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Application of Irradiation as Pretreatment Method in the Production of Fermented Fish Paste

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Abstract: Fermented fish paste, an important source of cheap protein in some regions, is one of the most consumed condiments in the Philippines. Two species of fish, dilis (*Stolephrus commersonii*) and galunggong (*Decapterus macrosoma*) were subjected to irradiation as pretreatment method prior to production of bagoong isda. Pre-treatment at 3 and 10 kGy resulted in lower microbial load of the fermented fish paste. Irradiation of dilis resulted in an improved proteolytic degradation as compared with galunggong. There was no significant change in pH for both species. Acceptability scores of treatment at 3 kGy showed general improvement in the quality of the fish paste for dilis but not for galunggong. However, at 10 kGy, sample texture and overall acceptability was generally lower for dilis. SDS-PAGE electrophoresis of the water-soluble proteins indicate that at higher dose of radiation, fish proteins may have been converted to higher molecular weight aggregates which has affected fermentation properties of the substrates. Irradiation of fish prior to fermentation may result to chemical changes in proteins such as fragmentation, cross-linking, aggregation and oxidation and may likewise affect the chemical nature, physical state and organoleptic property of the final product.

Key words:

INTRODUCTION

Fish paste or bagoong isda is one of the fermented fish products in the Philippines. It is a well-known saline product obtained by partial fermentation of fish and widely produced and consumed in comparison with fish sauce. It is primarily used as a condiment and as staple food in some regions[11]. Since it contains 8-25% protein, it is a good source of protein in the Filipino diet. The meat-flavored fermented fish paste (*bagoong isda*) in Southeast Asia is comparable to the soybean-based (sauces, paste or *miso*) in Japan and China[7]. *Stolephorus commersonii* (dilis) and *Decapterus macrosoma* (galunggong) are the two common fish species for production of fermented fish paste.

Typical problems encountered with the production of fermented fish paste include long fermentation time that usually takes from 3 weeks to 1 year[6] and high microbial/filth load. At present, the fermented fish paste industry is looking for a technique that could give high quality product with shorten fermentation time, high protein and low microbial load. In this technique, the problem of spoilage of fish due to its high perishability could be minimized. These developments in the processing of fermented fish paste production may help the country boost its export industry.

This study aims to demonstrate the utilization of gamma-radiation as a pretreatment step for the production of fish paste. Specifically, the objectives of this study are to characterize the physico-chemical and microbiological properties of the irradiated and non-irradiated fermented

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fish paste, determine its sensory attributes in terms of its color, aroma, consistency, texture, saltiness and over-all appeal and to determine the best radiation dose that could give the highest quality of fermented fish paste.

MATERIALS AND METHODS

Fish samples of *Stolephorus commersonii* (dilis) and *Decapterus macrosoma* (galunggong) were purchased from market and washed thoroughly with water. The samples were then divided into three lots for the different treatments (non-irradiated, 3 kGy and 10 kGy irradiated), sealed in a polypropylene bag and frozen overnight. The samples were then placed separately in a styrofoam boxes which were placed in single layer around the irradiator chamber. Dry ice was placed at the bottom to ensure low temperature. Irradiation was done in a Co-60 irradiator at Philippine Nuclear Research Institute (PNRI). One lot from each sample was not irradiated while the other lots were subjected to 3 kGy and 10 kGy irradiation.

After irradiation, the samples were thawed at room temperature for one hour and thoroughly washed with water. After draining, salt was added until a 3:1 fish:salt (w:w) ratio was obtained. The salted fish samples were then placed in sterilized sealed container and were allowed to ferment for 45 days at room temperature. The fermented samples were then thoroughly blended, stored at room temperature and then used for different tests to determine its quality and acceptability.

The different fermented samples were then analyzed for their physico-chemical properties namely protein content and pH using standard methods. Protein content was determined by Kjeldahl method and the pH using pH meter. Microbiological analysis was also done on the irradiated and non-irradiated raw fish paste samples using the pour plate technique (AOAC, 1993). Plates were incubated at 35°C for 72 hours and colony forming units (cfu) per gram was counted.

In addition, sensory evaluation of the fish paste samples by consumers was made to determine the acceptability of color, aroma, texture, consistency, saltiness and over-all appeal using a 9-point Hedonic scale. The samples were sautéed for two minutes before the test and were mixed together with unripe Indian mangoes. Panelists were instructed to rinse their mouth with water after tasting and eating each sample.

Statistical analysis using ANOVA (analysis of variance) was done to determine if significant difference was observed. For those that are significantly different, Duncan Multiple Range Test (DMRT) was then employed to determine which is different.

Lastly, the protein profile of the different treated samples of galunggong was determined. The total soluble protein content of galunggong was precipitated in 10% TCA and analyzed in SDS-PAGE Laemmli Gel Method[5] using 18% resolving gel and 4% stacking gel in 1.5 M Tris Buffer pH 8.8 (Sigma, St. Louis, MO, USA). A denaturant of sodium dodecyl sulfate and $-mercaptoethanol was mixed with the polyacrylamide and bis-acrylamide as crosslinker. Ammonium persulfate (APS) (50 µl of 10% APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) (5 µl of 10% TEMED) were added to start the polymerization. Immediately, the gel solution was casted into a Bio-Rad gel casting system. The protein fractions were then loaded into the wells of the gel at a concentration of 50 µg/ml and electrophoresed with 1X running buffer in a Mini-PROTEAN 3 CELL (BioRad Laboratories, Cambridge MA). A standard low range molecular weight marker (Sigma, St. Loius, MO, USA) was used to determine the molecular weight of the proteins form in isolated samples. After electrophoresis, the gels were stained with Coomassie Blue.

RESULTS AND DISCUSSIONS

The application of gamma radiation as a pre-treatment for the production of fermented fish paste was determined in this study. Irradiation was employed in the study because of its potential to answer the improvements of fermented fish paste in terms of shorter fermentation time, improvement of organoleptic properties and microbiological safety.

Observation of the samples immediately after irradiation and addition of salt showed no visible difference with that of the non-irradiated. This observation remained the same for three weeks. After 45 days of fermentation, it was observed that the dilis and galunggong fish paste samples irradiated at 10 k Gy have retained the original form of the raw material. Both samples irradiated at 10 kGy has less disintegration and thicker consistency upon blending than the other treated samples. As the radiation dose increases, the lighter is the color of the blended sample.

Results of physico-chemical analysis of the different samples in terms of pH and protein content are shown in Figure 1 and 2. Gamma-irradiation had no effect on different fish paste samples in terms of pH. No significant difference was observed in both non-irradiated and irradiated samples. Irradiation works mainly on water molecules present in the food matrix. It does not interfere the acidity or alkalinity of the food product as shown by the result where the pH of the samples did not vary significantly.

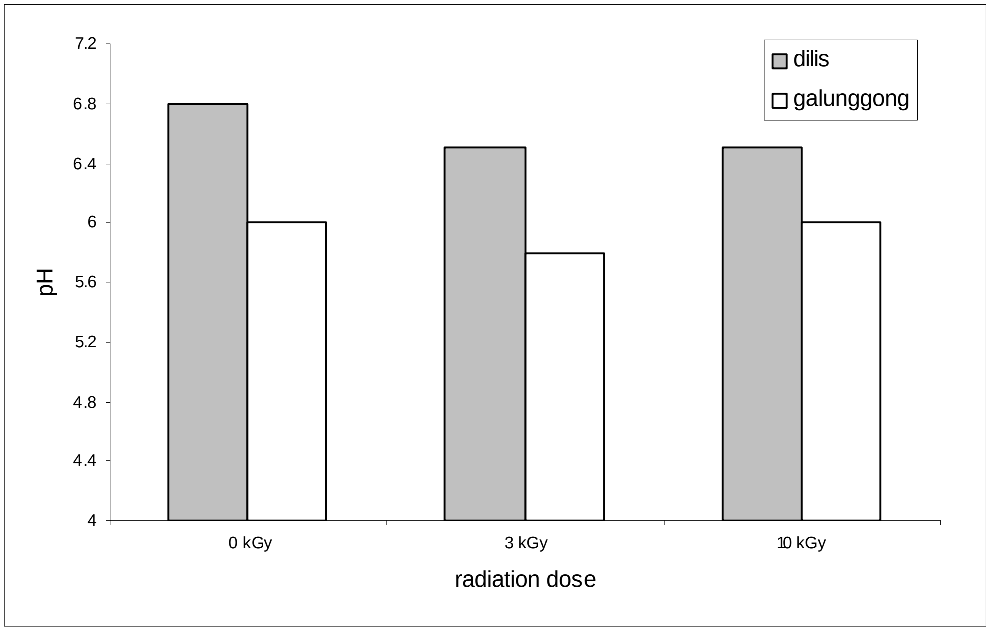

Fig. 1: Average pH of the fish paste in three treatments

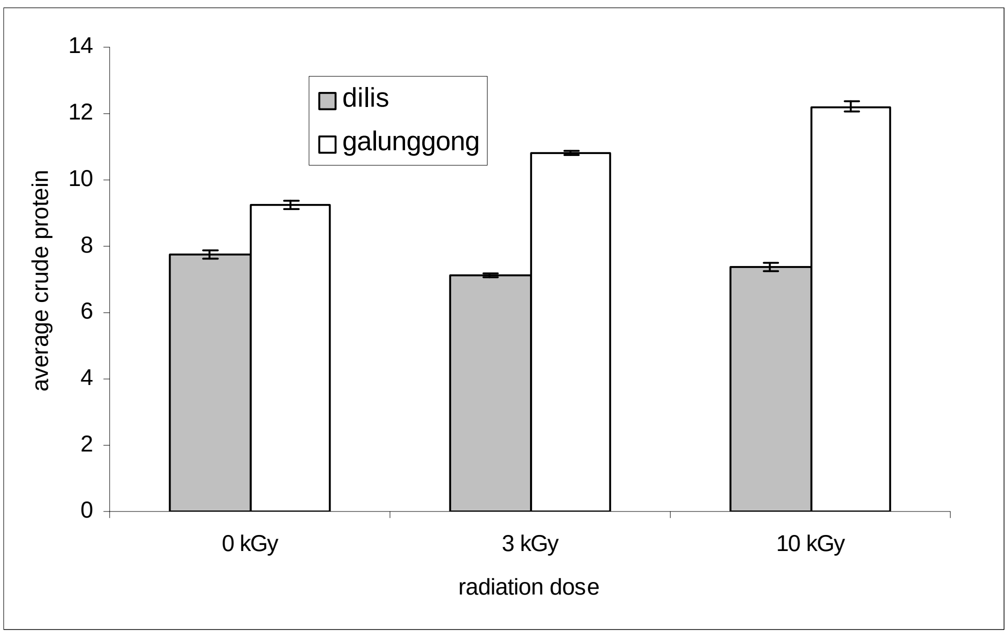

Fig. 2: Average crude protein (% wet basis) of the fish paste in three treatments

For the crude protein content, dilis fish paste sample did not vary significantly as gamma radiation is applied. However, this is not the case for galunggong fish paste sample, which was observed to significantly increase in its crude protein content as radiation is increased.

The main action of fermentation in fish paste production is partly liquefaction. Considerable moisture is extracted from the fish after salting because of high osmotic pressure. Proteins are hydrolyzed and dissolved in the brine as storage period continues. Therefore, the product contains hydrolytic products of the fish proteins as well as some unhydrolyzed substrate. Irradiation could lead proteins to multiple excitations/ionizations within the molecule[8]. The adsorbed energy could be transferred that may make one site more sensitive and thus may induce bond breakage thus leading to formation of more soluble

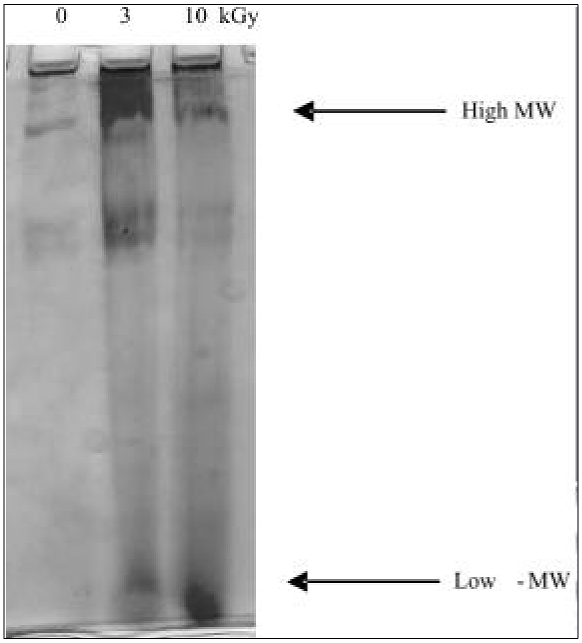

Fig. 3: SDS-PAGE profile of soluble protein of galunggong showing the formation of high and low-molecular weight protein aggregates.

protein. Therefore, the greater the radiation dose applied, the greater the protein that will be extracted.

However this mechanism could be offset or slowed down by the high salt concentration of the fish paste. At high concentrations, salts decrease the solubility of the protein molecule[10]. The salt molecules can shield the charged groups of protein, effectively making it a neutral dipole molecule. This is called the 'salting-out' process, which is the result of the salt effect on hydrophobic interactions of protein molecules.

Results from the crude protein content showed that the salting-out effect was not observed in dilis samples as no direct effect was noted as radiation is increased. However, for galunggong samples, the effect of radiation was seen more than the 'salting-out' effect because of the increase in protein content as radiation dose increases. Galunggong sample irradiated at 10 kGy had the highest crude protein in all samples.

The results on the microbiological test are shown in Table 1, which gives the weighted mean count of the total aerobic bacteria for both fish paste samples. There is no clear trend on the effect of radiation to microorganisms to both samples although results showed that the total plate count in both samples is still low. This could be attributed to the procedure used in the production of fish paste. After pretreatment with radiation, washing and draining of fish samples with water was still done that could have contributed to the microbial load. A further study on the microbial load of samples immediately after irradiation could be done to see the effect of irradiation on microorganisms, especially the lactic acid bacteria involved in fermentation.

The mean scores for acceptability of the fish paste samples using the Nine-Point Hedonic Scale of rating is tabulated in Table 2. Based on the results obtained, no significant difference was observed for all the attributes of the dilis sample. This could be due to the size of the fish, which is small that upon blending, no significant change could be observed in terms of texture and consistency. Salt could penetrate more evenly on the surface area with the small size of dilis samples. Color of the dilis fish paste sample was described as light gray while the aroma, texture and saltiness was 'just right' and consistency was halfway between thin and pasty. No significant difference was observed for all the attributes for the dilis fish paste sample.

However for the galunggong fish paste sample, the texture, saltiness and over-all acceptability of samples irradiated at 10 kGy was significantly different. Just like the other fish sample, size could be the reason for this. Blending of the samples may not have been equal for all parts of the fish thus the difference in texture. Salt may not have penetrated evenly on the sample for 10 kGy causing a more salty taste because of the undissolved rock salt. These noticeable differences for texture and saltiness could have affected the over-all acceptability of the galunggong fish paste sample. No significant difference could be observed for the rating of aroma. In terms of color, sample irradiated at 10 kGy was found to be lighter than the other two samples. In a study done by Johnson[3], irradiation was found to affect the color and flavor of ground beef patties wherein there is an increased in discoloration as radiation level increases. The non-irradiated sample has the thinnest concistency. This could be true as study have shown that globular proteins aggregate and could result to increase in viscosity which consequently affect texture and consistency as radiation is applied[9].

Finally, based on the SDS-PAGE profile, radiation pretreatment led to the appearance of low- and high-molecular weight aggregates in the soluble fractions of the galunggong (Figure 3). The chemical changes that generally occur in biopolymers when irradiation was applied are fragmentation, cross-linking, aggregation and oxidation by oxygen radicals generated in the radiolysis of the water[2]. Radiation causes the irreversible changes at the molecular levels by breaking the covalent bonds of the polypeptide chain. Exposure of proteins to oxygen radicals results in both non-random and random

Table 1: Total Plate Count (cfu/g) of the fish paste in three treatments.

	0 kGy	3 kGy	10 kGy
Dilis	1.2×10^{2}	3.6×10^{-1}	9.0×10^{-1}
Galunggong	1.0×10^{1}	2.5×10^{-1}	2.5×10^{-1}

Table 2: Summary of results for the acceptability test.

	Attribute	0 kGy	3 kGy	10 kGy
Dilis				
	Over-all	5.21	5.17	5.46
	Color	5.67	6.00	5.71
	Aroma	6.42	6.00	5.83
	Texture	6.08	5.75	5.75
	Consistency	5.88	5.79	5.92
	Saltiness	5.46	5.46	5.71
Galunggong				
	Over-all	5.84	5.68	4.28
	Color	6.60	5.96	5.52
	Aroma	6.40	6.24	5.96
	Texture	6.48	6.04	4.40
	Consistency	6.24	5.72	5.04
	Saltiness	6.64	5.84	4.20

fragmentation[4]. Fragmentation involves reaction of "-carbon radicals with oxygen to form peroxyl radicals that decompose to fragment the polypeptide chain at the "-carbon. At 3 kGy, crosslinking appears to be predominant, whereas at 10 kGy, fragmentation of the peptide chain is more obvious.

Conclusion: Pretreatment of fish samples using irradiation for the production of fish paste from dilis and galunggong were performed. Dilis samples irradiated at different doses did not give significant differences on all parameters tested. However, irradiation has an effect on the crude protein of galunggong and some sensory attributes like color, texture, saltiness and over-all acceptability.

Increased radiation dose increases crude protein of galunggong but its sensory attributes was also affected. The best radiation dose that gave the highest quality of galunggong fish paste by considering all factors was the galunggong samples irradiated at 3 kGy wherein a high protein content was obtained without compromising sensory attributes.

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