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Effect of irradiation dose and shelf life on microbial count in Asam Keumamah

Pengaruh dosis iradiasi dan umur simpan terhadap jumlah mikroba pada asam keumamah

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Abstract

Ready-to-eat foods are required immediately in disaster situations. Asam Keumamah is a ready-to-eat product that is not durable. Irradiation with asam keumamah extends the shelf life of the product, reduces microbial load, and maintains nutrition. This study aimed to determine the microbiological quality and food safety of Asam Keumamah irradiated at different doses. The experimental research used a Factorial Completely Randomized Design (CRD) on Asam Keumamah samples that had been irradiated at doses of 0, 20, 30, 40, and 50 kGy and stored for 0, 1, 3, and 6 months at room temperature. The irradiation of Asam Keumamah was carried out at the Merah Putih Gamma Irradiator Merah Putih in the Puspitek area, Serpong, South Tangerang. The total bacterial test on all samples using TPC was conducted at the Balai Perikanan Budidaya Air Payau Ujung Bate (BPBAP-UB) Aceh Besar Laboratory. Data were analyzed using factorial Anova test and Duncan's test at 95% CI. The results showed that the irradiation dose had a significant effect on the number of bacteria ($p<0,05$), the storage period had no significant effect on the number of bacteria ($p>0,05$), and the interaction between irradiation dose and storage period had a significant effect on the number of Asam Keumamah bacteria ($p=0,0001$). This study concluded that the higher the irradiation dose, the lower the number of bacteria and durability.

Keywords: Asam Keumamah, microbe, shelf life, irradiation dose

Abstrak

Makanan yang dapat langsung dikonsumsi sangat dibutuhkan pada situasi bencana. Asam keumamah dapat menjadi salah satu produk siap saji namun tidak dapat bertahan lama. Iradiasi memperpanjang umur simpan produk, mengurangi mikroba dan tetap mempertahankan kualitas gizi. Tujuan penelitian untuk mengetahui kualitas mikrobiologi dan keamanan pangan asam keumamah iradiasi dengan dosis yang berbeda. Desain penelitian eksperimental menggunakan Rancangan Acak Lengkap (RAL) Faktorial pada sampel Asam Keumamah yang diiradiasi pada dosis 0, 20, 30, 40, 50 kGy dan disimpan selama 0, 1, 3, dan 6 bulan pada suhu ruang. Iradiasi Asam Keumamah dilakukan Iradiator Gamma Merah Putih kawasan Puspitek, Serpong, Tangerang Selatan. Uji Total Bakteri terhadap seluruh sampel menggunakan metode ALT dilakukan di Laboratorium Balai Perikanan Budidaya Air Payau Ujung Bate (BPBAP-UB) Aceh Besar. Data dianalisis menggunakan uji Anova faktorial dan uji Duncan pada CI 95%. Hasil penelitian menunjukkan dosis iradiasi memiliki pengaruh yang nyata terhadap jumlah bakteri asam keumamah ($p<0,05$), masa simpan tidak memiliki pengaruh yang nyata terhadap jumlah bakteri ($p>0,05$). Interaksi dosis iradiasi dan lama penyimpanan menunjukkan pengaruh yang nyata terhadap jumlah bakteri Asam Keumamah dalam kemasan vakum ($p=0,0001$). Kesimpulan penelitian ini, semakin tinggi dosis iradiasi maka jumlah bakteri semakin berkurang dan tahan lama.

Kata Kunci: Asam Keumamah, mikroba, umur simpan, dosis iradiasi

Introduction

In an emergency, food is one of the types of assistance that disaster victims need. Food that can be consumed immediately (ready to eat), is practical to distribute, and nutritious is needed by refugees. An alternative food that can be provided to refugees is emergency food (Sumarto & Tajrifani, 2020).

Asam Keumamah is a processed product of skipjack tuna or tuna that has undergone the stages of cleaning, boiling, salting, and drying (Fajri et al., 2021). Asam Keumamah can be used as a variety of food menus, one of which is ready-to-eat Asam Keumamah, which can be consumed immediately or stored longer. Processed fish products and other fishery products can easily damage food items (Hadi et al., 2023).

Fish products that are stored for a long time are susceptible to the growth of microbes such as bacteria, fungi, and parasites. This may result in product damage and increase the risk of illness if the product is consumed (Safaeian & Khanzadi, 2018; Swastawati et al., 2020). Therefore, an appropriate postharvest technology is required to increase its shelf life. A post-harvest technology that can be applied to fishery products is irradiation (Pangestika et al., 2022).

Irradiation is a food-processing method that uses ionizing or non-ionizing radiation to reduce the number of pathogenic microorganisms, spoilage bacteria, and insects in food. By reducing these contaminants, irradiation helps to extend the shelf life of the product (Giteru et al., 2019). Irradiation is considered to be the most comprehensive or known form of radiation throughout the world, and it is used to improve food quality and safety. The main objective of food processing by irradiation is to reduce or eliminate spoilage bacteria and pathogens in food without causing organoleptic changes to the product (Putri et al., 2015). Irradiation technology is ionization radiation applied to food for certain purposes, such as preservation, controlling pathogenic microbes, or increasing shelf life. The irradiation dose used is important to consider because it can affect the physical, chemical, and biological properties of food ingredients. The irradiation dose to food varies depending on its intended use. Low doses (< 1 kGy) can be used to reduce the levels of pathogenic microbes, whereas high doses (> 10 kGy) are required to inactivate parasites or extend shelf life (Pillai and Shayanfar, 2018). Irradiation dose can affect the organoleptic quality (taste, aroma, and texture) of food ingredients. Therefore, dosage determination must consider the balance between microbial safety and sensory qualities (Lee & Kim, 2019).

Irradiation can reduce the number of pathogenic microbes; however, some microbes that are resistant to radiation may still be present in the product because of differences in the level of microbial resistance to irradiation (Irmanita et al., 2016). In particular, they are stored for a long time. Storage also affects the product quality. Storage of food products after irradiation plays an important role in maintaining their quality and freshness of these food products. Although irradiation helps to extend the shelf life of food, proper storage is still important. Irradiated products must be stored at appropriate temperatures and in secure packaging (Rai et al. 2020).

Temperature and humidity in the storage room affect the shelf life of the irradiated food. Maintaining proper temperature and humidity can prevent microbial growth and fat oxidation (Wani et al., 2016). Regular monitoring of the quality of irradiated food products during storage is essential. This can be achieved by periodically measuring the physical, chemical, and microbiological parameters of a product (Banerjee & Sharma, 2019).

Irradiated Asam Keumamah has the potential to be used as an emergency food and can be used as a supply for the Haj pilgrimage because it can be stored for 30 days at room temperature and retains the nutrients it contains. Irradiated Asam Keumamah with a low water content and an appropriate irradiation dose can be stored longer (Hadi et al., 2023). Therefore, this study aimed to determine the best combination of irradiation dose and storage time to maintain the quality of Asam Keumamah.

Methods

This experimental study used a Completely Randomized Design (CRD) with two factories, the first of which was irradiation treatment of 0kGy, 20kGy, 30kGy, 40kGy and 50kGy. The second factor is the storage time, which consists of 0, 1, 3, and 6 months at room temperature, and a Total Plate Count Test Analysis (general total bacteria test) was carried out. The variables examined in this study included total bacteria. This study was conducted between March and December 2023.

The main ingredients used were Asam Keumamah, Gamma Cobalt-60 rays, and microbial ALT testing using nutrient agar. The equipment used in this study was a vacuum sealer, glass beaker, test tube, measuring cup, dropper pipette, petri dish, oese, colony counter, autoclave, incubator, and irradiation device.

Cooked Asam Keumamah was placed in vacuum packaging and marked according to irradiation dose and shelf life. Asam Keumamah irradiation was carried out at the red and white gamma irradiators in the PUSPITEK area, Serpong, South Tangerang. Asam Keumamah Irradiation uses gamma rays and cobalt-60 (60Co). During gamma irradiation, the radiation source container is pulled out of the storage pool and placed in an irradiation chamber, a confined area with thick, leaded concrete walls, to prevent the release of gamma rays. The materials to be irradiated (including Asam Keumamah) were transported to the irradiation chamber by a conveyor system and exposed to gamma radiation for a predetermined period. Once the radiation process is completed, a mechanical pulley returns the radiation source to the storage pool, whereas the conveyor system moves the irradiated items out of the irradiation chamber (Hashim et al., 2024). Irradiated Asam Keumamah is stored for 0, 1, 3, and 6 months at room temperature.

The total bacterial test was carried out at the Ujung Bate Brackish Water Aquaculture Fisheries Center Laboratory (BPBAP-UB) Aceh Besar using the Total Plate Number method. Each 25 g sample was diluted in nutrient broth. Next, they were planted on nutrient agar and incubated at 27°C for 24 h. The number of colonies growing on the agar medium for each sample was counted using a colony counter multiplied by the dilution to determine the total number of bacteria in the sample. The number of bacterial colonies was converted to log 10 to facilitate the data analysis.

Data were analyzed using the factorial analysis of variance (ANOVA) RAL test and Duncan's test. This research has received approval and obtained Ethichal Clearance (EC), namely from the Health Assessment Ethics Commission, Faculty of Nursing, University of North Sumatra Number 2981/V/SP/2023.

Result and Discussion

Total Microbial Test Results

The results of the microbial ALT test for irradiated keumamah acid showed that the total number of microbes in keumamah acid without irradiation was (2,56 log cfu/g), which increased after stored for one month (5,56 log cfu/g), but decreased during storage. 3-6 months. Likewise, keumamh acid irradiated at 20 kGy did not show significant changes in bacteria. During storage, the surviving bacteria are able to adapt to the environment because keumamah acid still has a high water content. After the adaptation phase, growth occurs (log phase) and the maximum population is reached during storage at room temperature. Keumamah acid has good water and nutrient content for bacterial growth. However, after stored for 3-6 months, there was a decline because in the vacuum packaging condition, nutrients became increasingly depleted and the environment started to mix with the remaining bacterial metabolites so that the bacteria were unable to survive any longer (death phase). The results of the total microbial test are presented in Table 1.

Table 1. Bacterial count test results (mean ± SD) of irradiated keumamah acid with different shelf lives (log CFU/g).

Irradiation	Shelf Life (months)			
Dose	0	1	3	6
0 kGy	2,56± 1,83^{defg}	5,56± 0,41^{i}	3,62± 0,53^{f}	3,45± 0,46^{fg}
20 kGy	2,43± 0,61^{cdefg}	5,07± 1,25^{h}	2,93± 1,00^{efg}	3,31± 1,39^{fg}
30 kGy	1,72± 0,21^{bcde}	0,74± 0,86^{ab}	2,29± 0,75^{cdefg}	2,13± 0,25^{cdef}
40 kGy	2,50± 1,31^{fg}	0,73± 0,86^{ab}	0,69± 0,86^{ab}	1,51± 0,46^{abcd}
50 kGy	2,58± 0,79^{defg}	0,25± 0,52^{a}	0,50± 0,60^{ab}	1,12± 0,24^{abc}

Description: Data on average value and deviation. Different letters in the notation column indicate there is a difference ($p<0,05$)

Irradiation dose of 30-50 kGy is able inhibited microbial growth in Asam Keumamah. During storage at room temperature, there was a change in the total number of Asam Keumamah -producing bacteria. This amount is influenced by the effect of irradiation on the bacteria in food. Irradiation can inhibit bacterial growth in packaged food. Irradiation can damage bacterial DNA, causing tar to multiply and many bacteria to die. All Asam Keumamah samples that were irradiated at 30-50 kGy were safe to consume, even though they had been stored for 6 months, because the total number of bacteria was still below the SNI threshold.

Bacterial growth during storage is influenced by the ability of bacteria to adapt to the environment (Husna & Nurliana, 2020). Various studies have proven that various microbial contaminants, such as Salmonella spp., Staphylococcus aureus, Escherichia coli, Vibrio spp., Shigella spp., Listeria monocytogenes, Pseudomonas, Proteus spp., Streptococcus spp., and Bacillus cereus, are most often found in food, both in packaging and without packaging. Contamination can occur naturally through the food chain, production processes, distribution, and transportation. Poor hygiene and sanitation practices during food processing increase the risk of contamination, recontamination and food spoilage (Hashim et al., 2024).

Each microorganism has different sensitivity to gamma radiation. Some microorganisms are very difficult to inhibit or even kill with gamma radiation, but some microorganisms are easily killed by administering gamma radiation. The effect of radiation on living organisms is mainly related to chemical changes, which depend on the physical and physiological factors of the living organism. Physical parameters include the dose rate, dose distribution, and radiation quality. Physiological parameters include the temperature, water content, and oxygen concentration (Putri et al., 2015).

The results of the ANOVA test showed that the dose of Keumamah Acid irradiation had a significant effect on the number of microbes ($p<0,05$). Meanwhile, storage time (time) did not have a significant effect on the number of microbes ($p>0,05$). This explains why the higher the irradiation dose, the lower the number of microbes in the Keumamah Acid. The elimination of bacteria from food is generally related to the irradiation dose applied. Higher doses tend to eliminate more bacteria (Badejo and Afolabi, 2017). The higher the dose of irradiation, the lower the number of microbes. The decrease in the number of contaminating microbes is caused by the effect of irradiation, which can break chemical bonds in the bacterial DNA chain, thereby affecting the ability of bacterial cells to survive and reproduce (Sari et al., 2017).

The suitability of food ingredients that are safe for consumption is based on the microbial quality (Widyaningrum et al., 2022). Irradiation can extend the shelf life of food products by considering the principles of radurization, radiation, and adaptation, which must be considered. Radurization involves the use of low doses 0,1–1 kGy, which can inactivate parasites but do not completely eradicate microbial cells, thus potentially endangering food safety. Radicidation uses moderate doses of approximately 1–10 kGy, which are usually sufficient to eliminate harmful microorganisms in food, such as Salmonella spp. and Listeria monocytogenes. However, the resistance of microorganisms to radiation varies. Certain microorganisms, such as bacterial endospores, yeast, viruses, and fungi, are more resistant than the common microorganisms. Therefore, high doses are required to eliminate these microorganisms. Radapertization requires very high doses of 10 kGy or more, ranging from 30 to 50 kGy, which are generally used in food sterilization to destroy all microorganisms, including spores. This high dose of gamma irradiation is highly effective for completely sterilizing microorganisms. However, some researchers report a significant decrease in the sensory quality of food when exposed to high doses of Gamma irradiation (Hashim et al., 2024)

Storage time did not have a significant effect on the total microbes in Asam Keumamah. Microbial growth is inhibited by the ionization effect, which affects the ability of the bacterial cells to reproduce. Vacuum packaging also inhibits the growth of aerobic bacteria that contaminate foods. However, facultative anaerobic microbes such as lactic acid bacteria can develop. The decrease in the total number of microbial colonies in irradiated food was also caused by lactic acid bacteria inhibiting other microbes (Arham et al., 2019)

The growth of bacteria in fish is greatly influenced by temperature; the lower the temperature, the slower the growth of bacteria.

Furthermore, during storage, there are changes in decomposition, both by bacterial flora and proteolytic enzymes. The availability of oxygen also limits the growth of microbes, particularly aerobic bacteria (Kaiang et al., 2016).

The interaction between irradiation dose and storage time had a significant effect on the number of Asam Keumamah microbes (p= 0,0001). Asam Keumamah irradiated at 50 kGy can be stored longer; although there is an increase in the number of microbes during storage, it is not significant. The number of microbes in irradiated Asam Keumamah is still below the SNI; therefore, it is safe for consumption. The decrease in the number of microbes appears to be very significant; the greater the irradiation dose, the fewer the number of microbes, so that they can be stored longer. The irradiation dose applied to the food can significantly influence the number of microbes. High doses tend to kill or inactivate more microbes (Mahmoud & Al-Owainati, 2016).

Products with higher irradiation doses generally have a longer shelf life because of greater microbial reduction (Carocho & Morales, 2018). The storage of food products after irradiation affects the quality and freshness of food. Although irradiation helps to extend the shelf life of food products, proper storage remains crucial. Irradiated products must be stored at appropriate temperatures and in safe packaging conditions (Rai & Sinha, 2020).

A combination of appropriate irradiation doses and good preservation can produce products with a longer shelf life and very low microbial counts (Oliveira & Augusto, 2017). The interaction between the radiation dose and storage time can be complex. High doses of radiation can initially reduce the number of bacteria significantly; however, if the product is stored for a long time, bacteria can grow again if there is no additional preservation combination of irradiation and modified vacuum packaging (Memon et al., 2020). The total bacteria in processed fish is influenced by factors that are very important for maintaining the quality and safety of fish products. The quality of the raw fish used as raw material is the main factor influencing the number of bacteria in the processed products. Fish that have been previously contaminated will have higher bacterial counts (Kaiang et al., 2016)

The storage temperature is an important factor in controlling bacterial growth. Low temperatures can inhibit bacterial growth, whereas high temperatures accelerate bacterial growth. Cross-contamination during fish processing can increase the number of bacteria in a product. Therefore, strict hygiene practices during processing are essential. The use of preservatives, pasteurization, or other heat-processing methods can reduce the number of bacteria in the product. Processing methods, such as drying, also affect bacterial numbers (Sari et al., 2017).

Irradiation can affect the chemical components of food, including antioxidant compounds and vitamins (Matak et al., 2018). Irradiation at appropriate doses can reduce or even inactivate pathogenic microbes such as Salmonella and E. coli, which can cause disease in humans. This helps improve food safety (Strydom, 2020). Irradiation directly causes the breaking of chemical bonds in DNA and indirect effects of reactive oxygen due to water radiolysis on the cell membranes and chromosomes of microorganisms. Microbes have different levels of resistance to radiation, not only between different species but also between different strains within the same species. The resistance levels of gram-positive bacteria, such as Staphylococci, Enterococci, Clostridium, and Listeria, are slightly higher than those of gram-negative bacteria, such as Escherichia, Salmonella, Pseudomonas, Campylobacter, Yersinia, and Shigella. In addition, endospore microbes, yeast, viruses, and fungi are more resistant to radiation than are ordinary microbes. Therefore, a higher dose of irradiation is required to eliminate these microbes (Hashim et al., 2024).

This research still has limitations, namely the packaging process for Asam Keumamah only uses a vacuum sealer, so there is still a small amount of oxygen and water mixed with oil in the vacuum packaging, which affects the growth of microbes. Further research should be carried out to ensure that Asam Keumamah has low water and oil contents and is packaged using a device that can regulate the air content in the packaging (modified atmosphere packaging).

Conclusion

Asam Keumamah with irradiation has a real effect in eliminating the number of microbes, while storage time does not have a real effect on the number of microbes. The interaction between irradiation dose and storage time had a significant influence on the number of irradiated Asam Keumamah microbes. The higher the irradiation dose, the lower the number of microbes, and thus, the longer the storage time for Asam Keumamah. The combination of irradiation and vacuum packaging maintained the quality of Asam Keumamah during storage.

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