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Effects of Gamma Irradiation on Microbiological Quality, Protein and Amino Acid Profile of Edible Bird Nest Powder

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ABSTRACT

The effect of gamma irradiation was determined on the microbiological quality and amino acid profile of edible bird nest (EBN) powder. Seven doses of irradiation were used: 0.0 (control), 1.0, 2.0, 5.0, 7.5, 10.0, 20.0 and 30.0 kGy. The total plate count (TPC) of EBN samples that were inhibited or had TPC < 100 colony forming units (CFU).g^{-1} at 20.0 kGy was determined. An irradiation dose of 5.0 kGy was required to reduce the coliform and *Staphylococcus aureus* counts to below 100 CFU.g^{-1} and the yeast and mold counts to below 1,000 CFU.g^{-1}, while for *Escherichia coli*, 1.0 kGy was sufficient to inhibit or reduce the count to below 100 CFU.g^{-1}. *Salmonella* spp. was not detected in any sample. A minimum irradiation dose of 20 kGy was required to sterilize the EBN powder samples most effectively. The pH and water activity values of the samples were in the ranges 8.34–8.90 and 0.621–0.674, respectively. The microbiological quality changes of irradiated samples were caused by the gamma irradiation alone. Gamma irradiation at doses as high as 10.0 and 20.0 kGy did not produce significant changes in the amino acid profiles of the EBN samples compared to the non-irradiated samples. Gamma irradiation improved the microbiological quality of the EBN samples without affecting their amino acid profiles.
Keywords: amino acid profile, edible bird nest, gamma irradiation, microbiological quality, protein.

INTRODUCTION

There are more than 24 species of insectivorous, eco-locating swiflets distributed around the world, but only a few produce nests that are deemed edible by humans (Koon, 2000). The majority of edible bird nests (EBNs) traded worldwide come from two heavily exploited species, the White-nest swiftlet (*Aerodramus fuciphagus*) and the Black-nest swiftlet (*A. maximus*) according to Babji *et al*. (2011). EBNs used in this study were sourced from the swiftlet species commonly found in Malaysia (*A. fuciphagus*) which construct their nest with glutinous strands of starch-like saliva produced by a pair of salivary glands under their tongue; thereafter these birds mate and breed their young in the nest (Goh *et al*., 2001). According to Marcone (2005), the ascending order of composition in EBN is: lipid (0.14–1.28%), ash (2.1%), carbohydrate (25.62–27.76%) and protein (62–63%), making EBN a good source of protein. EBN contains

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18 types of amino acids needed by humans to produce energy in cells and to build the immune system through the production of globulin and antibodies, with aspartic acid and serine reported as the major amino acids (Marcone, 2005; Ma and Liu, 2012). The second largest concentration of nutrients in the EBN is carbohydrates (between 25.6 and 27.3%) consisting of 9.0% sialic acid, 7.2% galactosamine, 5.3% glucosamine, 16.9% galactose and 0.7% fucose (Kahtan and Weeks, 1969; Ma and Liu, 2012). The most abundant amino acids present are serine, threonine, aspartic acid, glutamic acid, proline and valine (Kathan and Weeks, 1969). In addition, many studies have indicated that various bird nests have abundant sialic acid-containing sugar chains (Martin *et al*., 1977; Pozsgay *et al*., 1987; Wieruszeski *et al*., 1987; Reuter *et al*., 1989; Kakehi *et al*., 1994). Sialic acid stimulates immune cells and enhances immune function (Dosher and Peng, 2010).

The drying process of EBN reduces the available water content for microbial usage and may cause the death of a small number of vegetative microorganisms, yeasts and molds, yet there remains a possibility of bacterial spores, yeasts and molds with high resistance surviving after the drying process (Miteva *et al*., 2008). Irradiation is widely emerging as a recognised safe and effective method of food preservation, being used to extend the shelf life of raw and processed foods in many countries worldwide. The main benefit of irradiation is widely accepted for eliminating microorganisms, insects or parasites capable of leading to food spoilage and toxicity and thus replacing chemical fumigants, without significantly affecting the physical state of the products (Delincee *et al*., 1998).

According to Food Irradiation Regulations (2011), no food except classes of food such as Class 1 (bulbs, roots and tubers), Class 2 (fresh fruits and vegetables), Class 3 (cereals and their milled products, nuts, oil seeds, pulses, dried fruits and their products), Class 4 (fish and fish products and frog legs), Class 5 (meat and meat products), Class 6 (dried vegetables, spices, condiments, dry herbs and tea); Class 7 (cocoa and cocoa products), Class 8 (dried food of animal origin) and Class 9 (other food not specified above), shall be treated with ionizing radiation for purposes specified in column (2) of the Third Schedule [Subregulation 8(2)] and the irradiation doses permitted for such treatment shall not be less than the doses specified in column (3) and more than the doses specified in column (4) of the said Schedule, respectively. No food shall be treated with ionizing radiation unless such treatment uses either one of the following sources: gamma rays from the radionuclides Cobalt-60 and Caesium-137; X-rays generated from irradiating apparatus operated at or below an energy level of 5 MeV; or electrons generated from an irradiating apparatus operated at or below an energy level of 10 MeV (Food Irradiation Regulations, 2011). Cobalt-60 and caesium-137 are two gamma energy sources used for food irradiation (Murano, 1995).

Gamma radiation has a higher energy compared with other radiation sources such as microwaves and ultraviolet and X-rays; thus, gamma radiation has a high penetration level and can penetrate into food easily (Riganakos, 2010). An irradiation dose for food at 10.0 kGy or less has been effective in destroying up to 99.9% of food-borne pathogens such as *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157: H7 and *Listeria monocytogenes* and thus is effective in reducing foodborne pathogens (Leonard, 2000). Trampuz *et al*. (2006) reported that the susceptibility of cells to irradiation is affected by several factors, among which are the rate of replication, intracellular water content, the amount of DNA, media composition, temperature, pH, oxygenation status and the ability of cells to repair DNA damage by irradiation. In 1980, the Joint FAO/IAEA/WHO Expert Committee on Food Irradiation concluded that the irradiation of food up to 10 kGy presents no toxicological hazard; hence, toxicological testing of foods so treated is no longer required and introduces no special

nutritional or microbiological problem (World Health Organization, 1981). However, irradiation at a dose of less than 10.0 kGy does not sterilize food. Irradiated foods still needs to be refrigerated and cooked before being eaten (Leonard, 2000). According to Murano (1995), dry food such as spices should be irradiated up to 30 kGy to remove microbial contamination and eliminate insect infestation. The current study was carried out to determine the effect of gamma irradiation on the microbiological quality, pH, water activity, protein and amino acid profile of EBN powder.

MATERIALS AND METHODS

Sample preparation

Raw, cleaned EBN samples collected from bird nest houses around Pahang and Terengganu, Malaysia were supplied by Koperasi Nest Excel Sdn. Bhd. (Kuala Lumpur, Malaysia). All the samples were ground using a universal cutting mill (Pulverisette 19; Fritsch GmbH; Idar-Oberstein, Germany) to a powder size of 0.25 mm. Approximately 5.0 g of the EBN powder samples was weighed and packed into separate polyethylene nylon packets.

Gamma irradiation

EBN powder was irradiated at doses of 0.0 (control), 1.0, 2.0, 5.0, 7.5, 10.0, 20.0 and 30.0 kGy using a cobalt-60 irradiator (Gammacell® 220 Excel; MDS Nordion; Ottawa, Canada) at a rate of 2.17 $kGy.hr^{-1}$. The times set for irradiation were 0.0, 0.4, 0.9, 2.3, 3.4, 4.6 and 9.2 hr.

Total plate count

The total plate count (TPC) was conducted using the method proposed by Roberts and Greenwood (2003) with some modifications. An EBN sample (2 g) was stomached with 18 mL maximum recovery diluents (MRD, Oxoid; Basingstoke, UK). Serial dilutions were carried out down to 1×10^{-7}. One mL aliquots of samples from each dilution were placed in Petri dishes in duplicates and mixed with molten plate count agar (PCA, Liofilchem; Roseto Degli Abruzzi, Italy). The solidified PCA plates/Petri dishes were incubated at 37 ˚C for 48 hr. Consecutive plates with numbers of colonies between 25 and 250 were counted after incubation and the colony forming units per gram ($CFU.g^{-1}$) were calculated using the formula stated in Roberts and Greenwood (2003).

Coliforms and *Escherichia coli*

Coliform and *E. coli* counts were performed according to the method proposed in Thermo Fisher Scientific Inc. (2001–2003) with some modifications. Sample dilution and pour plating methods similar to the above were used. A selective agar, *Brilliance E. coli/ Coliform* (Oxoid; Basingstoke, UK) was used to determine the coliforms and *E. coli* in the EBN samples. Each plate was examined for coliforms (pink colonies) and *E. coli* (purple colonies) after incubation at 37 °C for 24 hr. Consecutive plates with typical colonies below 150 were counted and the CFU per gram were calculated.

Staphylococcus aureus

A *Staphylococcus aureus* count was performed using the method proposed by Roberts and Greenwood (2003) with some modifications. Sample dilution and pour plating methods similar to the above were used. A selective agar (Rabbit Plasma Fibrinogen; Liofilchem; Roseto Degli Abruzzi, Italy) was used to determine the *S. aureus* in the EBN samples. Plates with black, grey or white colonies, surrounded by halo precipitation, were examined after incubation at 37 °C for 24 hr and the typical colonies below 150 were counted and the CFU per gram were determined.

Yeasts and molds

Yeast and mold counts were conducted referring to the method proposed in Roberts and Greenwood (2003) with some modifications. Sample dilution and spread plating methods

similar to the above were used. Aliquots (0.1 mL) of samples from each dilution were spread plated in duplicates on Dichloran rose bengal chloramphenicol agar (Liofilchem; Roseto Degli Abruzzi, Italy). The selective agar plates were incubated at 25 °C for 3 to 5 d. Plates containing less than 150 typical colonies were counted after incubation and the CFU per gram were calculated.

Detection of *Salmonella* spp.

Salmonella spp. detection was conducted referring to the method proposed by Roberts and Greenwood (2003) with some modifications. EBN samples (2 g) were each mixed with 18 mL of Buffered Peptone water (Liofilchem; Roseto Degli Abruzzi, Italy) and incubated at 37 °C for 18 ± 2 hr. Aliquot samples of 0.1 mL and 1.0 mL were added into 10 mL of sterile Rappaport Vassiliadis Soya (RVS, Liofilchem; Roseto Degli Abruzzi, Italy) and Selenite Cystine broth (SC, Oxoid; Basingstoke, UK), respectively. Incubation for the RVS and SC broths was at 42 °C and 37 °C, respectively, for 20-24 hr. The inoculated broths were streaked on xylose lysine deoxycholate (Liofilchem; Roseto Degli Abruzzi, Italy) agar and brilliant green agar (Liofilchem; Roseto Degli Abruzzi, Italy) using three-phase streaking in duplicate plates. The selective agars were then incubated at 37 °C for 20–24 hr. Five typical colonies were biochemically tested according to Roberts and Greenwood (2003) and the results were recorded as the presence or absence of *Salmonella* spp. in 2 g samples.

pH value determination

EBN samples (1.0 g) exposed at different gamma irradiation doses were dissolved in 5 mL of distilled water and the pH values were measured using a pH meter (S220; Mettler Toledo; Billerica, MA, USA).

Determination of water activity

The water activity (a_w) of EBN samples was determined using a water activity meter (CX-2; AquaLab; Pullman, WA, USA). Each EBN sample (1.0 g) was placed in a disposable container until the bottom of the container was fully covered before being placed in the machine to determine the a_w reading.

Determination of crude protein content

A modified Kjeldah method was used according to the Association of Official Analytical Chemists (1990). About 0.1 g of sample was weighed into an 800 mL Kjeldahl flask, following which 4–5 g of catalyst mixture made up of a ratio 10:1, potassium sulphate (K_2SO_4; Systerm®; Selangor, Malaysia):copper sulphate ($CuSO_4$; Systerm®, Selangor, Malaysia) were added. Then, 12.5 mL concentrated sulphuric acid (H_2SO_4; R&M Chemicals; Selangor, Malaysia) was added and the sample solution turned black immediately. The flask was placed on a heating block connected to a digestion rack and brought to boil at 420 °C for digestion to occur. The sample colour changed to blue-green indicating that the digestion process was completed. The sample solution was left to cool at room temperature.

At the same time, 25 mL of 4% boric acid (Sigma Aldrich; MO, USA) solution was prepared in an Erlenmeyer flask to which 75 mL of distilled water was added slowly to the cooled sample in the Kjeldahl flask. The end of the distillation tube was immersed in 4% boric acid solution to trap the distilled ammonia gas. 40% sodium hydroxide (NaOH; Sigma Aldrich; MO, USA) was added to neutralize the digestion products. During the distillation, the boric acid turned green. It was then titrated with 0.1 N hydrochloric acid (HCl; R&M Chemicals; Selangor, Malaysia) until the solution turned pink. The crude protein content of the EBN sample was calculated using the formulas as stated in AOAC (1990).

Determination of amino acid profile

Determination of each amino acid profile was carried out referring to the method proposed by Fontaine (2003). Amino acid profiles of EBN

samples were determined using high performance liquid chromatography (HPLC; Waters 2690; Waters Co.; Milford, MA, USA), a scanning fluorescence detector (Waters 470; Waters Co.; Milford, MA, USA) and an AccQ Fluoresence Reagent Kit (Waters Co.; Milford, MA, USA). Mobile phase A (AccQ Taq Eluent A) was prepared by diluting 100 mL of AccQ Taq Eluent A with 1,000 mL deionized water and filtered via a nylon cellulose membrane (size 0.45 µm). The diluted mobile phase A was later digested in an ultrasonic bath for 15 min. Mobile phase B was prepared by diluting 600 mL HPLC grade acetonitrile (Merck; Darmstadt, Germany) with 400 mL of deionized water and then filtered via a nylon cellulose membrane (size 0.45 µm). Internal standard (2.5 µmol mL^{-1} α-aminobutyric acid (AABAl; Sigma Aldrich; MO, USA) was prepared by diluting 0.5156 g AABA with 20 mL 0.1 N HCl (R&M Chemicals; Selangor, Malaysia) in a 100 mL volumetric flask. The solution mixture was added with deionized water to a volume of 100 mL to produce 50 µmol mL^{-1} AABA. About 0.5 mL of 50 µmol mL^{-1} AABA was pipetted in the 10 mL volumetric flask and was added with 0.1 N HCl to a volume of 10 mL to produce 2.5 µmol mL^{-1} AABA.

In order to determine all types of amino acid profile except for cysteine, methionine and tryptophan, a 0.003 g EBN sample was weighed in a hydrolysis tube and 15 mL 6 N HCl was added. The tube was tightened and inverted before placing it in an electric oven at 110 °C for 24 hr for hydrolysis processing. The sample was then cooled at room temperature before being filtered into a 100 mL volumetric flask using a Whatman No. 1 filter paper. About 400 µL of 50 µmol mL^{-1} of AABA standard solution was added into the volumetric flask and the sample was made up to a volume of 100 mL with deionised water. A 2 mL aliquot sample was filtered using a syringe through a cellulose acetate membrane (0.2 µm size) into a centrifuge tube (2 mL). A 10 mL sample was then injected into the HPLC instrument (Waters; Milford, USA).

The cysteine and methionine profiles were determined using the acid hydrolysis method (Wathelet 1999). A 0.003 g of EBN sample was weighed into a hydrolysis tube and placed in an ice bath. Two mL of newly prepared cold formic acid (Sigma Aldrich; MO, USA) was added into the tube before transferring into a cold room for 16 hr. Formic acid was prepared by adding formic acid (CH_2O_2) and hydrogen peroxide (H_2O_2; Sigma Aldrich; MO, USA) in the ratio 9:1. After 16 hr, 0.4 mL of hydrogen bromide (HBr; Sigma Aldrich; MO, USA) was added and placed in a cold room for 30 min. The samples were then dried in a hot air oven (Memmert; Schwabach, Germany) at 100 °C.

After the drying process, sample treatment was continued by adding 6 N HCl into the hydrolysis tube. The tube was tightened and inverted before placing it in the electric oven at 110 °C for 24 hr for the hydrolysis process. The tube sample was then cooled at room temperature before being filtered into a 100 mL volumetric flask using a Whatman No. 1 filter paper. About 400 uL of 50 umol mL^{-1} of AABA standard solution was added into the volumetric flask and sample was made to a volume of 100 mL with deionised water. A 2 mL aliquot sample was filtered using a syringe through a cellulose acetate membrane (0.2 um size) into a centrifuge tube (2 mL). A 10 mL sample was then injected into the HPLC apparatus.

Statistical analysis

The results obtained from the above tests were subjected to statistical analysis using the SPSS statistical software package (version 14; SPSS Inc; Chicago. IL, USA) to determine any significant differences among samples. Data analysis was carried out using one-way analysis of variance with Duncan's multiple range tests and a grand linear model using a multivariate test. Differences in means between the samples were determined at the 95% confidence level ($P < 0.05$).

RESULTS

Microbiological quality of edible bird nest

The TPC, coliform and *E. coli*, *S. aureus*, yeast and mold counts and detection of *Salmonella* spp. were determined on the non-irradiated and irradiated EBN samples. Eight levels of irradiation doses (including a control) were used to determine the appropriate dose for sterilization of the EBN samples.

According to Table 1, the TPC in both samples obtained from two different locations in the Malaysian Peninsular (Pahang and Terengganu), decreased significantly with an increasing dose of gamma irradiation. The TPC of EBN samples from Pahang and Terengganu irradiated at 10 kGy was reduced to 3.84 and 3.88 log CFU.g^{-1}, respectively. At an irradiation dose above 20.0 kGy, the TPC was no longer enumerated or was below 100 CFU.g^{-1}.

The coliform counts of the non-irradiated EBN sample from Pahang were significantly higher compared to the EBN samples from Terengganu with 5.95 and 5.61 log CFU.g^{-1}, respectively. At irradiation doses of 1.0 and 2.0 kGy, the coliform counts in the EBN samples from Pahang were significantly reduced compared to the samples from Terengganu. As shown in Table 1, the coliform counts of EBN samples from Pahang and Terengganu were significantly reduced by 2.79 and 2.08 log CFU.g^{-1}, respectively, after irradiation at 2.0 kGy. By increasing the irradiation dose to 5.0 kGy, the coliform counts in both samples were not enumerated or were below 100 CFU.g^{-1}.

For EBN samples from both Pahang and Terengganu, the *E. coli* counts were not enumerated or were below 100 CFU.g^{-1} at doses as low as 1.0 kGy. There was no *E. coli* colony detected in the EBN samples irradiated at 2.0 kGy and higher.

There was no significant difference in the *S. aureus* counts between the EBN samples from Pahang and Terengganu irradiated at doses of 0.0, 1.0 and 2.0 kGy. At 2.0 kGy, the *S. aureus* counts in the samples from Pahang and Terengganu were reduced by 1.96 and 1.88 log CFU.g^{-1}, respectively, and the pathogen was not enumerated or was below 100 CFU.g^{-1} for EBN samples at 5.0 kGy and higher.

Salmonella typhimurium strain (ATCC™ 14028®) was used as the positive control for the detection of *Salmonella* spp. The results showed

Table 1 Microbiological counts of irradiated edible bird nest powder at different irradiation doses.

Irradiation dose (kGy)	Total plate count (log CFU.g^{-1})		*Coliforms* (log CFU.g^{-1})		*E. coli* (log CFU.g^{-1})		*S. aureus* (log CFU.g^{-1})		Yeasts and molds (log CFU.g^{-1})	
	Pahang	Terengganu	Pahang	Terengganu	Pahang	Terengganu	Pahang	Terengganu	Pahang	Terengganu
0.0	7.64 ± 0.00 ^{aA}	7.66 ± 0.02^{aA}	5.95 ± 0.01^{aA}	5.61 ± 0.05^{aB}	2.47 ± 0.10^{A}	2.67 ± 0.10^{A}	4.55 ± 0.04^{aA}	4.66 ± 0.05^{aA}	5.10 ± 0.02^{aA}	4.80 ± 0.01^{aB}
1.0	7.09 ± 0.03^{bA}	7.10 ± 0.03^{bA}	3.80 ± 0.00^{bB}	4.64 ± 0.03^{bA}	< 2.0	< 2.0	3.72 ± 0.01^{bA}	3.77 ± 0.14^{bA}	4.74 ± 0.01^{bA}	4.11 ± 0.10^{bB}
2.0	6.29 ± 0.02^{cA}	5.90 ± 0.05^{cB}	3.16 ± 0.02^{bB}	3.53 ± 0.02^{bA}	< 2.0	< 2.0	2.59 ± 0.16^{bA}	2.78 ± 0.05^{bA}	3.39 ± 0.13^{cA}	2.94 ± 0.34^{cA}
5.0	4.90 ± 0.02^{dB}	5.06 ± 0.03^{cA}	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 3.0	< 3.0
7.5	4.24 ± 0.02^{dA}	4.50 ± 0.10^{cA}	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 3.0	< 3.0
10.0	3.84 ± 0.05^{dA}	3.88 ± 0.04^{cA}	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 3.0	< 3.0
20.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 3.0	< 3.0
30.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 3.0	< 3.0

Values represent mean ± SD of duplicate samples, n=2.

$^{a-d}$ = Different lowercase superscript letters in the same column indicate a significant difference between doses ($P < 0.05$).

$^{A-B}$ = Different uppercase superscript letters in the same row indicate a significant difference between locations for each microbial count ($P < 0.05$).

CFU = Colony forming units; < 2.0 and < 3.0 log CFU.g^{-1} = No microorganisms were detected on plates at dilution 1 ×10^{-2} according to the microbial counting rules.

that there were no *Salmonella* spp. detected in the irradiated EBN samples from both Pahang and Terengganu.

The yeast and mold counts of non-irradiated EBN samples from Pahang (5.10 log CFU.g^{-1}) were significantly higher than the EBN samples from Terengganu (4.80 log CFU.g^{-1}) but decreased significantly with increasing irradiation doses. Gamma irradiation at a dose of 5.0 kGy and above was effective in the inhibition of the growth or reduction of the yeast and mold counts (below 1,000 CFU.g^{-1}) in the dried EBN powder samples. This result also showed that the suspected molds present in the EBN samples from Pahang and Terengganu were *Aspergillus* spp. and *Penicillium* spp. (Figure 1).

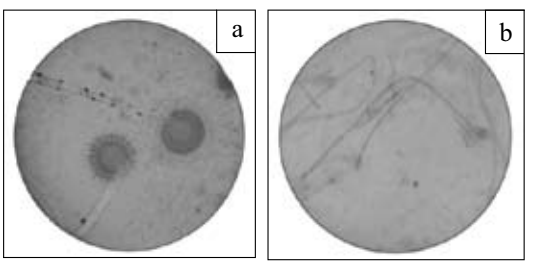

Figure 1 Microscopic view (× 100) of: (a) *Aspergillus* spp.; and (b) *Penicillium* spp. isolated from edible bird nest samples from Pahang and Terengganu, Malaysia.

pH values of edible bird nest

The pH value has a profound effect on the growth and viability of microbial cells (Ray and Bhunia, 2014). The pH value analysis was conducted to determine whether changes in the microbiological quality of the sample were entirely dependent on the gamma irradiation or due to the pH changes. The pH values of EBN samples from Pahang and Terengganu irradiated at 0.0, 1.0, 2.0, 5.0, 7.5, 10.0, 20.0 and 30.0 kGy ranged from 8.34 to 8.90 without any significant differences between location and the different irradiation doses applied (Table 2).

Water activity of edible bird nest

Physical stability and microorganisms in the EBN sample are closely related with the water activity which is one of the factors which affect microbial growth in food (Wareing. 2010). Table 3 shows the a_w values of EBN samples irradiated at different doses. The a_w values of EBN samples from Pahang ranged from 0.623 to 0.664, while samples from Terengganu ranged from 0.621 to 0.674. The a_w values differed significantly between doses and locations, but in general, there was no clear trend for an increasing or decreasing effect of a_w due to the irradiation dose.

Table 2 pH values of edible bird nest samples from Pahang and Terengganu irradiated at different doses of gamma irradiation.

Irradiation dose (kGy)	pH	
	Pahang	Terengganu
0.0	8.76 ± 0.00^{aA}	8.69 ± 0.04^{aA}
1.0	8.90 ± 0.04^{aA}	8.72 ± 0.08^{aA}
2.0	8.87 ± 0.14^{aA}	8.43 ± 0.18^{aA}
5.0	8.87 ± 0.11^{aA}	8.38 ± 0.17^{aA}
7.5	8.77 ± 0.10^{aA}	8.34 ± 0.13^{aA}
10.0	8.74 ± 0.10^{aA}	8.55 ± 0.08^{aA}
20.0	8.73 ± 0.11^{aA}	8.62 ± 0.04^{aA}
30.0	8.62 ± 0.00^{aA}	8.51 ± 0.04^{aA}

Values shown as mean ± SD of duplicate samples, n = 2.

$^{a–g}$ = Different lowercase superscripts in the same column are significantly different ($P < 0.05$).

$^{A–B}$ = Different uppercase superscripts in the same row are significantly different ($P < 0.05$).

Crude protein content of edible bird nest

Based on the results obtained from microbiological analysis, doses of 10.0 and 20.0 kGy were selected for the crude protein analysis. Samples which had been treated with the respective irradiation doses underwent crude protein analysis before amino acid profiling. Table 4 shows that only the irradiated EBN samples from Pahang had a significant increase in the crude protein content. There were no significant difference in the crude protein content between samples from Pahang and Terengganu.

Amino acid profile of edible bird nest

The EBN samples contained 18 types of amino acid (Table 5). The irradiation doses applied in this study did not change the amino acid profile of the EBN samples. The results indicated that gamma irradiation doses as high as 10.0 and 20.0 kGy did not significantly affect the amino acid profile of the EBN samples. Generally, there was no clear trend of an increase or decrease in the amino acid content profile of the samples with increasing gamma irradiation doses.

DISCUSSION

Microbiological quality of edible bird nest

From Table 1, the total plate count (TPC) in the EBN samples from both Pahang and Terengganu significantly decreased with increasing gamma irradiation doses. An irradiation dose of 20 kGy was needed to inhibit or reduce the TPC count in samples to below 100 CFU.g^{-1}. In 1986, Food and Drug Administration (1986) (FDA) approved a maximum dose of irradiation of

Table 3 Water activity of edible bird nest samples from Pahang and Terengganu irradiated at different doses of gamma irradiation.

Irradiation dose (kGy)	Water activity	
	Pahang	Terengganu
0.0	0.664 ± 0.000^{aB}	0.674 ± 0.001^{aA}
1.0	0.622 ± 0.001^{gB}	0.628 ± 0.000^{eA}
2.0	0.648 ± 0.000^{bA}	0.640 ± 0.001^{bB}
5.0	0.644 ± 0.000^{cA}	0.632 ± 0.000^{dB}
7.5	0.626 ± 0.001^{eB}	0.635 ± 0.001^{cA}
10.0	0.623 ± 0.000^{fA}	0.621 ± 0.000^{gB}
20.0	0.627 ± 0.001^{dB}	0.636 ± 0.000^{cA}
30.0	0.623 ± 0.000^{fA}	0.623 ± 0.001^{fB}

Values shown as mean ± SD of duplicate samples, n = 2.

$^{a–g}$ = Different lowercase superscripts in the same column are significantly different ($P < 0.05$).

$^{A–B}$ = Different uppercase superscripts in the same row are significantly different ($P < 0.05$).

Table 4 Crude protein content for edible bird nest samples from Pahang and Terengganu irradiated at different doses of gamma irradiation.

Irradiation doses (kGy)	Crude protein content (%)	
	Pahang	Terengganu
0.0	52.19 ± 0.25^{bA}	53.70 ± 0.88^{aA}
10.0	55.56 ± 0.22^{aA}	55.85 ± 0.81^{aA}
20.0	54.85 ± 1.32^{aA}	55.68 ± 0.05^{aA}

Values shown as mean ± SD of duplicate samples, n = 2.

$^{a–b}$ = Different lowercase superscripts in the same column are significantly different ($P < 0.05$).

^{A} = Different uppercase superscripts in the same row are significantly different ($P < 0.05$).

Table 5 Effect of irradiation doses on the amino acid profile of edible bird nest samples from Pahang and Terengganu.

Amino acid	Irradiation dose (kGy)					
	Pahang			Terengganu		
	0	10	20	0	10	20
Non essential amino acid (%)						
Aspartic acid	5.35 ± 4.62[a]	5.32 ± 0.05[a]	5.03 ± 0.49[a]	4.91 ± 0.10[a]	5.04 ± 0.27[a]	5.31 ± 0.56[a]
Serine	5.19 ± 0.24[a]	5.19 ± 0.17[a]	4.95 ± 0.50[a]	4.93 ± 0.17[a]	5.05 ± 0.32[a]	5.05 ± 0.11[a]
Glutamic acid	4.20 ± 0.18[a]	4.18 ± 0.25[a]	3.87 ± 0.36[a]	3.72 ± 0.09[a]	3.73 ± 0.22[a]	3.94 ± 0.28[a]
Glycine	2.23 ± 0.11[a]	2.20 ± 0.07[a]	2.11 ± 0.20[a]	2.14 ± 0.05[a]	2.17 ± 0.12[a]	2.16 ± 0.05[a]
Alanine	1.95 ± 0.06[a]	2.18 ± 0.07[a]	2.02 ± 0.25[a]	2.14 ± 0.02[a]	2.17 ± 0.24[a]	2.24 ± 0.26[a]
Proline	7.65 ± 3.97[a]	7.42 ± 2.57[a]	4.10 ± 0.18[a]	4.03 ± 0.09[a]	4.07 ± 0.17[a]	10.34 ± 9.03[a]
Tyrosine	2.71 ± 0.33[a]	3.09 ± 0.03[a]	2.98 ± 0.39[a]	3.05 ± 0.10[a]	3.13 ± 0.19[a]	3.05 ± 0.01[a]
Cysteine	1.34 ± 0.02[a]	1.43 ± 0.06[a]	1.46 ± 0.01[a]	1.76 ± 0.72[a]	1.54 ± 0.11[a]	1.60 ± 0.06[a]
Essential amino acid (%)						
Arginine	3.62 ± 0.37[a]	3.62 ± 0.21[a]	3.53 ± 0.50[a]	3.37 ± 0.08[a]	3.49 ± 0.18[a]	3.42 ± 0.13[a]
Histidine	2.09 ± 0.19[a]	2.11 ± 0.07[a]	2.05 ± 0.24[a]	2.01 ± 0.09[a]	2.03 ± 0.11[a]	1.99 ± 0.03[a]
Threonine	3.91 ± 0.17[a]	3.78 ± 0.16[a]	3.61 ± 0.38[a]	3.52 ± 0.09[a]	3.62 ± 0.20[a]	3.52 ± 0.06[a]
Valine	6.67 ± 0.57[a]	6.58 ± 0.23[a]	6.26 ± 0.63[a]	6.21 ± 0.13[a]	6.41 ± 0.35[a]	6.26 ± 0.07[a]
Methionine	0.32 ± 0.05[a]	0.35 ± 0.01[a]	0.33 ± 0.01[a]	0.32 ± 0.04[a]	0.32 ± 0.02[a]	0.32 ± 0.01[a]
Lysine	2.31 ± 0.13[a]	2.38 ± 0.09[a]	2.25 ± 0.29[a]	2.16 ± 0.06[a]	2.18 ± 0.11[a]	2.24 ± 0.14[a]
Isoleucine	2.07 ± 0.18[a]	2.01 ± 0.09[a]	1.94 ± 0.20[a]	1.90 ± 0.06[a]	1.92 ± 0.12[a]	1.90 ± 0.02[a]
Leucine	4.01 ± 0.34[a]	3.95 ± 0.18[a]	3.82 ± 0.41[a]	3.74 ± 0.09[a]	3.82 ± 0.23[a]	3.74 ± 0.01[a]
Phenylalanine	3.74 ± 0.34[a]	3.71 ± 0.16[a]	3.58 ± 0.40[a]	3.52 ± 0.09[a]	3.60 ± 0.20[a]	3.52 ± 0.02[a]
Tryphtophan	0.58 ± 0.02[a]	0.61 ± 0.10[a]	0.67 ± 0.07[a]	0.48 ± 0.00[a]	0.69 ± 0.00[a]	0.58 ± 0.00[a]
Total non essential amino acid (%)	34.24	34.63	30.05	30.05	30.39	37.11
Total essential amino acid (%)	25.70	25.48	24.51	23.86	24.59	24.07

Values shown as mean ± SD of duplicate samples, n = 2.

[a] = Different lowercase superscripts in the same row are not significantly different ($P < 0.05$).

30 kGy for dry products (spices and dry vegetable seasoning). To consider EBN as a dry product, the minimum 20.0 kGy of irradiation dose to sterilize the EBN powder was within the range set up by the FDA. This result was consistent with the studies conducted by Pezzutti *et al*. (2005), who reported that the TPC in onion flakes decreased to 3.0 log cycles with irradiation doses between 7.0 and 11.0 kGy and further reduced to below 10 cell.g^{-1} at radiation doses between 15.0 and 23.0 kGy.

A similar result was reported by Gezgin and Gunes (2007) where a total plate count of samples of *çiğ köfte* (an Armenian raw meat dish) decreased significantly with increasing gamma irradiation doses. Irradiation at 6.0 kGy has reduced the bacterial population in *çiğ köfte* to 6.0 log cycle. This phenomenon was due to the effect of ionizing radiation applied to the cell's genetic material of the bacteria and this likely resulted in cell death and a permanent change in the daughter cells thus retarding the ability of the bacteria to produce viable cells (Min *et al*., 2003; Magalhäes and Magagna, 2004).

The coliform counts of non-irradiated EBN samples from Pahang were significantly higher than in the EBN samples from Terengganu. At irradiation doses of 1.0 and 2.0 kGy, the coliform counts in the EBN samples from Pahang were significantly reduced compared to the samples from Terengganu. According to the Codex General Standard for Irradiated Foods, adopted in 1983 by the Codex Alimentarius Commission, the limits of irradiation doses for food were up to an overall average dose of 10 kGy (Anonymous, 1984). Gupta *et al*. (2011) reported that the irradiation doses needed to control the microorganisms in the sample were not only dependent on the microbial load in the sample but also on the resisting capability of the species. According to Table 1, the coliform counts in the EBN samples from Pahang and Terengganu were significantly reduced with increasing irradiation doses and were not enumerated or were below 100 CFU.g^{-1} at an irradiation dose of 5 kGy. Kumar *et al*. (2010) also reported a similar result, stating that an irradiation dose of 5.0 kGy was required to remove all coliforms in a sample of *gulva* and *chirata* herbs. This result was further reinforced by the data from Al-Bachir and Zeinou (2009) who stated that irradiation doses of 2.0, 4.0, and 6.0 kGy effectively reduced the coliforms present in camel meat to below 10 CFU.g^{-1}.

E. coli is sensitive to gamma irradiation, as a 1.0 kGy irradiation dose was sufficient to inhibit or reduce the count to below 100 CFU.g^{-1}. The *E. coli* populations decreased with an increased irradiation dose as the deactivation of the species was dependent on the dose applied, the phase of growth and the physiological state of the bacterial cell (Stapleton, 1995; Spoto *et al*., 2000; Gezgin and Gunes, 2007).

The counts of *S. aureus* in the samples from Pahang and Terengganu reduced with increasing irradiation doses and were inhibited or reduced to below 2.0 log CFU.g^{-1} in samples at doses above 5.0 kGy. This result was consistent with the studies conducted by Spoto *et al*. (2000) who reported that the counts of *S. aureus* in poultry meat decreased with increasing irradiation doses. The *S. aureus* count reduced to 2.0 and 6.0 log cycles at 2.0 and 4.0 kGy doses, respectively. The same trend was also shown by Arzina *et al*. (2012) who stated that irradiation doses at 2.0, 4.0 and 6.0 kGy reduced the counts of *S. aureus* in pizza by 2.0, 3.0 and 5.0 log cycles, respectively. *S. aureus* was not detected in the pizza sample irradiated at 8.0 kGy. Based on the study by Nortjé *et al*. (2006), irradiation doses ranging between 3.0 and 5.0 kGy were sufficient to inhibit *S. aureus*, even though the bacterial load present in the sample was high (1×10^5 to 1×10^6 CFU.g^{-1}).

EBN is classified as a dry product; therefore the presence of yeasts and molds in the samples was estimated to be high. The yeast and mold counts of non-irradiated EBN samples from Pahang were significantly higher compared to the EBN samples from Terengganu, but decreased significantly with increasing irradiation doses.

Gamma irradiation at a dose of 5.0 kGy and above was effective in inhibiting or reducing the yeasts and molds in the EBN samples to below 1,000 CFU.g^{-1}. The results obtained were supported by the data reported by Arichi *et al*. (2007) that the yeast and mold counts in black cumin decreased to 1.0, 2.0 and 3.0 log cycles with increasing irradiation doses of 6.0, 7.0 and 8.0 kGy, respectively. Additionally, Pezzutti *et al*. (2005) reported that the yeast and mold counts in onion flakes decreased by 2.0 log cycles at doses between 7.0 and 15.0 kGy. As shown in Figure 1, the molds that were present in the EBN samples (*Aspergillus* spp. and *Penicillium* spp.) were destroyed at irradiation doses of 1.0 kGy and above. According to Saleh *et al*. (1988), *Aspergillus* spp. and *Penicillium* spp. have similar relative resistances to a gamma irradiation dose of 1.7–3.0 and 1.7–2.5 kGy, respectively.

pH values of edible bird nest

There were no significant differences in the pH values between EBN samples from Pahang and Terengganu in all irradiation doses applied (Table 2). Kim *et al*. (2010) reported that there was no change in the pH of cheese slices and pizza by gamma irradiation at doses of 0.0, 1.0, 3.0 and 5.0 kGy. The same results were also reported by Ham *et al*. (2009) with plain yogurt as there was no change in the pH of samples irradiated at a dose of 10.0 kGy.

Generally, yeasts and molds are able to grow at a lower pH compared with bacteria. Gram-negative bacteria are more sensitive to low pH compared with Gram-positive bacteria. The suitable pH range for mold growth is 1.5–9.0, while 2.0–8.5 for yeast growth, 4.0–8.5 for the growth of Gram-positive bacteria and 4.5–9.0 for the growth of Gram-negative bacteria (Ray and Bhunia, 2014).

Water activity of edible bird nest

Generally, there was no clear trend in the increase or decrease of the a_w-dose irradiation. The a_w values of the EBN samples from Pahang and Terengganu ranged from 0.623 to 0.664 and from 0.621 to 0.674, respectively. A study conducted by Pezzutti *et al*. (2005) on onion flakes reported that at irradiation doses of 7.0, 9.0, 11.0, 15.0, 18.0 and 23.0 kGy showed no significant difference or trends in a_w changes.

Research by Byun *et al*. (2008) showed that β-glucan, a long-chain polymer composed of glucose units, after irradiation is broken into small granules. According to Tapia *et al*. (2007), microbial growth is inhibited at a_w 0.61 and below. The range of a_w in the EBN samples was from 0.621 to 0.674 and did not reach 0.61 or lower; thus, the inhibitory effect of microorganisms in the samples was believed not to be caused by changes in a_w.

Crude protein content of edible bird nest

The crude protein content was determined in the EBN samples before the amino acid profile analysis. Table 4 shows the crude protein contents of the EBN samples from Pahang and Terengganu. Gamma irradiation doses of 10.0 and 20.0 kGy significantly increased the crude protein content of the EBN samples from Pahang.

Based on the study by Marcone (2005), EBN contained 62.0–63.0% protein, which is higher than in the EBN samples in the current study. This could have been due to different sources of EBN samples. Marcone (2005) used EBN samples from caves and farms in Kuala Lumpur, Borneo and the Sumatran islands, while the farmed samples used for the current study were from Pahang and Terengganu.

Darfour *et al*. (2012) have reported that there are no significant changes in the protein content of cowpea (*Vigna unguiculta*, L. Walp) by gamma irradiation. However, studies by A/Azim *et al*. (2009) and Bamidele and Akanbi (2013) reported that the protein content in ground nuts (*Arachis hypogaea*) and pigeon pea (*Cajanus cajan*) flour) increases with increasing doses of gamma irradiation, respectively. A/Azim *et al*.

(2009) concluded that the effect of irradiation on the crude protein content in the sample is closely related to insects and microorganisms as well as genetic factors, and not due to the direct effects of irradiation.

Amino acid profile of edible bird nest

EBN contains 18 types of amino acids needed by humans, including 9 essential amino acids—histidine, isoleucine, leucine, lysine, methionine, phenylalanine, treonine, tryptophan and valine (Ma and Liu, 2012). The amino acid profiles of the EBN samples from Pahang and Terengganu were consistent with the range specified in the study by Marcone (2005) except for proline. The results showed that gamma irradiation at doses as high as 10.0 and 20.0 kGy did not significantly affect the amino acid profiles of the EBN samples from Pahang and Terengganu. The proline content in both the EBN samples from Pahang and Terengganu was higher than the range specified by Marcone (2005). The differences in the proline content may have been due to differences in the resources used in this study.

This study has shown that gamma irradiation at a dose as high as 10.0 and 20.0 kGy did not have a significant effect on the amino acid profile in the EBN samples. Generally, there is no clear trend in the increase or decrease in the content of the amino acid profile of samples with increasing doses of gamma irradiation. Matloubi *et al*. (2004) also proved that there was no significant difference in the amino acid profile in baby food protein with increasing gamma irradiation doses. There were only minimal changes in the amino acid profile due to the irradiation process; hence no significant effect was found on the nutritional value of the foods (Matloubi *et al*., 2004; Murano, 1995). The irradiation process does not create a serious problem from a nutritional standpoint because the amino acids themselves are protected from damage by irradiation due to their complex protein structure (Matloubi *et al*., 2004).

Based on Table 5, the total amino acid content was higher than the crude protein content. Heidelbaugh *et al*. (1975) compared three different methods to calculate the crude protein content in 68 types of food and found that the protein content in 40% of the samples varied depending on the methods of calculation. The calculation for the crude protein content in this study used a conversion factor of 6.25 from the Kjeldahl method. Multiplication of the Kjeldahl nitrogen content ranged from 5:30 to 6:38 depending on the type of food and the calculations based on the amino acid composition as determined by chemical analysis. The calculation of crude protein in the EBN samples using the conversion factor of 6:25 may have underestimated the protein content in the samples.

CONCLUSION

The total plate count for the EBN samples from Pahang and Terengganu decreased with increasing irradiation doses. At a dose of 20 kGy, the total plate count of the EBN samples was inhibited or reduced to below 2.0 log CFU.g^{-1}. Coliforms, *E. coli, S. aureus*, yeasts and molds were enumerated in the EBN samples. The isolated molds from the EBN samples were likely to be *Aspergillus* spp. *and Penicillium* spp. An irradiation dose of 1.0 kGy was required to reduce the *E. coli* count to a level that could not be enumerated, while for coliforms and *S. aureus* as well as for yeasts and molds, a 5.0 kGy irradiation dose was required to decrease these to undetectable levels. *Salmonella* spp. was not detected in the EBN samples from either location. Based on the microbiological tests, the effective dose to sterilize the EBN samples was 20.0 kGy. The crude protein content of the EBN samples from Pahang significantly increased at 0.0, 10.0 and 20.0 kGy. However there were no significant changes in the amino acid profiles of the EBN samples irradiated at 0.0 kGy, 10.0 kGy and 20.0 kGy from Pahang and Terengganu.

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