Oil Characterization and Aflatoxin Profile of Peanut Kernel Subjected to Gamma Irradiation

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Abstract—Peanuts are nutritious foods, however, following harvest and storage, these nuts are prone to mycotoxin contamination. Gamma irradiation is an effective technique in postharvest pest control to kill insects and inhibit mycotoxin biosynthesis. Thus, this study aimed to investigate the effect of doses of gamma ray on oil properties and mycotoxin profile of raw peanut seeds. Seeds were exposure to gamma radiation at 10 and 25 kGy and stored at ambient temperature for 6 months compared to nontreated seeds before storage. Color, hardness, oil content, total sugar, total protein content, peroxide value (PV), malonaldehyde (MDA) and aflatoxin analysis were assessed. According to this finding, no significant differences of color, hardness, oil content, PV, MDA were found among treatments. Aflatoxin inhibition and total sugars of treated gamma irradiation peanuts at 10 kGy were higher than treated peanuts at 25 kGy.

Index Terms—Gamma irradiation, peanut, aflatoxin, oil, lipid oxidation

I. INTRODUCTION

Peanuts have been considered as an extensively edible oil seeds which are a good source of healthy fat [1]. Much of peanut oil is in a beneficial form, 14% saturated fatty acids, 50% monounsaturated fatty acids with majority of oleic acid and linoleic [2]-[4]. In addition, peanuts are also rich in macro nutrient substances such as proteins and amino acids [4]. Although, it has been proved as a high nutritious value, peanut oxidizes and deteriorates easily due to a high content of unsaturated fatty acids (50%) as a consequence of a rancidity and a loss nutritional value during storage or display in the market [5]. Peanuts and peanut products also contain a high risk of mycotoxin contamination, especially aflatoxins [6], [7]. The mycotoxins are a serious threat for human health which has been reported as teratogenic effect, nephrotoxicity, tremorgenic toxin, nephrotoxicity, immunotoxin [1]. Therefore, it is important to pursue for a practical and safe sterilization method to inhibit or reduce the risk posed by aflatoxin contamination of peanuts.

The replacement of a chemical fumigants or a thermal treatment for the physical methods including gamma

irradiation becomes more acceptable worldwide. Gamma irradiation has been used as non-thermal treatments to preserve various products such as seeds and grains [8], food additives, meat, poultries, and seafoods [9]. This method is considered as the simple and effective decontamination technique [10]. It can inhibit mycotoxin [11], [12] and foodborne pathogens [13], [14]. According to reference [15], gamma irradiation at 10 kGY eliminated completely the fungi and reduced aflatoxin B1 in soybeans. Nevertheless, the gamma irradiation dose may affect the postharvest quality of seeds and grains. Irradiated nuts changed lipid component by increasing total saturated fatty acid and decreasing unsaturated fatty acid [16], because the liquid undergoes hydrolysis stage to produce more hyperoxides [4]. Conversely, there was no significant change in fatty acid composition of irradiated pine nuts [17] and in oxidation stability of soybean oil when the gamma irradiation dose was less than 20 kGy [15]. In addition, irradiation at low dose can maintain the nutritional components of peanut [14]. However, the contradictory on the effect of gamma irradiation on aflatoxin reduction has been revealed. Also, there are few reports using high doses of gamma irradiation to suppress aflatoxin in peanut seed during storage. Therefore, the purpose of this study was to investigate the physical properties oil characteristic and aflatoxin of raw peanut seeds treated with gamma irradiation after long term storage at ambient temperature.

II. MATERIALS AND METHODS

A. Sample Preparation

Peanut seeds were purchased from local market in Bangkok, Thailand and irradiated at Gamma irradiation plant at Thailand Institute of Nuclear Technology (TINT). In the comparison with the control (before gamma irradiation/non-treated peanuts), a half kilogram of shelled peanuts was irradiated at tentative doses of 10 and 25 kGy using GIC Multipurpose Irradiator (Power Plus System, UK). Dosimetry was performed using Harwell Red 4034 dosimeters (Harwell Dosimeters, UK). Control and irradiated seeds were stored at ambient temperature for 6 months. After the storage period, peanuts were sampled, rapidly turned into frozen forms by liquid nitrogen, and then stored at -20°C for the further analysis.

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B. Color Measurement

The color of testa and cotyledon were measured by L^* and *hue* angle based on CIE LAB system using handheld colorimeter (CR-400, Konica Minolta, Osaka, Japan).

C. Hardness

Hardness analysis was determined by using texture analyzer (TA.XT. plus, UK). Running model was set as compression mode with 1 mm/sec pre-test speed, 2 mm/sec test speed, 5 mm/sec post-test speed. Setting of trigger force started for detection at 0.049 N with 1 mm of the puncture. Hardness was expressed in max compression force.

D. Oil Content

Oil content was analyzed by AOAC [18]. Total finishing lipid product was collected to calculate the percentage of lipid and stored at 4° C for the further analysis of peroxide value, meanwhile the pellet was stored at -20° C for total protein and total sugar content.

E. Total Sugar Content

Total sugar content was determined by phenol sulfuric acid method describing by Dubois [19].

F. Total Protein Content

Total protein content was analyzed according to Bradford [20].

G. Malonaldehyde (MDA)

MDA was examined based on Health and Packer method [21]. Briefly, 1 g of peanut sample was mixed into 5 mL trichloroacetic acid 0.1 % w/v. The mixture was then homogenized and centrifuged. 0.5 mL supernatant was added with 1 mL 0.5% w/v thiobarbituric acid in 20% trichloroacetic acid then incubated in the water bath at 95°C for 30 min. Then, samples were placed in ice bath for 5 min and centrifuged again. The absorbance was calculated at both 532 nm and 600 nm. The MDA value was determined by using the extinction coefficient as $155 \text{ M}^{-1}\text{cm}^{-1}$.

H. Peroxide Value (PV)

PV was measured according to the method described by IDF standard 74A:1991 that has been discovered by Shantha and Decker [22]. 0.004 g of a crude oil was weighed and then mixed with 9.8 mL of working solvent (chloroform and methanol 7:3 v/v). After well mixing by vortex, 50 μ L of ammonium thiocyanate was added. Adding 50 μ L iron II solution into the solution was carried out before incubation for 5 min at room temperature under dark condition. The absorbance was read at 500 nm. Whole process was conducted in the subdued light within 10 min. The PV was expressed in meq peroxide per kilogram by formula (1) below:

$$PV = \frac{(As - Ab) \times m}{55.84 \times m_0 \times 2}.$$
 (1)

where: m – slope of the Fe³⁺ standard curve; m_0 – mass in gram of sample; A_s – absorbance of sample; A_b – absorbance of blank; 55.84 - atomic weight of Fe³⁺.

I. Aflatoxin Extraction

Twenty grams of the grinding peanut sample was extracted by 100 milliliters of 70% methanol with agitation at 100 rpm for 30 minutes. The suspension was filtrated through Whatman paper No.4.

J. ELISA Analysis

The aflatoxin content was analyzed by using the ScreenEZ Aflatoxin ELISA Kit (Siam Inter Quality Co., Ltd.; NANA-169450). Briefly, 1 mL of the sample suspension was diluted into 3 mL of the washing buffer. 50 µL of samples then was added into ELISA wells before adding 50 µL enzyme conjugation suspension (AFB1-HRP). Incubation was carried out under dark condition at 37°C for 30 min. Three times extra rinses were performed. After the last wash, 100 µL substrate was added into each well before the second incubation at 37°C for 10 min. All reactions inside ELISA wells were stopped by using stop reaction agents. Finally, ELISA wells were transferred into ELISA reader and the absorbance at 425 nm was utilized to detect aflatoxin. The results were expressed by comparing with aflatoxin B1 standard at the concentration of 0 - 2 ppb.

K. Statistical Analysis

All experimental works were proceed using a completely randomized design with 4 replications and the results were collected as averages and standard errors. The use of one-way analysis of variance (ANOVA) was run using Tukey's tests to divide means at P < 0.05 by Minitab Statistical Software Release 14 (Minitab Inc., PA, USA).

III. RESULTS AND DISCUSSION

A. Color

The effects of gamma irradiation on color of testa and peanut cotyledon are shown in Fig. 1 and Fig. 2. In the comparison with the control, L^* of irradiated peanuts was decreased slightly, but these values revealed no significant difference (P > 0.05). Similar to L^* value, no significant difference was found in *hue* angle on testa, while *hue* angle of cotyledon was reduced slightly after gamma irradiation. Reference [12] explained that a few changes in peanut color after irradiation (at 10 kGy) because a generated free radical during water radiolysis can accelerate oxidation that causes a darker color but no statistical differences.

B. Hardness

The results in Table I showed the reduction of hardness on irradiated samples once increasing irradiation doses. Despite of a decreasing trends, there was no statistical significance (P > 0.05). According to the previous study from reference [23], the textural attributes of carrots, potatoes, and beetroots truly changed once the exposures to irradiation doses from 3 - 10 kGy. Irradiation has been reported as alternating macromolecules such as starches, proteins in foods [24]. The technique hydrolyzes their chemical bonds of plant matters and degrades starches into dextrins. That reactions cause texture deformation. A loss in texture profile is unavoidable, but this influence is unimpaired in low water content sample [17].



Figure 1. Changes in L^* value of testa and cotyledon of peanut seeds irradiated with gamma irradiation at 10 and 25 kGy and stored for 6 months compared to non-irradiated seeds.

C. Oil Content

The impact of gamma irradiation on the percentage of oils presented in Table I. The oil content of irradiated peanuts was lower than that of non-irradiated samples with no significant differences among treatments. The similar findings were obtained by reference [25] who shown no effect of gamma rays at 2 kGy on stored peanuts. Reference [14] also reported the same observation and explained that a low moisture content of dehydrated peanut, especially commercial products, did not provide enough of water to promote the action of free radicals. It was therefore the unchanged of crude fat content was detected.

D. Total Sugar Content

Total sugar of gamma irradiated peanut at different doses is illustrate in Table I. It was noticed that the increase of the total sugar from 0 to 10 kGy. This outcome was contrary to some previous studies e.g. reference [14], [4]. In their reports, they concluded that no significant impact of gamma ray on total sugar of finished products. Obviously, the polysaccharides undergo the degradation during irradiation processes, particularly compounds: cellulose, starch, pectins, and gums [26]. Gamma irradiation interrupts polysaccharides resulting in glycosidic bonds breakage. A breakage in polysaccharide structures is in order to produce more reducing sugar content. However, according to reference [27], they explained that the relation between polysaccharide degradation and radiation doses is not a linearly proportionate. At low-dose of irradiation, the higher degree of degraded polysaccharides was detected than those under high-dose of irradiation. This finding accords with the earlier observation [26], which showed the depolymerization of cotton cellulose after gamma exposure. During introduction of gamma rays, cellulose is competent to acid hydrolysis therefore cell wall constituents are degraded and converted to sugars, whereas, total protein contents in peanut seed irradiated with gamma ray at 25 kGy did not differ with that of nontreated seeds (Table I). High doses of irradiation affect to enzyme activity such as α -amylase. Gamma irradiated at 2.0 kGy suppressed the activity of α -amylase in wheat seeds due to pH of seeds decrease to acidic condition [28]. On the other hand, under the high doses of gamma irradiation, the depolymerization shifts into alkaline condition, and slows down the polysaccharide degradation [26]. Thus, using high dose of irradiation (25 kGy) may inactivate enzyme activity, resulting in preventing carbohydrate degradation.

TABLE I. HARDNESS, OIL CONTENT, TOTAL SUGARS, TOTAL PROTEIN OF IRRADIATED PEANUT SEEDS AT 10 AND 25 KGY IN COMPARISON WITH CONTROL (NON-IRRADIATED PEANUT SEEDS).

Properties	Before irradiation	10 kGy	25 kGy	F-test
Hardness (N)	15.24 ±1.21	13.36 ±0.42	12.76 ±0.63	NS
Oil content (%)	51.15 ±2.19	44.45 ±1.83	47.25 ±0.78	NS
Total sugars (mg/gFW)	40.54 ± 3.31^{b}	55.62 ±2.22 ^a	39.46 ±2.24 ^b	**
Total protein (mg/gFW)	27.80 ± 0.84^{a}	22.54 ± 0.51^{b}	23.75 ± 0.60^{b}	**

Results are means $(n = 4) \pm \text{standard errors}$. Within a row, means annotated with different letters show significant differences between means (P < 0.01).



Figure 2. Changes in *hue* angle (H°) of testa and cotyledon of peanut seeds irradiated with gamma rays at 10 and 25 kGy and stored for 6 months compared to non-irradiated seeds.

E. Total Protein Content

As shown in Table I, the amount of proteins of irradiated peanut at 10 or 25 kGy was notably lower than non-irradiated seeds (P < 0.01). Results were similar to those previous reports by references [4], [29] who demonstrated that protein contents decreased with the increasing gamma irradiation doses. Gamma irradiation affected the solubility of proteins [29]. Reference [4] revealed that gamma rays adjust in protein structural such as disrupted the disulfide bones. This finding revealed that high irradiation dose caused protein degradation in peanut seeds.

F. Malonaldehyde (MDA)

The MDA is a low molecular weight compounds that is synthesized by the end of lipid peroxidation [30]. The result showed that the MDA content of irradiated seeds was higher than non-irradiated samples with no significant differences (Fig. 3). This result confirms the previous study by reference [4], the MDA content increased markedly in peanut seeds irradiated with gamma irradiation from 5 kGy to 10 kGy. Lipid peroxidation has been reported to be involved in the generation of reactive oxygen species (ROS). Gamma irradiation can create the reactive species including ROS and reactive nitrogen species (RNS) resulting peroxided lipid. The study of reference [31] explained the oxidative effect from ionizing radiation, especially gamma rays, can immediately modify cellular redox reactions within days and months after the initial gamma exposure treatment.

G. Peroxide Value (PV)

PV is method that uses to detect a primary product of lipid oxidation, hydroperoxides (ROOH). In this study, PV of peanut at 10, 25 kGy were 4.766 and gamma irradiated 4.819 meq peroxide per kilogram, respectively (Fig. 3), but, surprisingly, these results were lower and no significant difference with the control. Conversely, PV of gamma irradiated peanuts was raised with increasing gamma doses [17], [32], [16]. However, according to reference [33], their study found that PV value of irradiated almond skins reduced after storage period. And the decrease of this value by timing depended on the breakdown of primary initiation products of oxidation. In reference [32], they re-confirmed that hydroperoxides are unstable compounds which tend to be decomposed into secondary products such as aldehydes, ketones and hydrocarbons.



Figure 3. Changes in MDA and PV of peanut seeds irradiated with gamma rays at 10 and 25 kGy and stored for 6 months compared to nonirradiated seeds.

H. Aflatoxin Analysis

Aflatoxin of irradiated peanuts at 10 and 25 kGy were 4.73 and 33.01 ppb, respectively. In comparison with the non-irradiated samples, a value was 23.02 ppb, it indicated that using a moderate dose is better than highirradiation dose. Even though gamma irradiation is considered as a promising tool in postharvest pest controlling, high irradiation dose might cause metabolic oxidative stress to plant tissue and might reduce survival properties of plants. According to reference [34], they identified an adverse effect of using high gamma irradiation doses from 12.5 to 20 kGy on Moluccella seeds. The survival percentage of Moluccella reduced sharply after the gamma exposure. In here, at 25 kGy of gamma irradiation, peanuts were injured that can bring out the higher change for aflatoxin contamination or provides a suitable condition for fungus growth. According to Food and Drug Administration regulations,

the maximum level of aflatoxin which is found in peanuts and peanut products should be lower than 20 ppb. However, the trickier regulation belongs to EU Commission No. 165/2010 that was passed through in 2010. It mentioned that the total maximum residues of aflatoxin (B1, B2, G1, G2) in peanuts have to be under 15 μ g/kg, especially Aflatoxin B1 residues have to below than 8 μ g/kg. Gamma irradiation at 10 kGy showed the high effectiveness to inhibit the aflatoxin contamination (4.73 ppb), in the comparison to FDA and EU standards. This effectiveness broadly supports the previous works [12], [15].

IV. CONCLUSION

The present study set out to investigate the effect of gamma rays on lipid content and mycotoxin profile on peanuts and those findings have shown a useful of moderate-irradiation dose to preserve the raw peanuts. High treated gamma dose (25 kGy) changes the carbohydrate, the protein content, and injures peanuts as consequences of higher mycotoxin contamination. On the other hand, the moderate irradiation dose (10 kGy) is suitable for stored peanuts at the ambient temperature in order to inhibit mycotoxin and preserve macronutrient compounds as well. There is a change of MDA and PV content after gamma ray treatment, but no significant difference. To combine all analysis parameters, gamma irradiation at 10 kGy could be the optimum dose for stored peanut at the ambient temperature up to 6 months. A future study could assess the long-term effect of the combination of the use of gamma irradiation and packages.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ngoc X. B. Nguyen carried out the experiment. Apiradee Uthairatanakij and Pongphen Jitareerat developed the manuscript and the experimental design. Chainarong Rattanakreetakul performed the aflatoxin analysis. Natta Laohakunjit designed and carried out chemical analysis section, while Nattapon Kaisangsri processed the physical parameters.

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