, Brawijaya University.

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