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Enhancing Gamma Aminobutyric Acid (GABA) Content in Germinated Thai Hom Mali Brown Rice by Low Dose Electron Beam Irradiation

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ABSTRACT

Gamma-aminobutyric acid (GABA) produced by germination of brown rice, is a non-proteinogenic amino acid, which acts as a neurotransmitter in the brain, induces hypotension and inhibits cancer-cell proliferation. In this study, the GABA content of germinated Thai Hom Mali (*Oryza sativa* L.) brown rice irradiated at a dose of 1.0 kGy with 10 MeV electron beam and 5 MeV X-ray irradiation was determined by high performance of liquid chromatography (HPLC), after 0.5, 4, 8 and 12 months of storage. The content of bioactive compounds such as gamma oryzanol, total phenolic content and antioxidant activity were measured. The results showed that at 4-12 months of storage, the 10 MeV electron beam can consistently enhance GABA content with 1.9-2.1 times higher yield than non-irradiated groups at 4 and 8 months of storage, respectively. Conversely, GABA was not affected by X-ray irradiation. Neither electron beam irradiation nor X-ray irradiations affected gamma oryzanol, total phenolic content and antioxidant activities such as ferric reducing ability potential and DPPH free radical scavenging assay. These results reveal that electron beam irradiation at a dose of 1.0 kGy, energy of 10 MeV is an effective method for increasing GABA content in Thai Hom Mali germinated brown rice for up to 12 months of storage.

Keywords: Electron beam; GABA; Germinated brown rice; *Oryza sativa* L.; X-ray irradiation

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1. Introduction

Amino acids in rice grains are major nutrition sources required for metabolism and growth for the people in Asian countries where rice is the main primary food. Despite the flexibility and popularity of white rice, there has been an increase in consumption of brown rice because of its high values of bio-functional components such as dietary fiber, vitamins B1, B3, B5, B6, magnesium, phosphorus, manganese, iron, selenium, protein and carbohydrates. Germinated brown rice (GBR) is produced by soaking brown rice grain with water to promote germination. GBR could become a popular healthy food option, because the germination process in brown rice could induce digestion of grain protein, resulting in amino acids, peptides and accumulation of high amount of bioactive compounds, such as gamma aminobutyric acid (GABA), gamma oryzanol, tocopherol; antioxidant activities that would potentially improve health promoting functions in humans [1]. Glutamic acid is a non- essential amino acid, which can be found in some protein-rich plant foods such as beans, wheat, and cereals. During the germination process in brown rice, glutamic acid is catalyzed by enzyme glutamate decarboxylase, and GABA is produced via the decarboxylation process [2]. GABA is distributed mainly in the brain of the mammals and plays a role as an inhibitory neurotransmitter, which has relaxing and tranquillizing effects [3]. Furthermore, many studies have reported that GABA also has medicinal effects such as inducing antihypertensive effects, ameliorating blood flow in the brain, regulating sleep, enhancing memory and controlling the proliferation of tumor cells, [4-7].

Food irradiation is a technology that improves the quality, and extends the shelf life and safety of foods, by exposing food to ionizing radiation, such as gamma proton, X-rays generated from the machines at below energy of 5 MeV and accelerated electron generated by machines at below energy of 10 MeV [8]. Electron beam irradiation is a well-known sterilization process that acts through electron generation of cathode by the use of commercial electricity under vacuum conditions [9]. Moreover, electron beams from an electron accelerator can generate physical, chemical, and biological changes, which can delay maturation, inhibit sprouting, and promote materials conversion, achieving the purpose of food preservation. Electron beam irradiation has been widely used as a technology to improve protein quality and modify the structure of food protein such as wheat germ protein, pea protein hydrolysates and rice protein [10-12]. In the radiation process, proteins are affected by direct and indirect effects of ionizing, which results in conformational and structural changes. The most commonly occurring changes of proteins are polymerization and fragmentation or depolymerization of food protein [13].

Nowadays, irradiation is speculated as an effective pretreatment for maintaining the quality of white rice during storage. However, the results on early study showed that gamma irradiation at doses of 1.0 and 2.0 KGy influenced cooking quality of 3 brown rice varieties (medium grain and long grain) from Louisiana, USA., increased water uptake and amount of starch in residual cooking liquid with increasing dose levels. This suggests that the variety of rice contributes to cooking quality results from gamma irradiation [14]. The report of a recent study indicated that irradiation dose for improving quality of Chinese white rice grain should be less than 2.30 kGy, and the best cooking quality of rice will be kept when irradiated with 0.83 kGy [15]. For the purpose of infestation, an electron beam irradiation at low dose of 1.0 kGy can kill some pestilent insects and their eggs, such as rice weevil, red flour beetle, and *Rhizopertha dominica* in grains during storage. The study concludes that low doses of high-energy electron beam irradiation could be an effective method of pretreatment for

improving the quality and infestation of rice during storage [16].

The purpose of this work was to study the effect of high energy electron beam and X-ray irradiation at low dose of irradiation on maintaining and/or activating of GABA content- nonprotein amino acids incorporated in protein and the properties of antioxidant activity of germinated Thai Hom Mali brown rice (*Oryza sativa* L.) during storage. This rice cultivar has been globally recognized for its high quality. Whole rice grain enriched with GABA could be exported with the higher value. This study aimed to promote a new method that can increase value added and improve the quality of the Thai rice cultivar product.

2. Materials and Methods

2.1 Materials

Thai Hom Mali brown rice (*Oryza sativa* L.) was obtained from Sakon Nakhon province, in the northeastern region of Thailand. The brown rice was harvested in October 2021. The germination process was performed by soaking brown rice samples at 40 °C for 4 h and wrapping them in filter clothes for long incubation at 40 °C 20 h, 90% RH. During germination, the samples were washed every 6 h to prevent off-odor. The post-incubated samples were steamed in autoclave at 100 °C for 10 min. The GBR was dried at 80 °C for 180 min [10]. After drying, the GBR sample was packed at weight of 1.0 kg/packet in polyethylene (PE) bag with vacuum (13.5 × 17 × 3.5 cm). Samples were transferred and irradiated at Irradiation Center, Thailand Institute of Nuclear Technology (Public Organization), Techno-polis, Pathum Thani province, Thailand. The irradiated samples were re-packed with quantities of 2 packets /box with the sizes of 20 × 29 × 11 cm. per box. Six boxes of each treatment were performed with non-irradiated, electron beam treatment and X-ray irradiation treatment, for a total of 18 boxes. All samples were kept at room temperature (25 ± 3°C).

2.2 Electron beam and X-ray irradiation process

The GBR was irradiated at dose of 1.0 kGy, with electron beam and X-ray irradiation. The non-irradiated served as control. The sample was exposed to electron beam irradiation at a dose of 1.0 kGy, beam at energy of 10 MeV, pulse repetition frequency (RPF) setting 80 and conveyor speed of 10.560 m/min. The irradiation was performed on both sides for horizontal setting. The minimum and maximum absorbed doses were measured using B3 radiochromic dosimeter. The uniformity of X-ray irradiation was obtained in vertical setting at energy of 5 MeV, with the conveyor speed 0.503 m/min. The absorbed dose was measured by alanine blister dosimeter. After irradiation process was completed, the samples were stored at room temperature (25 ± 3°C).

2.3 Sample collection

Every 0.5, 4, 8 and 12 months of storage, the treatments of non-irradiated, electron beam and x-ray irradiated GBR samples were collected with the triplicates. GABA content, gamma oryzanol, total phenolic content, antioxidant activity such as ferric reducing ability potential (FRAP) and DPPH free radical scavenging assay were measured for all the samples. The color characteristics were measured with Hunter *L a b* color system.

2.4 GABA content

First, the sample was collected with weight at 300 g in the triplicate of samples at interval of storage periods. The GABA content assay was modified from that described in the previous report [17]. All GBR was ground to fine powder with the blender. One gram of the powdered sample was suspended with 0.3% sulfosalicylic acid and stirred with a magnetic stirrer for 30 min. Then, the extract was centrifuged and the supernatant was pipetted and added with 0.01 M sodium hydrogen carbonate ($NaHCO_3$)

and 3.98 mM Dabsyl-Cl. All samples were heated in water bath at 70 °C for 10 min. The solution was cooled down to room temperature. After that, the sample was pipetted and added with ethanol (HPLC grade) and 0.025 M potassium dihydrogen phosphate (KH_2PO_4). The extracts were filtered with a nylon syringe filter. The quantification of GABA content was analyzed by using high performance of liquid chromatography (HPLC) method. The HPLC separation of GABA content was performed with Supelcosil LC-DABS column. The mobile phase was gradient of 70% 0.025 M sodium acetate (pH 6.8) and 30% methanol (HPLC grade). The content of GABA was determined and calculated with the following formula:

$$GABA(mg) = \frac{CV}{10W}, \qquad (2.1)$$

where C is concentration of solution extract from HPLC in g/ml, V is final volume in ml., W is weight of sample in g.

2.5 Gamma oryzanol

The gamma oryzanol content was evaluated by the previous method for analysis of rice bran oil [18]. The GBR sample was ground and weighted accurately at 0.02 to 2.0 g so prepared in to 25 ml, of volumetric flask, marked with n-hexane. After that, the solution was sonicated for 15 min. The extract was centrifuged at 4,000 RPM, 25 °C for 5 min, then the supernatant was collected. Adjust the volume with n-heptane, and measure by UV-Vis spectrophotometer at the wavelength of 315 nm. The extinction values recorded must lie with the range 0.3-0.6. If not, the measurements must be repeated using more concentrated or more diluted solutions as appropriate.

The content of gamma oryzanol was calculated using Eq. (2.2).

$$\gamma\text{-oryzanol},\% = \frac{25A}{wE}, \qquad (2.2)$$

where w is mass of sample in g, A is extraction (absorbance) of the solution, E is specific extinction E1% 1cm = 359.

2.6 Total phenolic content and antioxidant activity

2.6.1 Preparation of the extracts

GBR samples were collected in 3 replicates (100 g each) at different intervals during the storage period. All samples were ground with a blender. Then, 0.5 g of the GBR sample was suspended in 10.0 ml of 60% ethyl alcohol. The extract was set at room temperature for 24 hrs. Then, the extract was filtered. The filtrate was concentrated by rotary evaporator under reduced pressure at 40 °C. After complete evaporation, the residue was weighed and determined for the total phenolic content and antioxidant activity.

2.6.2 Total phenolic content

The total phenolic content of the extract was estimated using the Folin-Ciocateau assay by Velioglu et al. [19]. The total phenolic content was determined using spectrophotometer at absorbance of 725 nm. The standard calibration curve was plotted using gallic acid as standard reagent. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g of sample.

2.6.3 Ferric reducing ability potential

The ferric reducing ability potential assay (FRAP) of the extract was measured by using a method of reduction ferric ions to form ferrous tripyridyltriazine complex described by Benzie and Strain [20]. The ferric reducing ability was measured by using UV-Visible spectrophotometer at absorbance of 596 nm. The antioxidant potential of the GBR samples was analyzed based on a calibration curve plotted using $FeSO_4.7H_2O$ as standard reagent. A ferric reducing ability potential was expressed as μmol $FeSO_4$/g.

2.6.4 DPPH free radical scavenging assay

The free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) assay was carried out to evaluate antioxidative activity. The assay method was slightly modified from the one described by Khattak, et al. [21]. The DPPH antioxidant activity was examined by using optimal UV-Visible spectrophotometer at wavelength of 517 nm. The free radical-scavenging activity was expressed as mg ascorbic acid equivalent (AAE)/g of sample.

2.7 Color characteristics

The GBR sample was collected and weighted at 100 g in 3 replicates during the storage period. The color characteristics were determined using Hunter *L a b* color system by colorimeter (Konica Minolta CR-300 Chroma Meter, Osaka, Japan). D65 was used as the standard average daylight source. Each treatment sample was measured with 10 replicates. The data was analyzed by analysis of variance (ANOVA) to compare color values of *L* scale: light to dark, *a* scale: green to red and *b* scale: blue to yellow between the non-irradiated and both of irradiated samples.

2.8 Statistical analysis

The data was collected and the data analysis was performed by one way ANOVA to test significant difference among the treatment at $p < 0.05$ and followed up by Duncan's new multiple range test with SPSS software.

3. Results and Discussion

3.1 GABA content

Regarding to the GABA content results (Fig.1), it was found that when the storage time increased from 4 to 12 months, GABA content of germinated Thai Hom Mali brown rice can be constantly enhanced by electron beam irradiation at a dose of 1.0 kGy, energy of 10 MeV. The enriched electron beam irradiated GBR sample contained 13.30 ± 0.21 to 9.84 ± 0.94 mg/100g of GABA at 4 and 8 months of storage, respectively, which was significantly higher yield than non-irradiated samples at 1.9-2.1 folds. Conversely, the results of X-ray irradiated sample showed that irradiated at 1.0 kGy, energy of 5 MeV with increasing time 4 to 8 months of storage did not significantly increased GABA content when compared with non-irradiated sample, but the increasing value was found at 12 months.

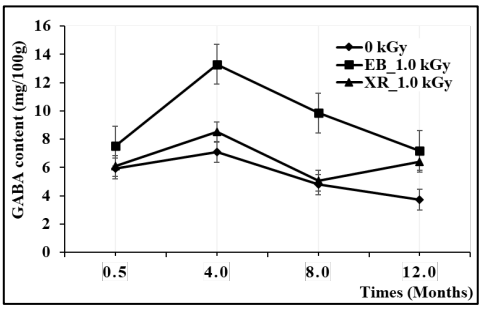

Fig. 1. GABA content of non-irradiated, electron beam and X-ray irradiated GBR samples.

Ionizing radiation is radiation with enough energy to remove tightly bound electrons from the orbit of an atom, causing that atom to become charged or ionized, which occurs in two forms: waves or particles. Electromagnetic (EM) radiation, the high frequency portion of the electromagnetic spectrum that includes X rays and gamma rays, is ionizing. Particulate radiation is a form of ionizing radiation. This consists of atomic or subatomic particles (electrons, protons, etc.) that carry kinetic energy, or mass in motion [22].

X-rays are similar to gamma rays. X-rays are produced by reflecting a high-energy stream of electrons off a target substance (usually one of the heavy metals) into food. X-rays are also widely used in medicine and industry to produce images of internal structures. An electron beam is a stream of high-energy electrons propelled from an electron accelerator into food, produced by the acceleration and conversion of electricity [23].

Irradiation can cause subsequent changes in food components, especially in carbohydrates, protein, lipids and vitamins.

When ionizing radiation passes through matter such as food, it loses energy. At the same time, the energy is absorbed and consequently leads to the ionization or excitation of the atoms and molecules of the matter [24].

GABA accumulation in plants has been reported via two pathways. In the first, the active BADH2 enzyme catalyzes γ-aminobutyraldehyde (GABald) to form GABA. In the second, the production of GABA occurs through the GABA shunt via irreversible alpha decarboxylation of L-glutamic acid. A recent study in 2017 showed that the accumulation of 2-acetyl-1-pyrroline (2AP) in Thai upland rice has been synthesized via L-proline metabolisms by inactive betaine aldehyde dehydrogenase (BADH2), which activates 2 AP accumulation. Meanwhile, active BADH2 inhibits 2 AP accumulation but activates γ-aminobutyric acid (GABA) content. The results show that the 2 AP content of gamma irradiated Thai upland white rice (germinated with a 24-h duration) was higher than non-irradiated rice for all gamma doses (20, 40, 60, 80, 100, 150, 200, 250 and 300 Gy), particularly at 20 Gy, which showed the highest level of 2 AP than non-irradiated rice. The reduction of the GABA content of irradiated rice was cause by an increase in gamma dose. However, some volatile compounds appeared in the irradiated rice at gamma doses of 60, 80, 100 and 300 Gy. The results concluded that GABA content is sensitive to gamma irradiation conditions. [25].

On the other hand, varied results were reported in 2018. Electron beam irradiation at doses of 5 kGy can reduce harmful microorganisms in germinated brown rice flour and GABA content was not affected, but *β*-sitosterol (one of several phytosterols with chemical structures similar to that of cholesterol) was affected and the content decreased [26].

GABA accumulation in rice is affected by several factors, such as salt concentration, duration of seed germination, rice genotype, rice cultivar and the reduction of exposure to solar intensity [25]. The results of this study indicated that the type of irradiation also affects GABA content of germinate rice.

This study revealed that electron beam irradiation at low doses of 1.0 kGy and the storage time was more significant to GABA content of germinated Thai Hom Mali brown rice than the X-ray irradiated rice and the non-irradiated.

3.2 Gamma oryzanol content

Gamma oryzanol is a natural useful antioxidant that is found in rice bran, germ and rice bran oil. It is a group of ferulic acid esters of phytosterols and triterpene alcohols [27]. γ-oryzanol has been reported to inhibit gastric secretion, reduce serum and cholesterol absorption, increase HDL cholesterol and inhibit platelet aggregation [28]. Many studies have reported that γ-oryzanol of different parts of rice during germination process at different incubation periods and temperatures was increased with greater level than non-germinated brown rice [29-30]. In this study, the results showed that gamma oryzanol was found at high values for all the storage periods. The range of gamma oryzanol was from 34.48 ± 1.0 to 45.14 ± 3.49 mg/100 g of dry weight.

This revealed that low dose of electron beam and X-ray irradiation do not affect gamma oryzanol content of germinated Thai Hom Mali brown rice for the preservation period of 12 months (Fig. 2).

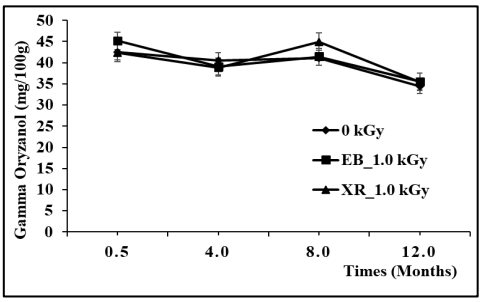

Fig. 2. Gamma oryzanol content of non-irradiated, electron beam and X-ray irradiated GBR samples.

3.3 Total phenolic content and antioxidant activity

In this study, the total phenolic content of non-irradiated and irradiated GBR was varied from 0.64 ± 0.02 to 0.27 ± 0.04 mg GAE/100g. Ferric reducing ability potential (FRAP) and DPPH free radical scavenging were in range of 6.59 ± 0.08 to 2.88 ± 0.38 μmol$FeSO_4$/g, 0.32 ± 0.01 to 0.19 ± 0.02 mgAAE/g, respectively (Fig. 3). Both electron beam and X-ray had no effect on total phenolic content and antioxidant activities of GBR. However, storage time decreased the total phenolic content and antioxidant activities.

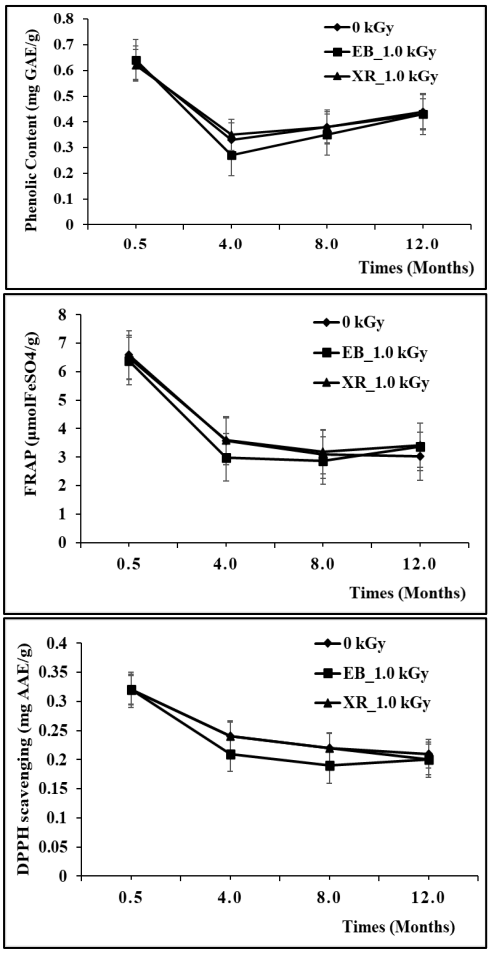

Fig. 3. Total phenolic content (above), ferric reducing ability potential (FRAP) (center) and DPPH scavenging (below) of non-irradiated sample for all storage durations.

3.4 Color characteristics

The non-irradiated and irradiated GBR samples were examined for their color values in Hunter *L a b* system as shown in Fig. 4. The colors, and the nutrition value of food are critical factors and the most significant quality for customer acceptance of the food product. In regards to lightness (*L* value), the results show that *L* values of electron beam irradiated samples significantly decreased when compared with non-irradiated sample after 0.5 and 4 months of storage. This revealed that electron beam irradiation could change the darker color of GBR samples. On other hand, the X-ray irradiated samples did not have *L* values change when compared to the non-irradiated sample.

The Hunter *a* values (*a*– : greeness, *a*+: redness) show that the redness values of electron beam irradiated sample significantly increased when compared with X-ray irradiated and the non-irradiated samples. In contrast, for X-ray irradiated GBR samples, the *a* values are similar to the non-irradiated sample at 0.5 to 8 months of storage. However, at 12 months of storage the result showed that the redness of X-ray irradiated sample was slightly higher than the non-irradiated sample.

The Hunter *b* values indicate that (*b*-: blueness, *b*+: yellowness) the yellowness of electron beam irradiated samples show higher values than the non-irradiated and x-ray irradiated sample at 4 to 12 months of storage peroids.

These results implied that electron beam irradiation at the dose of 1.0 kGy could change the color characteristics of GBR samples. Similar results were found by comparing natural potato starch with irradiated potato starch [31]. There were two potential reasons for these results. First, is that the caramelization reaction of monosaccharides cleaved from starches polysaccharides under irradiation [32]. Second, there is the Maillard reaction between protein residues and sugars [33].

4. Conclusion

Electron beam irradiation at low dose of 1.0 kGy, energy of 10 MeV and the increasing times of storage enhanced the bioactive compound such as gamma aminobutyric acid (GABA) in germinated Thai Hom Mali brown rice (GBR), while total phenolic content, gamma oryzanol and antioxidant activities were comparable to non-irradiated GBR. Electron beams also affected the color value of GBR, suggesting the formation of brown polymers by the Mailard reaction or caramelization reaction. On the other hand, X-ray irradiation at comparable doses of 1.0 kGy, 5 MeV does not affect on GABA content and bioactive compounds and color value of GBR sample. This shows that an electron beam at low dose of irradiation is an efficient method to enhance GABA content of germinated Thai Hom Mali brown rice (GBR) and might be considered as a suitable technique allowing long-lasting conservation of GBR.

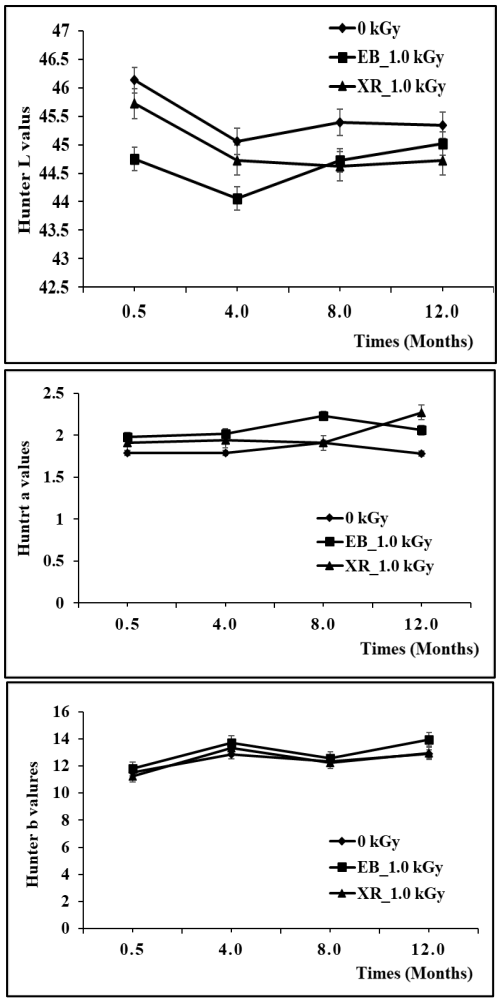

Fig. 4. Color characteristics in Hunter *L a b* system of non-irradiated, electron beam and X-ray irradiated GBR samples.

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