

## Increasing $\gamma$ -Aminobutyric Acid in Mixed Germinated Brown Rice Via Electron Beam Irradiation

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### Abstract

Germinated brown rice (GBR) is considered a functional food due to its rich health promoting bioactive compounds such as  $\gamma$ -aminobutyric acid (GABA),  $\gamma$ -oryzanol, phenolics, and antioxidants. In this study, different germinated brown rice varieties were treated with electron beam irradiation (EBI) and x-ray irradiation to assess the effects on these compounds. Food irradiation technology endorsed by the US-FDA and WHO for improving food safety and extending shelf life was utilized. Germinated black fragrant brown rice (Hom Nil brown rice, *Oryza sativa* L.), and germinated brown rice (GBR) mixed with 3 varieties of brown rice (Thai Hom Mali brown rice, red cargo brown rice, and black fragrant brown rice) were treated with EBI and x-ray irradiation at a dose of 1 kGy, energy levels of 10 and 5 MeV, respectively. The results showed that EBI at the specified dose could significantly increase GABA content in a mixed GBR sample from 3.76 mg/100 g (dried weight) in a non-irradiated sample to 6.44 mg/100 g (dried weight) in an EBI sample, which was a 1.7 fold increase. Moreover, both irradiation methods did not impact any other health promoting compounds such as  $\gamma$ -oryzanol, total phenolic content, or antioxidant activity. However, x-ray irradiation decreased anthocyanin activity in germinated black fragrant brown rice. The Hunter Lab system was used to determine color characteristics. The results showed that EBI slightly increased the yellowness value of a mixed GBR sample. In addition, EBI and x-ray irradiations can reduce microbial contamination of total aerobic plate count and total yeast and mold count in both GBR samples to low levels of  $1.30 \times 10^2 - 1.30 \times 10$  and  $< 10 - 1.8 \times 10$  CFU/g, respectively. The results demonstrated that EBI can improve the nutritional quality and enhance consumer food safety in GBR products.

**Keywords:** Germinated brown rice, *Oryza sativa* L., GABA content,  $\gamma$ -oryzanol, Antioxidant activity  
Anthocyanins activity, Health promoting compounds, Food irradiation

### Introduction

At present, brown colored rice varieties have received increasing attention from consumers due to

their high nutritional values. Brown rice is a whole grain rice that provides a good source of fiber, vitamins B1,

B2, iron, potassium, phosphorus, and zinc. It is also associated with a wide spectrum of several bioactive compounds, including  $\gamma$ -aminobutyric acid (GABA),  $\gamma$ -oryzanol, phenolic acid, and flavonoids, that exhibit antioxidant activity, although almost all of these phytochemicals and vitamins are mainly located in the bran layers of brown rice [1]. In addition, the germination process has been demonstrated to increase levels of health promoting compounds. Germinated brown rice (GBR) exhibits greater potential health benefits compared to non-germinated brown rice (non-GBR). Many studies indicated that the GBR provided more amounts of GABA after 24 - 48 h of incubation and showed an increase in antioxidant activity than non-GBR [2], [3]. The GBR extracts containing GABA have been demonstrated to offer many health benefits, including improving blood flow in the brain, and possessing anti-obesity, anti-inflammatory, anti-allergic, and anti-tumor properties [4]. Studies reported that during the germination process, microbial fermentation occurred. It was found that the microbial contamination of enteropathogenic bacteria such as *Bacillus cereus*, including several species of *Bacillus* spp., aerobic bacteria such as lactic acid bacteria (LAB), as well as yeast and mold increased to high levels [5],[6]. The impact of the drying process is to extend GBR's shelf life by reducing the proliferation of microorganisms via the germination process. However, some studies reported that the content of GABA decreased with the increase in drying temperatures, indicating its sensitivity to the steaming and subsequent high temperatures dryings [7],[8].

Food irradiation is a food safety technique that involves exposing food to ionizing radiation to ensure safety and extend its shelf life. Rice, dried spices, herbs, fish, nuts, and fruits have been commercially irradiated to prevent infestation and control foodborne diseases. There are 3 types of ionizing radiation that were permitted by the US-FDA and WHO for exposure to foods and agricultural products; gamma rays from a  $^{137}\text{Cs}$

Cs or  $^{60}\text{Co}$  radioactive isotope source, electron beam, and x-rays irradiation. Recently, gamma irradiation was applied to induce bioactive compounds in Thai pigmented germinated paddy rice. The newly pigmented paddy rice was exposed to gamma irradiation at low doses of 5 to 1,000 Gy using a  $^{137}\text{Cs}$  radioactive isotope of the gamma ray source. Results indicated that the phenolic, flavonoid, carotenoid, and chlorophyll content of germinated paddy rice increased after gamma irradiation [9]. Another study indicated that gamma irradiation from a  $^{137}\text{Cs}$  isotope source at doses of 5 to 60 Gy can induce the  $\gamma$ -oryzanol content of germinate white rice varieties, which was approximately 1.27 folds higher than the non-gamma irradiated one [10]. These studies suggest that low doses of gamma radiation can stimulate and increase the bioactive compounds of germinate rice samples and improve their quality.

At the commercial level, most irradiated food and agricultural products are processed in facilities that utilize  $^{60}\text{Co}$  as the source of gamma radiation. The amount of  $^{60}\text{Co}$  available worldwide is limited and it difficult to supply large amounts of radioisotopes poses security and logistical challenges. Nowadays, electron accelerators that use electricity to generate electron beams and x-ray irradiation have been developed for food irradiation. These are alternative technologies that use electricity to generate ionizing radiation eliminate the need for acquisition, transport, disposal, and safeguard of radioisotopes. The US-FDA and WHO permit electron beams generated from machine sources operated at or below an energy level of 10 MeV and x-rays operated at or below an energy level of 7.5 MeV for used in food and agricultural products in the purpose of food safety [11].

Most commercially manufactured GBR in the food market is typically sold in dried form to extend shelf life. Until now, the study of the effect of EBI on the bioactive compounds of germinated rice products in the food market has rarely been reported. In the previous study, EBI and x-ray irradiation were applied to treat

GBR after the drying process. The result showed that EBI at a dose of 1 kGy, and an energy level of 10 MeV can enhance GABA content after prolonged storage for 4 and 8 months. The EBI samples had 1.9 to 2.1 times higher GABA content compared to the non-irradiated sample; however, the x-ray irradiated sample was not altered [12].

In this study, EBI and x-ray irradiation were applied to treat dried GBR products originally produced from black, red, and brown rice varieties in a single form and a mixed form. The influence of pigmented rice varieties and the suitable formula of germinated rice for enhancing the high availability of bioactive compounds were investigated. Therefore, it is important to find technique that can control microorganisms in GBR while ensuring GABA content and other bioactive compounds are minimally affected. The primary aim of the study was to assess the efficacy of machine sources for radiation treatment of agricultural and food products. The findings demonstrated that EBI and x-ray irradiation can be useful and effective methods for maintaining the high quality of germinated rice products and enhancing food safety for the consumer.

## Materials and methods

### Sample preparation

At first, the brown rice samples were obtained from the northeastern region of Thailand in an early state within 1 - 2 months after harvest. Initially, a single formula of a black fragrant GBR sample and a mixed GBR sample were prepared. Equal ratios of black fragrant brown rice and mixed GBR formulas, including Thai Hom Mali brown rice, black fragrant brown rice or Hom Nil brown rice, and red cargo brown rice, were used. Each formula was washed and soaked in water at 1:2 ratio. After that, it was incubated at  $3 \pm 1$  °C for 6 - 8 h. Then, it was washed every 3 h to reduce unpleasant odors. The brown rice was wrapped with white cloth and incubated at  $40 \pm 1$  °C for 18 - 24 h in the germination process. After that, the GBR occurred. The GBR was

washed and steamed in the autoclave at 100 °C for 10 min. The steamed GBR samples were removed and dried in an oven at temperature of 40 °C for 24 h [8]. After the drying process, all GBR samples were sealed and vacuum packed in polyethylene bags each weighting  $1.00 \pm 0.05$  kg and stored at ambient temperature at  $30 \pm 3$  °C.

### Irradiation procedure

The GBR samples were packed into the cupboard box with each box containing two 1-kg packets. All 19 boxes were prepared for each kind of GBR sample. Each GBR sample was divided equally into 3 treatments, a non-irradiated sample, electron beam irradiated and x-ray irradiated. All samples were kept at an ambient temperature. The samples were irradiated at the Irradiation Center, Thailand Institute of Nuclear Technology (Public Organization), Pathum Thani province, Thailand. The irradiated procedure was performed with electron beam irradiation and x-ray irradiation at a dose of 1 kGy, energy levels of 10 and 5 MeV, respectively. After irradiation, the absorbed dose was measured using a B3 radiochromic and alanine blister dosimeters.

### $\gamma$ - aminobutyric acid (GABA) content analysis

The GBR sample were collected after 5 days of irradiation. Each sample was collected with  $300 \text{ g} \times 3$  replicates. Each replicate was blended into the powder with the blender and weighed with 1 g of the sample. GABA content was analyzed and followed by a modified method in the previous study [13]. The sample was immersed in 0.3 % sulfosalicylic acid and stirred for 30 min. After centrifugation, the supernatant was filled with 0.01 M sodium hydrogen carbonate ( $\text{NaHCO}_3$ ) and 3.98 mM Dabsyl-Cl. The solution was heated to 70 °C for 10 min in a water bath and cooled down to ambient temperature. Then, the sample was suspended with ethanol (HPLC grade), and 0.025 M potassium dihydrogen phosphate, ( $\text{KH}_2\text{PO}_4$ ) and the filtrate was

filtered. After that, GABA content was analyzed by HPLC column with gradient elution, 0.025 M sodium acetate (pH 6.8), and 30 % methanol (HPLC grade) as the mobile phase. The concentration of GABA content was determined using the following equation.

$$\text{GABA}(\text{mg}) = CV/10W \quad (1)$$

where  $C$  is the concentration of solution extract (g/mL),  $V$  is the final volume (mL),  $W$  is the sample weight (g).

#### $\gamma$ - oryzanol analysis

The analysis of  $\gamma$  - oryzanol was performed using a modified method of rice bran oil in the previous study [14]. The sample was blended, weighed at 2.0 g, then suspended with n-hexane. The suspension was sonicated for 15 min. After centrifugation, the supernatant was collected and the absorbance spectra were measured by UV-VIS spectrophotometer at a wavelength of 315 nm. For adjusting the volume, n-heptane was used for evaluation. The extinction values achieved of the lie with the range of 0.3 to 0.6 was recorded. The content of  $\gamma$ -oryzanol was determined by the following formula.

$$\gamma\text{-oryzanol}(\%) = 25 \times (1/w) \times (1/E) \quad (2)$$

where  $A$  is the absorbance of the solution,  $w$  is the mass of the sample (g) and  $E$  is the specific extinction  $E^{1\% 1 \text{ cm}} = 359$

#### Anthocyanins activity

The anthocyanin activity assay was determined using a modified method from a previous study [15]. Each 1-g sample was weighted, immersed in buffer solution at pH 1.0 and 4.5. The suspension was adjusted to a volume of 50 mL. The infusions were filtered with Whatman No.1 filter paper. After that, the filtrate was determined by using a UV-VIS spectrophotometer at wavelengths of 520 and 700 nm. The absorbance spectra of the anthocyanin pigment (cyanidin-3-glucoside

equivalents, mg/L) was recorded and calculated using the following equation.

$$\text{Anthocyanin pigment (mg/L)} = A \times MW \times 1000 / \epsilon \times l \quad (3)$$

where  $A = (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 1.0 - (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 4.5$

$MW = 449.2 \text{ g/mol}$  (cyaniding-3-glucoside weight)

$E = 2,690$  molar extinction coefficient

$L = \text{pathlength in cm}$

#### Extraction procedure for total phenolic content and antioxidant activity

The GBR samples were blended into powdered form and collected in 3 replicates (100 g each). The 1 g sample was weighted and suspended in 10.0 mL of 60 % ethyl alcohol. The suspension was stored at ambient temperature for 24 h. Then, it was filtered and evaporated with the rotary evaporator under reduced pressure at 40 °C. After the procedure, the residue was weighted and determined for total phenolic content and antioxidant activity.

#### Evaluation of total phenolic content

The total phenolic content of the GBR extracts was determined using the Folin-Ciocateau assay [16]. The extracts were detected using a UV-VIS spectrophotometer at 725 nm and compared with a standard curve of gallic acid. Results were expressed as mg gallic acid equivalent (GAE)/g of sample.

#### Ferric reducing ability potential

The ferric reducing ability potential (FRAP) assays in this study followed the method of reducing ferric ions to form a colored ferrous tripyridyl triazine complex at low pH [17]. The FRAP assay directly measures antioxidant that inhibits free radicals. The FRAP assay of the GBR sample was conducted by comparing it with the standard curve of ferrous ions using a UV-VIS spectrophotometer at an absorbance of 596 nm.

### **DPPH free radical scavenging assay**

The free radical scavenging assay using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay was slightly modified from a previously described method [18]. Free radical scavenging activities were determined using a UV-VIS spectrophotometer at a wavelength of 517 nm, and the percentage inhibition was calculated. The results were expressed as mg of ascorbic acid equivalent/g of dry sample.

### **Color characteristics analysis**

The powder samples were collected and weighed in 3 replicates of 100 g. The ColorFlex EZ spectrophotometer with 45 % measurement geometry to measure the color of GBR samples. The measurement of color in colorimetry was determined using the Hunter L (brightness), a (redness), b (yellowness) system with D65 illuminant. Each sample was measured 5 times and the average values of Hunter L, a, b were calculated. The statistical analysis of variance (ANOVA) was used to determine the changes in color characteristics.

### **Microbial reduction**

The GBR samples were collected with 50 g portion with 3 replicates per treatment. The microbial safety of electron beam and x-ray irradiation on GBR samples was determined by performing a total aerobic

plate count [19] and a total yeast and mold count [20] with 3 replicates of each sample.

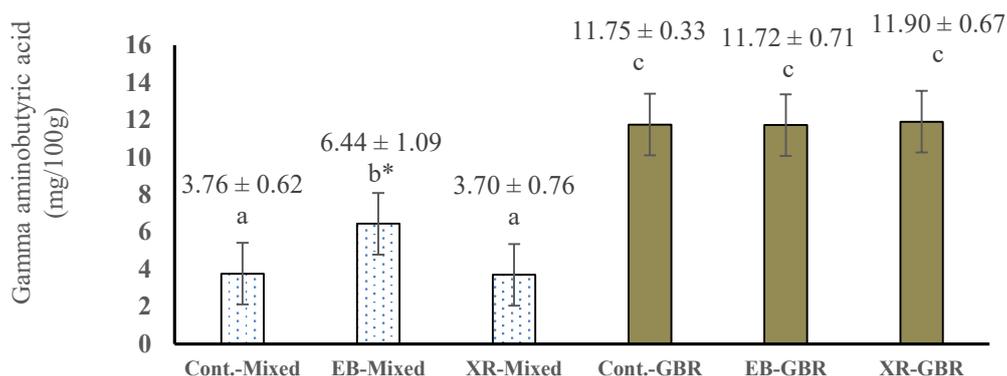
### **Statistical analysis**

The effects of irradiations were determined using analysis of variance (ANOVA) statistical analysis. There were 3 replicates per treatment. Duncan's new multiple rang test with SPSS software was used to compare differences between treatments, with a significant level of  $p < 0.05$ .

## **Results and discussion**

### **GABA content**

GABA is a non-protein amino acid synthesized during the germination process of rice via the decarboxylation of L-glutamic acid, catalyzed by glutamate decarboxylase [21]. The GABA content was evaluated as shown in **Figure 1**. The findings show that after the germination process was performed at 24 - 32 h, the GBR was steamed in the autoclave at 100 °C for 10 min, and the drying process was done in an oven at 40 °C for 24 h. The dried mixed GBR samples, which were treated with EBI at a dose of 1 kGy, at an energy of 10 MeV achieved a GABA content 1.7 times higher than non-irradiated samples. ( $6.44 \pm 1.09$  mg/100 g in the EBI sample,  $3.76 \pm 0.62$  mg/100 g in the non-irradiated sample). However, the GABA content in x-ray irradiated GBR samples was not significantly altered ( $3.70 \pm 0.76$  mg/100 g).



**Figure 1**  $\gamma$  - aminobutyric acid (GABA) content (mg/100 g) of the irradiated GBR.

The highest level of GABA content was found in a single formula of black fragrant GBR sample, ranging from  $11.72 \pm 0.71$  to  $11.90 \pm 0.67$  mg/100 g. The results revealed that GABA content in black fragrant GBR was not affected by irradiation. According to previous findings, a low dose of EBI at 1 kGy, an energy of 10 MeV, and an increased storage time can enhance GABA content in Thai Hom Mali GBR. The brown rice samples were germinated and steamed in the autoclave at 100 °C for 10 min. and dried in an oven at a temperature of 80 °C for 3 h. Results showed that after 15 days of irradiation, the GABA content in EBI irradiated samples of Thai Hom Mali GBR significantly increased from  $7.52 \pm 3.59$  mg/100 g at 15 days of storage to  $13.30 \pm 0.21$  mg/100 g at 4 months of storage, whereas the levels of GABA content in non-irradiated samples were not influenced by storage time ( $5.93 \pm 0.25$  mg/100 g after 15 days of storage to  $7.09 \pm 1.21$  mg/100 g at 4 months of storage); this suggests that EBI and the increased in storage time significantly enhanced the GABA content of irradiated samples to 1.9 folds compared to the non-irradiated samples. However, x-ray irradiation had no effect [12]. The results revealed that after the germination process, black rice had a higher yield of GABA content than brown rice and a mixed formula of black, red, and brown rice, respectively. GABA is mainly enriched through plant metabolism and microbial fermentation. The different amounts of

GABA content found among the rice varieties are mainly caused by their genetic constitution [21]. The results suggest that a low dose of EBI affects biomolecules and increases the GABA content in dried GBR samples. However, this discrepancy might be attributed to different rice varieties, germination procedures, temperature and drying process, sources of irradiation and dose, structural and functional relationships of biomolecules such as proteins and amino acids, and time intervals after storage of irradiated GBR products.

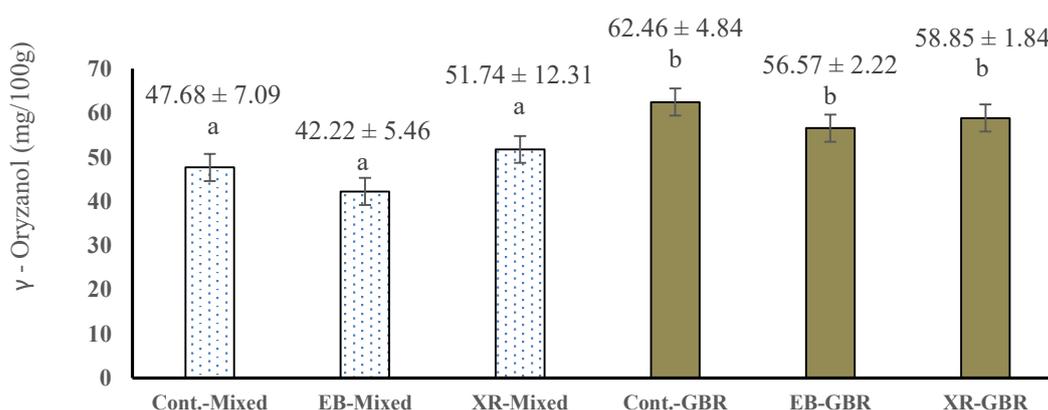
According to the study, low-dose EBI treatment at 1, 2, 3, and 4 kGy changed water distribution in newly harvested rice with high moisture and dried rice with low moisture. After irradiation, the free water content decreased. The proportion of reduction shown by the free water content of low moisture rice (LM) was higher than that of the free water content of high moisture rice (HM). Therefore, free water in LM dried rice was influenced by irradiation and mainly turned into free radicals and thermal energy. As free water was decomposed by EBI, the radiation also cleaved the double helixes of starches and proteins, and partially oxidized protein and fat [22]. Li *et al.* [23] reported that the amino acid content of rice protein was increased by enzymatic hydrolysis, but the amino acid content of the rice protein hydrolysates (RPHs) was decreased after being irradiated with EBI at a high dose of 50 kGy. EBI

induced the amino acid oxidation as well as destroyed the secondary structure and microscopic morphology of the hydrolysates. However, the amino acid content of the irradiated hydrolysates was higher than the amino acid content of the intact protein at a lower irradiation dose of less than 10 kGy.

#### $\gamma$ -oryzanol content

The  $\gamma$ -oryzanol content was evaluated as shown in **Figure 2**.  $\gamma$ -oryzanol is a well-known natural antioxidant that is extracted from rice bran layer of brown rice. Due to its wide spectrum of biological activities, such as high antioxidant activity and playing

a role in anti-inflammatory activity and anti-cancer, it showed a potential impact on reduction of total plasma cholesterol and increased in HDL cholesterol level. 80 % of gamma oryzanol related components include mixtures of cycloartenyl ferulate, phytosterylferulates, methylenecycloartenyl ferulate, and campesterol ferulate [24]. Many studies reported that due to the germination process of brown rice, the level of GABA content and  $\gamma$ -oryzanol could increase [24,25]. In this study, results indicated that a single variety of black fragrant GBR showed a higher  $\gamma$ -oryzanol level than the mixed GBR sample.



**Figure 2**  $\gamma$ -oryzanol content (mg/100 g) of the irradiated GBR.

Chinvongamorn and Sansenya indicated that black rice varieties seem to have higher  $\gamma$ -oryzanol content, which contained 1.55 and 2.03 folds more higher yield than red rice varieties, and non-colored rice varieties, respectively. The results showed that gamma irradiation at doses of 5 to 60 Gy can induce  $\gamma$ -oryzanol content in 4 newly germinated rice varieties. These mean the highest  $\gamma$ -oryzanol levels was found in the irradiated Pathum Thani germinated white rice with approximately 1.27 folds higher than the non-irradiated samples. However, the lowest  $\gamma$ -oryzanol content of the rice varieties was observed at 300 Gy. Results indicated low doses of gamma irradiation can induce plant growth and

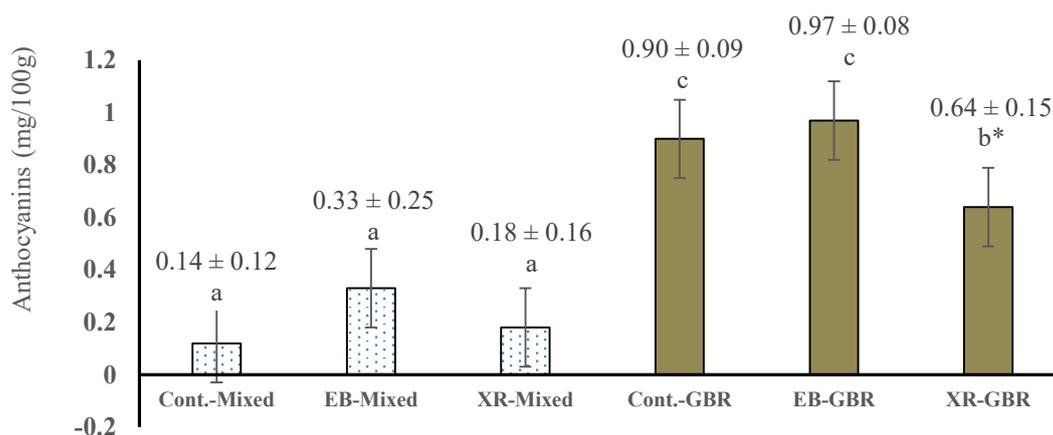
tolerance, whereas decreased plant survival and decreased plant biochemical compounds are found in high doses of irradiation [10]. In addition, the results of this study showed that both EBI and x-ray irradiation at a dose of 1 kGy, an energy of 10 MeV and 5 MeV, respectively, did not affect  $\gamma$ -oryzanol levels in dried GBR samples. Nowadays, the mechanisms of EBI and x-ray irradiation for activating  $\gamma$ -oryzanol levels in GBR products have rarely been reported.

#### Anthocyanin content

Anthocyanins have been widely used as health benefits-bioactive compounds, including their role in

the preventing disease associated with oxidative stress, cardiovascular issues, neurodegenerative disorders, diabetes and cancer [26]. The anthocyanins content in this study was determined as shown in **Figure 3**. The black fragrant GBR sample exhibited the highest anthocyanins activity. Results indicated that the anthocyanin activity level of black fragrant GBR samples was 2.9 to 6.4 times higher than that of mixed GBR samples. EBI did not affect anthocyanin activity, but x-ray irradiation slightly decreased anthocyanins in black fragrant GBR samples. According to a previous report, the total anthocyanin content of black colored rice varieties is higher than that of red colored rice varieties. Results showed that at gamma doses of 80 to

300 Gy, the total anthocyanin content increased approximately twice compared with non-irradiated rice, whereas at gamma doses of 500 and 1,000 Gy anthocyanin activity was slightly decreased. This suggests that the low doses gamma irradiation seem to stimulate total anthocyanin content and rice growth rate, whereas high gamma doses inhibit the total anthocyanin content and rice growth rate [27]. In this study, the effect of x-ray irradiation on total anthocyanin in GBR samples are similar to those of gamma rays in rice samples. X-ray irradiation at a dose of 1 kGy, an energy of 5 MeV suppressed total anthocyanin activity in black GBR samples.



**Figure 3** Anthocyanin content (mg/100 g) of irradiated GBR.

#### Total phenolic content and antioxidant activity

The total phenolic content and antioxidant activity were determined as shown in **Table 1**. Results indicated that black fragrant GBR samples have the highest total phenolic content, ferric reducing ability potential and DPPH radical scavenging. Both EBI and x-ray irradiation did not affect the black fragrant GBR samples or the mixed GBR samples. The total phenolic

content and antioxidant activity of GBR samples depended on rice varieties. According to the study, Chiang Mai black rice locally in the northern part of Thailand is richer in phenolic acid, anthocyanins, flavonoids, and DPPH free-radical scavenging than Hom Mali brown rice and Suphanburi-1 brown rice varieties [28].

**Table 1** Total phenolic content, FRAP value and DPPH radical scavenging of the irradiated GBR.

Sample	Dose	Total phenolic	FRAP	DPPH
		content (mg AAE/g)	( $\mu\text{mole FeSO}_4/\text{g}$ )	radical scavenging (mg GAE/g)
Mixed GBR	0	$0.64 \pm 0.02^{\text{ns}}$	$6.16 \pm 0.04^{\text{ns}}$	$0.44 \pm 0.02^{\text{ns}}$
	EBI 1 kGy	$0.65 \pm 0.00^{\text{ns}}$	$6.17 \pm 0.02^{\text{ns}}$	$0.43 \pm 0.00^{\text{ns}}$
	XR 1 kGy	$0.65 \pm 0.00^{\text{ns}}$	$6.18 \pm 0.20^{\text{ns}}$	$0.44 \pm 0.01^{\text{ns}}$
Germinated	0	$1.77 \pm 0.03^{\text{ns}}$	$27.93 \pm 0.53^{\text{ns}}$	$1.05 \pm 0.13^{\text{ns}}$
black fragrant	EBI 1 kGy	$1.83 \pm 0.09^{\text{ns}}$	$28.61 \pm 0.05^{\text{ns}}$	$1.12 \pm 0.19^{\text{ns}}$
brown	XR 1 kGy	$1.78 \pm 0.05^{\text{ns}}$	$28.26 \pm 0.46^{\text{ns}}$	$1.07 \pm 0.15^{\text{ns}}$
Rice				

Notes; ns = non-significant ( $p > 0.05$ ).

### Color characteristics analysis

The color characteristics of GBR samples are shown in **Table 2**. The physicochemical color characteristic was evaluated with Hunter L a b system. In a mixed GBR sample, results revealed that EBI and x-rays at dose of 1 kGy, energy of 10 MeV and 5 MeV, respectively did not altered L and a value. However, EBI significantly increased the b value of a mixed GBR sample resulting in a slight increased the yellowness value of the product ( $p < 0.05$ ). However, the colors of irradiated black fragrant GBR sample were not altered. According to a previous study, in which Thai Hom Mali GBR samples were irradiated with EBI at a dose of 1 kGy, energy of 10 MeV, and kept at ambient

temperature for 4 to 12 months, the irradiated samples exhibited higher GABA content than non-irradiated sample, results also showed the yellowness of EBI samples was significantly increased compared to the non-irradiated and x-ray irradiated samples [12]. Another study reported that the germination process can increase GABA content of Indian rice varieties, and the yellow medicinal brown rice variety called Njavara showing the superior GABA content compared to the other indigenous Indian rice varieties [29]. However, a slightly decreased amount of anthocyanin in x-ray irradiated black fragrant GBR sample did not affect the color characteristics.

**Table 2** Color characteristics of the irradiated GBR.

Sample	Hunter L a b system	Dose		
		0	EBI 1 kGy	XR 1 kGy
Mixed GBR	L	$56.76 \pm 0.90^{\text{ab}}$	$56.38 \pm 0.63^{\text{a}}$	$57.01 \pm 0.39^{\text{b}}$
	a	$5.52 \pm 0.08^{\text{a}}$	$4.96 \pm 0.04^{\text{a}}$	$5.09 \pm 0.15^{\text{a}}$
	b	$12.27 \pm 0.47^{\text{a}}$	$13.29 \pm 0.22^{\text{b}}$	$12.34 \pm 0.46^{\text{a}}$
Germinated black fragrant brown rice	L	$19.12 \pm 0.56^{\text{a}}$	$19.36 \pm 0.51^{\text{a}}$	$19.40 \pm 0.46^{\text{a}}$
	a	$3.33 \pm 0.22^{\text{a}}$	$3.43 \pm 0.21^{\text{a}}$	$3.47 \pm 0.25^{\text{a}}$
	b	$1.78 \pm 0.40^{\text{a}}$	$2.08 \pm 0.42^{\text{a}}$	$2.08 \pm 0.34^{\text{a}}$

Notes: The small different letters in the row are significantly different according to Duncan's multiple range test at  $p < 0.05$ .

### Microbial reduction

The microbiological quality of GBR samples is shown in **Table 3**. The results of total aerobic plate count (TAPC) showed that EBI and x-ray irradiation at a dose of 1 kGy, energy of 10 and 5 MeV can decrease TAPC in mixed GBR and black fragrant GBR samples at rate of 1.04 - 1.2 and 1.31 - 1.45 log cycles, respectively. They also eliminated the total yeast and mold count in the range of 2.01 and 0.74 - 0.92 log cycle, in mixed GBR and a black fragrant GBR samples, respectively. This demonstrated that the application of ionizing radiation is an effective method to reduce microbial contamination in dried GBR samples. The ionizing irradiation such as ions or gamma rays or x-rays inactivate the microorganisms without significantly increasing the temperature of the food product. The application of EBI technology using an accelerator

beam in an electrical field to control flow of electrons showed that the high intensity of electrons also depends on the energy of radiations. While, the utilization of high energy EBI (5 - 10 MeV) technology includes the phytosanitary treatment, disinfection and preservation of food products, the sterilization of medical products and also material modification [30]. Some studies on food safety have shown that x-ray is an appropriate alternative to the gamma rays and EBI for microbial loss in food products [31]. In yeasts, molds, and bacteria, the irradiations induces genomic, biochemical and physiological changes. The main effect of irradiations is damaged to macromolecules such as nucleotides by breaking single and double bonds of DNA and destroying other cellular materials such as membranes and key enzymes, finally resulting in cell death [30].

**Table 3** Microbiological safety of the irradiated GBR.

Sample	Microbes	Dose		
		0	EBI 1 kGy	XR 1 kGy
Mixed GBR	Total aerobic plate count (CFU/g)	$6.50 \times 10^3$ <sup>a</sup>	$4.30 \times 10^2$ <sup>b</sup>	$3.40 \times 10^2$ <sup>b</sup>
	Total yeast & mold count (CFU/g)	$9.20 \times 10^a$	$< 10^b$	$< 10^b$
Germinated black fragrant brown rice	Total aerobic plate count (CFU/g)	$3.70 \times 10^2$ <sup>a</sup>	$1.80 \times 10^b$	$1.30 \times 10^b$
	Total yeast & mold count (CFU/g)	$1.0 \times 10^2$ <sup>a</sup>	$1.2 \times 10^b$	$1.8 \times 10^b$

Notes: The small different letters in the row are significantly different according to Duncan's multiple range test at  $p < 0.05$ .

In addition, EBI processing is quite easy for low density and thin products. However, the penetration of EBI is limited through packages with high densities [11]. In this present study, a specific high energy of 10 MeV at a dose of 1 MeV would also benefit, due to the fast delivery of the treatment time. The short irradiation duration decreases the negative effects on the bioactive compounds, but activates the GABA content of the GBR sample. The operational parameters such as a specific

packaging configuration, and the undertaking of dose mapping need to be carefully considered to ensure that the food products are irradiated correctly. In further study, the effect of EBI at various doses on sensory characteristics and the overall quality of irradiated GBR samples were investigated. Therefore, the suitable dose of EBI for good cooking quality will be applied.

## Conclusions

In this study, a dried black fragrant GBR sample and a dried mixed GBR brown rice sample consisting of Thai Hom Mali brown rice, red cargo brown rice, and black fragrant brown rice, which are rich in GABA content,  $\gamma$ -oryzanol, and antioxidant activity were treated with 10-MeV electron beam irradiation and 5-MeV x-rays, and the changes were analyzed. A low dose of EBI was identified as a suitable alternative method to enhance GABA content and maintain the antioxidant activity of the dried mixed GBR sample, ensuring high availability levels. It also had minimal effects on the viability level of bioactive compounds in the black fragrant GBR sample. Additionally, EBI and x-ray irradiation significantly improved the microbial quality by reducing the total yeast and mold count and the total aerobic plate count in all GBR samples. The effects of EBI and x-ray irradiation on bioactive molecules varied depending on the rice varieties, types of biomolecules, type and dose of irradiation, and formula of the GBR product. However, x-rays irradiation at a dose of 1 kGy, energy of 10 MeV is limited and suppress total anthocyanin in black fragrant GBR samples. Results indicated that EBI could be green, safe, and serve as alternative food safety technologies to gamma irradiation.

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