



## THE APPLICATION OF GAMMA IRRADIATION TECHNOLOGY AND FROZEN STORAGE FOR DECREASING TOTAL BACTERIA IN SOME LOCAL FRUITS

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### ABSTRACT

Fruit is one of food product that have a relatively short time of storage because it is very susceptible to microbial contamination. one of the efforts that can be done is by using a combination of gamma irradiation and frozen storage. The gamma irradiation is one of the food processing technology that aims to reduce the number of microbial by damaging the DNA in bacteria. To optimize the gamma irradiation process can be carried out also with a combination of frozen storage which can inhibit the activity of enzymes and chemical reactions in the bacterial cell so as to prevent bacterial DNA repair after irradiation. Irradiation dose used in the process of preservation of fruits and a decrease in the number of bacteria around 2-10 KGy.

Key words: frozen storage, gamma irradiation, preservation of fruits

### INTRODUCTION

Food is the primary need for human which must always available enough in amount and have the good quality. Foods is needed by human for the growth and survival because it is the source of nutrition for human. Generally perishable foodstuff either due to weather influence, budding, root, germination, insect and microbe patogen which can produce the deadly toxin (Akrom, 2014; Fabryana, 2015). Food which contaminated of bacterium patogen can generate the disease, such as Foodborne disease and it's become the serious problem in Indonesia. The Council for Agricultural Science and Technology (CAST) show the data of 6 - 33 million case of diarrhal disease and about 9000 death every year because of bacterium patogen (Thayer Et al., 1996). Like Salmonella, Campylo-Bacter, Escherichia, Coli, Vibrio, and Toxoplasma gondii and the other parasite ( Archer And Kvenberg, 1985 in Farkas, 1998).

The usage of nuclear Technology in the field of food have been proven can assist to solve various nitation sproblems. Some examples of the application of nuclear technique for this purpose have been developed for preservation, food security, and sterilization of foodstuff (PDIN, 2010). Food irradiation is physical process which can be used to preserve and enhance food security. The type of radiation that used for is high energy radiation called ionizing radiation. This Irradiation technology have many advantage compared to conventional preservation process, such as can reduce post harvest

loss, improving security and food trade, and and reduce pollution or environmental damage due to the use of chemicals.

The effect of food irradiation process is the breaking of phosphodiester and hydrogen bonds in the DNA chain microbe that cause the microbial growth is inhibited. However, microbial cells will be able to repair it's DNA so that it will grow back. Therefore, to increase the effectiveness of the irradiation treatment should be combined with other treatment that called frozen storage (Molins, 2001).

The radiation dose is given, it may affect to the results of post-harvest, such as reduced storage losses, the longer shelf life and food safety of microbial pathogens and parasites (Farkas, 2006). Low doses (0.4 to 2.5 kGy) used for the purpose of inhibiting the germination and maturation and eradicate the insect, moderate doses (1-10 kGy) can already be used to kill pathogens, and high dose (30-50 kGy) used for kill all types of bacteria present. By limiting the irradiation dose and the maximum energy from the radiation source, the food preserved by irradiation does not become radioactive (ICGFI, 1999). Safety test of irradiated foods for human consumption known as wholesomeness tests, including toxicology test, macro and micro nutrients and microbiological and sensory tests. (Ward and Hackney, 1991).

Codex Alimentarius Commission FAO/WHO recommends a dose of irradiation may be used in food irradiation does not exceed 10 kGy (Gould, 1995). The amount of energy is actually very small, equivalent to the amount of heat required to increase the temperature of the water 2,4<sup>0</sup>C. Therefore, food irradiated with doses below 10 kGy only very small changes as well as safe for human consumption (Zubaidah, 2007)..

The radiation source used is a radioisotope Cobalt-60 (60Co), cesium-137 (137Cs), each of which produce gamma rays, electron beam machines and machines X-ray generator (Zubaidah, 2006). Gamma rays emitted by the radioisotope 60Co and 137Cs are the source of ionizing irradiation has been widely used for food preservation commercial applications (Zubaidah, 2007). Generally, the gamma rays are used for radiation is the result of the decay of atomic nuclei 60Co as 60Co has a greater radiation energy so as to have great penetrating power. 60Co emits two gamma rays with energies of each of 1.17 MeV and 1.33 MeV which has a half life 5.27 years (Fabryana, 2015).

Red guava fruit (*Psidium guajava* Linn) and red chili (*Capsicum annum* L.) is one of the local food commodities from Indonesia. Red guava fruit contains vitamin C two times more than oranges. However, red guava fruit and chili have the same period short enough storage in free space and easy to attacked by parasites. Therefore, it takes irradiation to inhibit the growth of microbes that cause red guava fruit and chili becomes faster rot and also to kill pathogenic bacteria that cause disease in humans.

When gamma rays that have enough energy hits a material will cause excitation, ionization and chemical changes. Excitation occurs when a living cell in red guava fruit and chilli being sensitive to outside influences. While ionization occurs when compounds or macromolecular complexes into fractions or ion free radicals that cause the microbes die. Chemical changes occur as a result of excitation, ionization and chemical reactions that take place when the radiation is completed. When chemical changes occur in living cells, it will inhibit the synthesis of DNA which causes the cell division process or a normal life processes in the cells will be disrupted and biological effects occur (Maha, 1985).

The gamma rays in the process of food irradiation interacts with the material so produce in pair production, this occurs when energy photons (gamma rays) of more than 1.02 MeV (Meredith, 1977). Pairs production is the process of formation of a positron and an electron pairs when photons interact with matter (Beiser, 2003).

In the process of food irradiation, gamma rays hitting material so as to produce an ion pair (ionization). This ionization that causes changes in the physical characteristics and function of cells. irradiation on the organism can provide two effects, namely the effects of direct and indirect effects (Figure 1). Direct effects are

the result of a direct collision energy radiation or electron in a microbe that causes the breakdown of DNA chains and affect the cell's ability to reproduce and survive. Indirect effects occur when the radiation of the water molecule that is a major component in the cell so as to form the radiolysis water molecules and free radicals (Adams, 2008).

Disruption of growth and microbial synthesis process, will inhibit the metabolism of food, this will lead to a process of maturation or decay is slow. Even bacteria that cause disease in humans would be reduced in number.

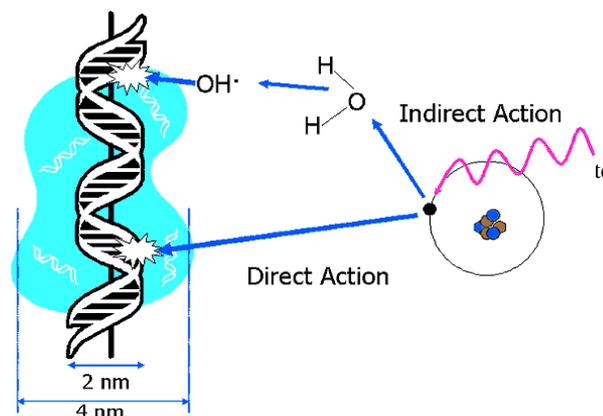


Figure 1. Direct and indirect effects of radiation on biological systems (Source: Oseuxtendedcampus Oregon State University)

## METHODS

This research sample is red guava and chili. Samples selected according to size, mass and maturity level that is considered relatively equal. Each sample are grouped according to the irradiation dose given. The dose given was 2.5 kGy; 5 kGy; 7.5 kGy; and 10 kGy and the other used as a control sample were not irradiated. This research through several steps, that is:

### Process the Gamma Irradiation

The process of irradiation used Natural Rubber irradiators (Irka) / Latex Irradiator with 60Co gamma source, with radioactive activity is 18003.683 Ci, and dose rate of 0.73771 kGy / hour in September 2014, in PATIR - BATAN (Isotope Technology Application Center and Radiation - National Nuclear Energy Agency) Pasar Jumat, South Jakarta. Red guava fruit and chili were irradiated at a controlled dose variation, that is: 2.5 kGy; 5 kGy; 7.5 kGy; and 10 kGy.

### Frozen Storage

Samples that have been irradiated, then stored in a frozen state. then testing the amount of microbes on day 4, 9, 15 and 20 to the sample approached the foul.

### Calculating Total Microbes

Plate Count Agar (PCA) or often referred to as Standard Methods Agar (SMA) is a medium growth of microorganisms, which are commonly used to count the number of total bacteria (all types of bacteria) contained in each sample foods: milk, waste water and other samples which also usually using Total Plate Count (TPC). Plate Count Agar (PCA) is a solid medium, the media that will be solid when cool. It was prepared by dissolving all the ingredients to form a suspension of 23.5 g / L then sterilized in an autoclave. Composition Plate Count Agar (PCA) containing: 0.5% Tryptone, 0.25% yeast extract, 0.1% glucose, 1.5% agar. Plate Count Agar (PCA) containing glucose and yeast extracts are used to grow all kinds of bacteria. It is containing the nutrients provided by Tryptone, vitamins of yeast extract, and glucose is used as an energy source for microorganisms that support the growth of bacteria. Plate Count Agar (PCA) is not a selective media because the media is not only covered by a specific type of microorganisms.

The making Plate Count Agar (PCA) can be done by mixing 23,5g into 1L of distilled water, then heated to boiling to dissolve the whole medium. As well as sterilization using an autoclave at a temperature and time set is 121°C for 15 minutes. Media will be in inoculation with microorganisms certainly before solidifying needs to cool down the room at a temperature up to 47-50 °C. If the media is too hot, microorganisms will be grown will die. After the media becomes solid and has been sterilized, the media is left exposed to air for 15 minutes for inoculation with microorganisms. Inoculation is planted inokula aseptically into sterile media both in liquid media, semi-solid, or solid. Inokula are materials that contain microorganisms either in liquid or solid state. Inoculation goal is to purify, identify, rejuvenate, and storing microorganisms.

Analysis of microorganisms used to determine the type and number of microorganisms contained in foodstuffs. The analytical method using Standard Plate Count (SPC), to explain about how to count the colonies on plates as well as how to choose the existing data to calculate the number of colonies in some foodstuffs. colony counting procedures are as follows: 1. Cawan chosen and counted are those containing the number of colonies between 30 to 300. 2. Some colonies were merged into one is a collection of large colonies where the number of doubtful colonies can be counted as one colony. 3. A chain of colonies is seen as a thick line is counted as one colony. The formula to calculate the number of colonies per ml is as follows:

$$\text{The number of colony per ml} = \frac{\text{the number of colony per cup} \times (1/Fp)}{1}$$

by Fp [is] thinning factor.

### RESULT AND EXPLANATION

In Figure 2 and Figure 3 explains that the larger the dose of radiation is given, the number of microbes will be reduced. however, in the process of storage, from 4 to 20 days, the number of microbes actually increased. This is probably caused by: microbe can repair its soft DNA structure and the entry of bacteria into the sample. the entry of bacteria is estimated at the time of distribution of BATAN Jakarta to UNNES campus Semarang and during the storage process is not sterile and is not in freezing conditions. In addition, the process of packing the sample into a container which is not sterile, contribute significantly to contamination of the sample. so that the radiation dose given is inversely proportional to the total microbes die.

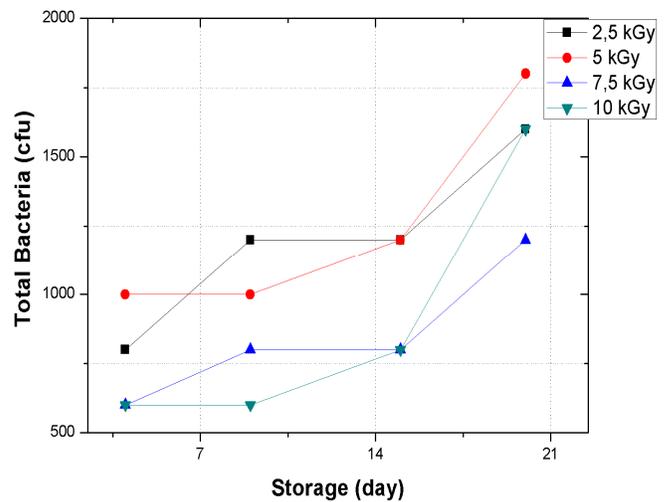


Figure 2. Graph the relationship between the storage time to the total bacterial of red guava (*Psidium guajava* Linn)

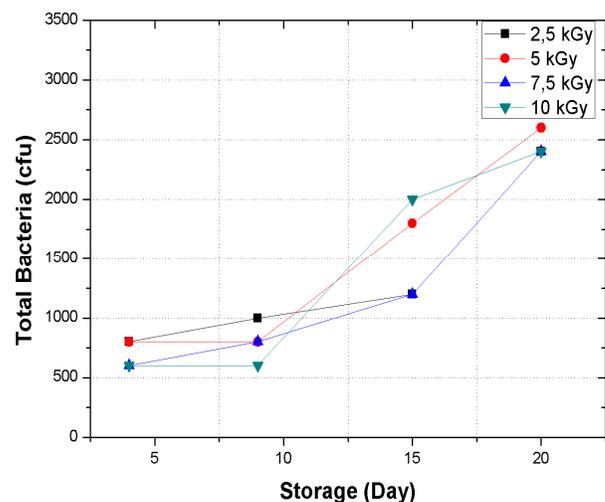


Figure 3. Graph the relationship between the storage time to the total bacterial of chilli (*capsicum annum* L.)

To improve the situation can be made with frozen storage. Frozen storage causes water availability decreases, so the activity of enzymes and microbes can be inhibited or stopped and mencegaah chemical reactions so as to maintain the quality (taste and nutritional) food (Fabryana, 2015). Although frozen storage can reduce the number of microbes that are very large but can not make food into sterile of microbes. Frozen storage will increase the concentration of electrolytes in the microbial cells, because the water will freeze forming ice crystals, damaging the colloidal system of protoplasm (eg colloidal systems of proteins) and cause the denaturation of proteins in microbial cells (Cahyani et al., 2015). Frozen storage can cause death or sublethal damage in some cells

Some changes in physical-chemical properties that occur, due to irradiation can lead change and the loss of nitrogenous bases, breaking the hydrogen bonds, termination of the sugar chain phosphate of each polinuklida of DNA (single strand break), termination of the chain adjacent to both poly nucleotides of DNA (double strand break), and the formation of intra-molecular crosslinking (base damage). Most microbes are able to single strand break repair. Some literature suggest that microbes are sensitive can not fix double stand breaks, while microbes showed higher resistance has the capacity to repair double-strand break. Results repair or rearrangement of the DNA can be the same or different from the original (Fabryana, 2015).

## CONCLUSION

The process of food irradiation using gamma rays with a radiation dose of 2.5 to 10 kGy is able to kill most microbes in food and inhibits the physiological processes of microbes so that food is more durable. but the process of packing and storage are not appropriate, will lead to the emergence of bacteria again

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