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Gamma Irradiation and Exogenous Proline Enhanced the Growth, 2AP Content, and Inhibitory Effects of Selected Bioactive Compounds against α-Glucosidase and α-Amylase in Thai Rice

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ABSTRACT: Exogenous proline can improve the growth, aroma intensities, and bioactive compounds of rice. This study evaluated the effects of gamma irradiation under proline conditions on the 2-acetyl-1-pyrroline (2AP), phenolic, and flavonoid contents of rice. Moreover, the bioactive compounds of gamma-irradiated rice under proline conditions that inhibited α-glucosidase and α-amylase were evaluated by in silico study. A low gamma dose (40 Gy) induced the highest rice growth under 5 mM proline concentration. The highest 2AP content was stimulated at a gamma dose of 5–100 Gy under 10 mM proline concentration. At 500 and 1,000 Gy gamma dose, the highest flavonoid and phenolic contents of rice were stimulated. 1-(2-Hydroxy-5-methylphenyl)-ethanone, which had the highest binding affinity (−7.9 kcal/mol) against α-glucosidase, was obtained at 500 and 1,000 Gy gamma dose under 5 and 10 mM proline concentrations. Meanwhile, 6-amino-1,3,5-triazine-2,4(1H,3H)-dione, which had the highest binding affinity (−6.3 kcal/mol) against α-amylase, was obtained under 10 mM proline concentration in non-gamma-irradiated rice. The results indicate that using a combination of gamma irradiation and exogenous proline is suitable for producing new rice varieties. Moreover, the bioactive compounds that were obtained in new rice varieties exhibited health benefits, especially for diabetes mellitus treatment (inhibition of α-glucosidase and α-amylase).

Keywords: 2-acetyl-1-pyrroline, bioactive compounds, digestive enzymes, exogenous proline, gamma irradiation

INTRODUCTION

Rice is a staple food worldwide. Rice seeds contain high levels of macro- and micronutrients (Lin et al., 2014). Moreover, they are rich in bioactive compounds (Verma and Srivastav, 2020). These compounds exhibit various biological activities, including antioxidant activity (Goufo and Trindade, 2014), anticancer activity (Yousif et al., 2022), antibacterial activity (Yoshida et al., 2022), anti-inflammatory activity (Vichit and Saewan, 2016), and anti-diabetes mellitus activity by inhibiting α-amylase and α-glucosidase (Sansenya et al., 2021). Black rice extracts (Thai native-colored rice) show mixed-type inhibition against α-amylase and α-glucosidase similar to the anti-diabetic drug acarbose (Sansenya and Nanok, 2020). In addition, the compounds from the pericarp of black rice, including cyanidin-3-glucoside and 6'-*O*-feruloyl-sucrose, exhibit high binding affinity against α-glucosidase (−92.47 and −58.91 kcal/mol, respectively) (Bhuyan et al., 2022).

Based on differences in the *Badh2* gene, 2-acetyl-1-pyrroline (2AP) content, and aroma intensities, rice can be classified into two types: fragrant and non-fragrant rice cultivars (Bradbury et al., 2005; Chen et al., 2008). 2AP is the characteristic compound for fragrant rice cultivars. However, 2AP has also been found in some non-fragrant rice cultivars, albeit in small amounts (Hinge et al., 2016). Rice contains more than 100 volatile compounds, leading to mixed aroma intensities (Widjaja et al., 1996). Some volatile compounds of fragrant rice cultivars exhibit biological activities. 2-Pentylfuran, vanillin, and guaiacol are the major compounds that contribute to the aroma intensities of rice, and these compounds exhibit inhibitory activities against α-glucosidase and α-amylase (Nanok and Sansenya, 2021). Moreover, these aroma compounds can inhibit the tyrosinase enzyme (Sansenya et al., 2021). Various factors affect the 2AP content and volatile compounds of rice, including salinity, drought

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stress, and proline content. According to Yoshihashi et al. (2004), drought stress particularly affects the 2AP content. In another study, salinity affected the increase of the 2AP content of rice (Poonlaphdecha et al., 2012). 2AP is synthesized via L-proline metabolism in rice. Thus, exogenous proline has been reported to increase the 2AP content in fragrant rice cultivars (Luo et al., 2020).

Gamma irradiation is one of the techniques used for plant mutation. This technique can improve the physiological and biochemical characteristics of plants (Kiani et al., 2022). Gamma irradiation has also been used to produce new rice mutant lines. Researchers reported that gamma irradiation at low doses stimulates rice growth. However, a high gamma dose inhibits rice growth (Sansenya et al., 2019). Gamma irradiation also stimulates the synthesis of biochemical compounds in rice. Hwang et al. (2014) reported that gamma irradiation induces tocopherol accumulation. Moreover, gamma irradiation at low gamma doses induces γ-oryzanol in germinated rice (Chinvongamorn and Sansenya, 2020). In a previous study, gamma irradiation increased the phenolic, flavonoid, and antioxidant activities of five popular Thai rice cultivars (rice extract) and induced the production of some biological compounds related to rice growth, including proline (Archanachai et al., 2021). Thus, the gamma irradiation technique and combined with exogenous proline might be important for generated the new rice line with including of specific characteristics. In the present study, we determined the effects of gamma irradiation under exogenous proline conditions on the growth, 2AP content, and bioactive compounds of Thai rice. Moreover, we investigated the inhibitory effects of selected bioactive compounds against α-glucosidase and α-amylase through in silico study.

MATERIALS AND METHODS

Chemical reagent

Standard 2AP with a purity of 95% was obtained from BOC Sciences. Chemical reagents used for determining the total phenolic and flavonoid contents were obtained from Sigma-Aldrich.

Plant materials

Rice samples (*Oryza sativa* L.) were harvested from farmers in Pathum Thani province (rice season 2022). The rice seeds were sterilized with 0.1% NaClO and distilled water. Thereafter, the sterilized seeds were dried in a hot air oven at 40°C until the moisture content was less than 13%. The rice seed samples were kept in a desiccator until gamma irradiation.

Gamma irradiation and growth condition

Approximately 100 g of rice seed samples was packed in a polyethylene bag. Then, the samples were exposed to gamma ray (^{137}Cs) with a dose of 0 (control), 5, 40, 100, 500, and 1,000 Gy. The gamma irradiation experiment was conducted at the Gamma Irradiation Center and Nuclear Technology Research Center, Faculty of Science, Kasetsart University.

Twenty-five grams of gamma-irradiated rice seeds (5, 40, 100, 500, and 1,000 Gy) and non-gamma-irradiated rice seeds (0 Gy) were germinated on germinating paper for 7 days at room temperature. During germination, the samples were sprayed with 50 mL of proline solution (0, 1, 5, and 10 mM) daily until harvested. At day 7, the rice plant was harvested, and the rice shoot was measured. The rice samples were kept at −20°C until further experimentation.

Total phenolic and flavonoid contents

Rice samples (gamma-irradiated and non-gamma-irradiated rice) were extracted with methanol in a 1:3 ratio (rice samples 50 g:150 mL of methanol) for 3 days. The extraction solution was centrifuged at 1,411 *g* to discard rice residues. The supernatant was filtered using a 0.45-μm syringe filter. The solvent was evaporated in the hot air oven at 50°C. The crude extract was kept in a desiccator until used.

The total phenolic and flavonoid contents were calculated in accordance with the modified method of Archanachai et al. (2021). About 100 μL of sample solution (1 mg/mL), 100 μL of methanol, and 200 μL of 10% (v/v) Folin-Ciocalteu reagent were combined and agitated for 5 min to determine the total phenolic content. The combination solution was then added with 600 μL of 1 M sodium carbonate. The reaction mixture was incubated at room temperature in the dark. The absorbance of the final product was measured using a spectrophotometer at 760 nm. The calibration curve of gallic acid (micrograms of gallic acid equivalent per gram of dry weight) was used to determine the total phenolic content of samples.

About 500 μL of sample solution (1 mg/mL), 340 μL of deionized water, and 30 μL of sodium acetate (1 M) were combined and incubated for 5 min to determine the total flavonoid content. After shaking for 5 min, 30 μL of $AlCl_3$ (1 M) was added to the reaction solution. Then, 200 μL of NaOH (1 M) was added, and the mixture was incubated at 30°C for 15 min. Finally, the absorbance of the final product was determined using a spectrophotometer at 415 nm. The calibration curve of quercetin, which measures the amount of quercetin equivalent in micrograms per gram of dry weight, was used to determine the total flavonoid content of the sample solution.

Determination of 2-acetyl-1-pyrroline (2AP) content

Rice samples (gamma-irradiated and non-gamma-irradiated rice) were homogenized to fine pieces using a CryoMill with liquid nitrogen cooling. One gram of samples was weighed into 20-mL headspace vials and capped immediately.

The standard 2AP concentration (between 0.05 mg/mL and 2.50 mg/mL) was prepared from a 5 mg/mL stock solution by diluting with methanol-toluene (1:1 ratio). The 2AP content of all rice samples (gamma-irradiated and non-gamma-irradiated rice) was determined following the method of Sansenya et al. (2017). The headspace approach using the Agilent GC autosampler 120 was utilized to evaluate the 2AP content of rice samples. A gas chromatography-mass spectrometry system (GC-MS; Agilent 7890A GC-7000 Mass Triple Quad) equipped with a DB-Wax capillary column (60 m, 0.25 mm i.d., 0.25 m film thickness, J&W Scientific) and a quadrupole mass detector was used to separate the volatile compounds. With a collision energy of 5 v, the precursor ion at *m/z* 111 and the product ion at *m/z* 83 were chosen for 2AP. The dwell time for MS detection was 30 ms. The amount of 2AP was quantified by measuring the peak area of the ion at *m/z* 83, and its quantities were derived using its calibration curve.

Profile of bioactive compounds

The volatile compounds of rice extracts were determined following the method of Pattarathitiwat et al. (2021). Four grams of rice extracts was weighed into 20-mL headspace vials and capped. A solid phase microextraction fiber (50/30m DVB/CAR/PDMS, Supelco) was used to extract volatile chemicals for 20 min after the sample was preheated at 50°C for 10 min. The fiber was then desorbed in the GC injector port at 250°C for 5 min. A GC-MS system (Agilent 7890A GC-7000 Mass Triple Quad) equipped with a capillary column (DB-WAX, 60 m, 0.25 mm, 0.25 μm, J&W Scientific) and a quadrupole mass detector were used to separate the desorbed volatiles. The split ratio used for the injector operation was 5:1. The carrier gas was helium, which flowed at a steady rate of 0.8 mL/min. The temperature of the GC oven was set at 32°C for 10 min, increased to 40°C at 3°C/min and maintained for 15 min, then increased to 160°C at 3°C/min, and finally increased to 230°C at 4°C/min and maintained for 5 min. The mass spectrometer was operated in the electron ionization mode with the ion source temperature and ionization energy set at 230°C and 70 eV, respectively. The scan range was 25 – 400 *m/z*, and scan mode was used. Data analysis was performed using the Agilent MassHunter Qualitative Analysis B.04.00 software. Volatile compounds were identified by comparing the mass spectra to NIST mass spectral libraries (National Institute of Standards, 2011 version). The content of volatile components was determined based on the peak area.

Docking study and inhibition (K_i) calculation

There are two enzyme targets in this study: α-glucosidase and α-amylase. Their three-dimension structures were obtained from the Protein Data Bank (PDB) with PDB codes of 3A4A for α-glucosidase and 7TAA for α-amylase. The crystal structures of both enzymes were cleaned by removing complexed water molecules and ligands using AutoDockTools (Morris et al., 2009). Afterward, polar hydrogen atoms were added. All ligand structures were collected from the PubMed database.

Docking calculations were performed using AutoDock Vina (Trott and Olson, 2010). The grid box was set at 6 nm×6 nm×6 nm with space point of 0.0357 nm. The visualizations of the molecular docking results were illustrated using the Visual Molecular Dynamics program (Humphrey et al., 1996). The interaction types of all enzyme-ligand complexes were investigated using the Protein-Ligand Interaction Profiler webserver (Adasme et al., 2021).

The K_i values of each compound were calculated using the following formula: $K_i = \exp(\Delta G/RT)$, where ΔG is the binding energy, R is the gas constant (1.985×10^{-3} kcal mol^{-1} K^{-1}), and T is the temperature (298.15 K).

Statistical analysis

The results on rice growth and 2AP, phenolic, and flavonoid contents in rice samples are expressed as means± standard deviations. Statistical significance was assessed using one-way analysis of variance. Then, post hoc analysis using Duncan's multiple-range test comparisons was carried out. Statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Effects of gamma irradiation and proline on the growth of gamma-irradiated rice

The effects of gamma irradiation and proline on the growth of gamma-irradiated rice (5 – 1,000 Gy) and non-gamma-irradiated rice (0 Gy) are shown in Supplementary Table 1. Proline (5 and 10 mM) increased rice growth by 8.03±0.45 and 9.83±0.75 cm, respectively, compared without (5.13±0.45 cm). At 0 mM proline concentration, rice growth was induced by gamma irradiation at a dose of 5 – 100 Gy (8.63±0.55 to 9.93±0.61 cm) compared with the control (5.13±0.45 cm). Rice growth at 1 and 5 mM proline concentrations showed a similar trend to that at 0 mM proline concentration with gamma irradiation at a dose of 5 – 100 Gy (7.07±0.35 to 10.37±0.55 cm for 1 mM proline and 8.00±0.26 to 10.67±0.32 cm

for 5 mM proline concentration). Moreover, the rice growth rate seems to be higher in samples exposed to 1 Gy gamma irradiation and 5 mM proline concentration and 5 – 40 Gy gamma irradiation and 5 mM proline concentration than in samples exposed to the same gamma dose and 0 mM proline concentration. However, the growth rate of gamma-irradiated rice (5 – 1,000 Gy) at 10 mM proline concentration was lower than that of non-gamma-irradiated rice. A gamma dose of more than 500 Gy inhibited the growth rate of gamma-irradiated rice compared with non-gamma-irradiated rice under all proline concentrations.

Proline is a traditional amino acid that plays a beneficial role in plants exposed to non-stress and various stress conditions. In non-stress conditions, the proline content is correlated with plant growth and development (Kavi Kishor et al., 2015). Proline stimulates cell wall synthesis and plant development, including root and pollen development. Our findings also indicate that exogenous proline promoted rice growth in the absence of stress conditions. Exogenous proline also affects plant stress depending on the concentration. For example, low proline concentration enhances stress tolerance (Kaur and Asthir, 2015). Moreover, during stress conditions, exogenous proline promotes plant growth (Kaur and Asthir, 2015; El Moukhtari et al., 2020). Plants under radiation treatment such as ultraviolet B (UV-B) can be alleviated by proline concentration because proline scavenges the free radicals generated by UV-B (Saradhi et al., 1995; Arora and Saradhi, 2002; Kaur and Asthir, 2015). Gamma irradiation can promote and inhibit plant growth at low and high doses, respectively (Archanachai et al., 2021). Gamma rays can also generate reactive oxygen species (ROS), which cause DNA damage and affect plant growth and development (Roldán-Arjona and Ariza, 2009; Qi et al., 2015). Our results showed that a low gamma dose stimulated rice growth, but a high gamma dose inhibited rice growth. The proline condition also affected the rice growth rate (Supplementary Table 1). For example, 1 and 5 mM proline concentrations can alleviate gamma irradiation at 5 and 40 Gy, respectively. Our results supported a previous study showing that exogenous proline can alleviate various stress conditions, including radiation stress.

Effects of gamma irradiation and proline on the 2AP content of gamma-irradiated rice

Table 1 shows that the 2AP content of non-gamma-irradiated rice increased with increasing proline concentration (8.17±0.55 to 12.47±0.59 μg/g). At 0 mM proline concentration, the 2AP content of gamma-irradiated rice was affected by gamma dose at 5 – 100 Gy (10.13±0.95 to 10.40±0.56 μg/g) compared with the control (8.17±0.55 μg/g). At 1 mM proline concentration, the 2AP content of gamma-irradiated rice continuously decreased after 5 Gy of gamma dose. However, the 2AP content of gamma-irradiated rice at 5 and 5 – 100 Gy increased under 5 and 10 mM proline concentrations, respectively. Interestingly, the 2AP content of gamma-irradiated rice at 500 – 1,000 Gy decreased under all proline concentrations (Table 1).

Proline is the precursor of the 2AP biosynthesis pathway by inactivating betaine aldehyde dehydrogenase (BADH) (Bradbury et al., 2005; Chen et al., 2008). Exogenous proline can induce 2AP accumulation in *indica* and *japonica* fragrant rice grains. Luo et al. (2020) reported that increasing exogenous proline concentration decreased the transcription level of *BADH2* and activities of BADH enzyme. Gamma irradiation also affects 2AP accumulation in fragrant rice. Sansenya et al. (2017) reported that gamma rays induce the 2AP content in germinated fragrant rice at low gamma dose (20 – 60 Gy). Our results also show that increasing exogenous proline concentration stimulates the 2AP content in rice. In addition, low gamma dose (5 – 100 Gy) promotes 2AP production, whereas high gamma dose (500 – 1,000 Gy) inhibits 2AP production in rice. At the same time, the combination of gamma irradiation and exogenous proline condition also stimulated 2AP accumulation in rice, especially at 10 mM proline concentration and 5 to 100 Gy gamma dose. However, the 2AP content still decreased at high gamma dose under all proline concentrations, which might be explained by the role of proline in scav-

Table 1. 2AP content of gamma-irradiated and non-gamma-irradiated rice under proline condition

Gamma dose (Gy)	2AP content (μg/g)			
	0 mM	1 mM	5 mM	10 mM
0	8.17±0.55^{b}	9.17±0.45^{a}	10.60±0.30^{b}	12.47±0.59^{c}
5	10.13±0.95^{a}	9.53±0.45^{a}	11.37±0.45^{a}	16.90±0.60^{a}
40	10.17±0.65^{a}	7.47±0.31^{b}	9.27±0.45^{c}	16.23±1.27^{ab}
100	10.40±0.56^{a}	5.20±0.40^{c}	6.70±0.50^{d}	15.53±0.67^{b}
500	3.73±0.65^{c}	2.70±0.20^{d}	3.03±0.35^{e}	3.60±0.20^{d}
1,000	3.17±0.45^{c}	2.47±0.23^{d}	2.57±0.45^{e}	3.27±0.45^{d}

Different letters (a–e) within the column indicate significant differences in 2AP content (mg/g) in rice samples under different proline conditions (P<0.05).
2AP, 2-acetyl-1-pyrroline.

enging large amounts of ROS produced by the high gamma dose.

Effects of gamma irradiation and proline on the phenolic and flavonoid contents of gamma-irradiated rice

Table 2 shows that the flavonoid content of non-gamma-irradiated rice under proline condition (1 to 10 mM) was lower than that under 0 mM proline condition. The flavonoid content of gamma-irradiated and non-gamma-irradiated rice under 0 mM proline condition ranged from 26.86±1.50 μg QE/g dw to 112.27±5.03 μg QE/g dw. Gamma-irradiated rice at a dose of 100 – 1,000 Gy had higher flavonoid content than non-gamma-irradiated rice. By contrast, gamma-irradiated rice at a dose of 5 – 100 Gy under 1 mM proline condition had higher flavonoid content than non-gamma-irradiated rice and gamma-irradiated rice at a dose of 500 – 1,000 Gy. Interestingly, the flavonoid content of gamma-irradiated rice (5 to 1,000 Gy) under 5 and 10 mM proline condition was higher than that of non-gamma-irradiated rice. Moreover, the flavonoid content of gamma-irradiated rice under 10 mM proline condition increased when the gamma dose was increased from 5 Gy to 1,000 Gy.

The phenolic content of gamma-irradiated rice under proline condition varied at different gamma doses (5 – 1,000 Gy) and compared with 0 Gy. At 0 mM proline concentration, the highest phenolic content was observed in gamma-irradiated rice at a dose of 1,000 Gy. The highest phenolic content of gamma-irradiated rice under 1 mM proline condition was observed at a dose of 100 Gy. Meanwhile, the highest phenolic content of gamma-irradiated rice under 10 mM proline condition was identified at a dose of 5 – 40 Gy. However, under 5 mM proline condition, the phenolic content of gamma-irradiated rice (5 – 1,000 Gy) was lower than that of non-gamma-irradiated rice (Table 2).

Phenolic and flavonoid compounds are closely related to plant defense mechanism under stress conditions, including salinity, drought, and UV radiation (Mandal et al., 2010; Kumar et al., 2020). Proline is a critical amino acid in plant abiotic stress and is also related to phenolic and flavonoid contents (Kaur and Asthir, 2015; Gao et al., 2023). Archanachai et al. (2021) reported that the phenolic and flavonoid contents of rice were stimulated by gamma irradiation at 60 Gy. This research also reported that gamma rays induced the antioxidant activity of gamma-irradiated rice. In our study, the phenolic and flavonoid contents in gamma-irradiated rice increased when the gamma dose was increased. Moreover, increased proline concentration induced phenolic and flavonoid contents in gamma-irradiated rice. Thus, our results, including those of the previous study, suggested that the phenolic and flavonoid compounds in plants are closely related to the proline content under stress conditions, including gamma irradiation.

Effects of gamma irradiation and proline content on the volatile compound profile of rice

Supplementary Table 2 shows the volatile compounds of gamma-irradiated and non-gamma-irradiated rice under proline conditions. Volatile compounds, including 2-methyl-propanal, glycerin, n-hexadecanoic acid, and (Z,Z,Z)-9,12,15-octadecatrienoic acid, were found in both gamma-irradiated and non-gamma-irradiated rice. Moreover, some volatile compounds, including acetic acid, 2,3-butanediol, [R-(R*,R*)]-2,3-butanediol, 1,2-cyclopentanedione, palmitic acid, ethyl oleate, linoleic acid ethyl ester, ethyl-9,12-octadecadienoate, dihydro-4-hydroxy-2(3H)-furanone, ethyl 9,12,15-octadecatrienoate, octadecanoic acid, and oleic acid, were found in abundance in gamma-irradiated and non-gamma-irradiated rice. Volatile compounds, including γ-carboethoxy-γ-butyrolactone and heptadecanoic acid, were only found in non-gamma-irradiated rice without proline condition. Meanwhile, volatile compounds, including pentanoic acid, (R)-1-ethyl-2-pyrrolidinecarboxamide, 2-hydroxy-2-cyclopenten-1-one, 3-methyl-butanamide, 1-methyl-1H-pyrazole-4-carboxylic acid, tetrahydro-3-furanol, ethyl 9-hexadecenoate, and stearic acid, were found in gamma-irradiated and non-gamma-irradiated rice without proline condition. Under 1, 5, and 10 M proline condition, volatile compounds, including 2-methyl-butanal, cyclopentanol, 2,2,6,6-tetra-

Table 2. Total phenolic and flavonoid contents of gamma-irradiated rice under proline condition

Gamma dose (Gy)	Total flavonoid content (μg QE/g dw)				Total phenolic content (μg GAE/g dw)			
	0 mM	1 mM	5 mM	10 mM	0 mM	1 mM	5 mM	10 mM
0	50.49±0.92^{c}	26.14±1.32^{cd}	26.80±1.57^{e}	33.01±1.67^{c}	223.57±4.11^{d}	206.06±2.80^{b}	328.64±6.78^{a}	323.25±6.78^{b}
5	31.36±0.83^{d}	27.95±1.17^{bc}	29.71±1.16^{d}	68.13±1.67^{b}	406.32±10.97^{b}	203.36±6.07^{b}	74.49±4.04^{f}	369.50±4.04^{a}
40	26.86±1.50^{e}	29.71±1.35^{b}	56.09±1.86^{a}	69.89±2.85^{b}	173.28±5.10^{e}	211.44±7.42^{b}	175.07±4.73^{d}	364.11±7.50^{a}
100	47.19±1.51^{c}	32.52±1.16^{a}	32.35±1.48^{cd}	66.43±1.66^{b}	249.17±14.40^{c}	263.98±4.11^{a}	317.86±4.11^{b}	275.21±49.16^{c}
500	61.64±1.10^{b}	24.77±1.49^{d}	33.72±1.69^{c}	106.44±2.67^{a}	257.24±6.91^{c}	175.52±8.83^{c}	114.91±5.39^{e}	221.32±6.74^{d}
1,000	112.27±5.03^{a}	25.37±1.60^{d}	39.93±1.74^{b}	107.10±1.26^{a}	459.31±6.78^{a}	93.80±4.73^{d}	227.61±4.73^{c}	305.29±4.33^{bc}

Different letters (a–f) within the column indicate significant differences in total flavonoid and phenolic contents (mg/g) in rice samples under different proline conditions (P<0.05).
QE, quercetin equivalents; GAE, gallic acid equivalents.

methyl-4-piperidinone oxime, beta-pyridylmethyl carbinyl benzoate, (E)-9-octadecenoic acid ethyl ester, 2-octylcyclopentanone, and 1-heptacosanol, were identified in gamma-irradiated and non-gamma-irradiated rice. However, volatile compounds, including 2-(formyloxy)-1-phenyl-ethanone, 3-hydroxy-2-butanone, (E)-2-heptenal, 2,5-dimethylpyrazine, dimethyl trisulfide, 3-hydroxy-propanoic acid, butanoic acid, 3-furanmethanol, 3-methyl-butanoic acid, 2,4-dimethyl-2-oxazoline-4-methanol, 5-methyl-1H-1,2,4-triazol-3-amine, (E,E)-2,4-decadienal, 3,3-dimethyl-2-pentanol, isobutyric acid, isophytol, (S)-2(3H)-furanone, dihydro-3-hydroxy-4,4-dimethyl, 1,16-hexadecanediol, ethyl caprate, 5-heptyldihydro-2(3H)-furanone, 4-[(tetrahydro-2H-pyran-2-yl)oxy]-1-butanol, 5-hydroxymethyldihydrofuran-2-one, methyl acetoxyacetate, hexan-2,4-dione, enol, 1-methyl-2,4-imidazolidinedione, heneicosane, 1-(2-hydroxy-5-methylphenyl)-ethanone, 4-hydroxy-2-methylacetophenone, ethyl 3-hydroxy-4,4-dimethypentanoate, 3,5-dihydroxy-2-methyl-4-pyrone, 2,3-dihydro-benzofuran, tetracosane, indole, methyl stearate, 2-pentylcyclopentanone, (S)-5-hydroxymethyl-2[5H]-furanone, linolenic acid, 11-eicosenoic acid, methyl ester, butyl 11-eicosenoate, tetracosane, 1,1-diethyl-2-(1-methylpropyl)-hydrazine, 4-(methylthio)cyclohexanone, methyl-α-D-lyxofuranoside, squalene, ethyl tetracosanoate, ethyl α-D-glucopyranoside, maltol, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF), were only found in gamma-irradiated rice under 1, 5, and 10 mM proline conditions. Moreover, (Z)-14-methylhexadec-8-enal was identified in non-gamma-irradiated rice under 1 mM proline condition. In comparison, 6-amino-1,3,5-triazine-2,4(1H, 3H)-dione and 9,12-octadecadienal were obtained in non-gamma-irradiated rice under 10 mM proline condition.

Gamma irradiation can activate the synthesis of phenolic compounds in plants and some compounds beneficial to human health (Oufedjikh et al., 2000). Moreover, gamma irradiation can stimulate the flavonoid content and antioxidant activity of plants (Patil et al., 2018). Chinvongamorn and Sansenya (2020) reported that gamma irradiation stimulates γ-oryzanol synthesis in both colored and non-colored rice. In another study, the contents of proline and other compounds beneficial to human health, including ascorbic acid, α-tocopherol, and retinol, in first- and second-generation fenugreek (*Trigonella foenum-graecum* L.) were increased by gamma irradiation (Hanafy and Akladious, 2018). Furthermore, a previous study found that gamma irradiation affected the physiological and biochemical profiles of second-generation mutant plants. Moreover, various chlorophyll pigments in the second generation of wheat (*Triticum turgidum* ssp. *durum*) were affected by gamma irradiation (Ahumada-Flores et al., 2021). Thus, our findings, including those of previous reports, indicate that the physiological and biochemical properties of first-generation plants stimulated by gamma irradiation could be transferred to other generations.

Our results reveal that volatile compounds, along with their biological activity, were also stimulated by gamma irradiation under exogenous proline conditions (Supplementary Table 2). 2,5-Dimethylpyrazine, which exhibits antimicrobial activity, was stimulated by gamma irradiation at doses of 40 and 100 Gy under 10 mM proline concentration (Cherniienko et al., 2022). Maltol was stimulated by a high gamma dose (500 – 1,000 Gy) under 1, 5, and 10 mM proline concentrations. This compound is a natural food flavor enhancer and exhibits broad biological activities, including antimicrobial activity, antioxidant activity, and anti-inflammatory activity (Ziklo et al., 2021; Ahn et al., 2022). Interestingly, maltol has been reported to prevent diabetic peripheral neuropathy (DPN) in diabetic rats (Guo et al., 2018). Isophytol is used in the fragrance industry as an intermediate for vitamin E and K synthesis. This compound, which has been reported to exert antibacterial and antifungal activities, was stimulated in gamma-irradiated rice at doses of 40 and 100 Gy under 5 and 10 mM proline concentrations (Tao et al., 2013). HDMF, one of the aroma compounds found in many fruits (Schwab, 2013), was stimulated by gamma irradiation under 1, 5, and 10 mM proline concentrations. HDMF is widely used in the food industry (Xiao et al., 2021) and has been reported to exert biological activity, including antioxidative activity, against lipid peroxidation (Koga et al., 1998). Thus, the results suggest that gamma-irradiated rice under proline conditions can be used as a source of bioactive compounds that are beneficial to human health and some of these compounds may be valuable in the food industry.

Molecular docking of heterocyclic compounds of gamma-irradiated and non-gamma-irradiated rice in the active site of α-amylase and α-glucosidase

Table 3 shows the results of docking study on the heterocyclic compounds of gamma-irradiated and non-gamma-irradiated rice in the active site of α-amylase and α-glucosidase. The results reveal that the binding affinities of heterocyclic compounds with α-amylase and α-glucosidase were −3.5 to −6.3 and −4.0 to −7.9 kcal/mol, respectively. Moreover, the inhibition constants (K_i) of these compounds for α-amylase and α-glucosidase were 24 – 2,702 and 2 – 1,161 μM, respectively. The highest binding interaction between heterocyclic compounds with α-amylase and α-glucosidase was obtained from 6-amino-1,3,5-triazine-2,4(1H,3H)-dione (−6.3 kcal/mol) and 1-(2-hydroxy-5-methylphenyl)-ethanone (−7.9 kcal/mol), respectively. This was consistent with the low K_i of 6-amino-1,3,5-triazine-2,4(1H,3H)-dione and 1-(2-hydroxy-5-methylphenyl)-ethanone, which inhibited α-amylase (24 μM for K_i) and α-glucosidase (2

Table 3. Binding affinity and inhibition constant (K_i) (at T=298.15 K) of bioactive compounds of gamma-irradiated and non-gamma-irradiated rice in the active site of α-amylase and α-glucosidase

Compound name	Binding affinity (kcal/mol)		Inhibition constant (K_i) (μM)	
	α-Amylase (7TAA)	α-Glucosidase (3A4A)	α-Amylase (7TAA)	α-Glucosidase (3A4A)
Acarbose	−7.6	−8.2	3	1
2-(Formyloxy)-1-phenyl-ethanone	−5.7	−6.4	66	20
Ethyl α-D-glucopyranoside	−5.3	−6.3	129	24
Methyl-α-D-lyxofuranoside	−4.7	−5.5	356	92
Butyrolactone	−3.5	−4.2	2,702	828
2,5-Dimethylpyrazine	−4.1	−4.8	980	300
5-Methyl-2(5H)-furanone	−4.1	−4.8	980	300
3-Furanmethanol	−3.9	−4.4	1,374	590
(R)-(+)-1-Ethyl-2-pyrrolidinecarboxamide	−4.4	−5.2	590	153
Phenylethyl alcohol	−5.3	−5.6	129	78
Maltol	−4.7	−5.3	356	129
1-(1H-pyrrol-2-yl)-ethanone	−4.4	−4.9	590	254
1-Methyl-1H-pyrazole-4-carboxylic acid	−4.9	−5.2	254	153
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	−5.0	−5.7	214	66
6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	−6.3	−6.6	24	14
5-Heptyldihydro-2(3H)-furanone	−4.9	−5.9	254	47
Cyclopentanol	−3.6	−4.8	2,282	300
2,2,6,6-Tetramethyl-4-piperidinone oxime	−5.9	−5.9	47	47
5-Hydroxymethyldihydrofuran-2-one	−4.6	−5.2	421	153
1-Methyl-2,4-imidazolidinedione	−4.2	−5.0	828	214
Tetrahydro-3-furanol	−3.5	−4.0	2,702	1,161
1-(2-Hydroxy-5-methylphenyl)-ethanone	−6.2	−7.9	28	2
4-Hydroxy-2-methylacetophenone	−5.9	−6.4	47	20
1-Butyl-2-pyrrolidinone	−4.3	−5.0	699	214
3,5-Dihydroxy-2-methyl-4-pyrone	−5.1	−5.8	181	55
2,3-Dihydrobenzofuran	−5.4	−6.5	109	17
2-Pentylcyclopentanone	−4.9	−5.8	254	55
2-Octylcyclopentanone	−5.3	−5.8	129	55
(S)-5-Hydroxymethyl-2[5H]-furanone	−4.5	−5.4	499	109
4-(Methylthio)cyclohexanone	−4.3	−4.6	699	421
2,4-Dimethyl-2-oxazoline-4-methanol	−4.3	−5.2	699	153
2-Hydroxy-2-cyclopenten-1-one	−4.4	−5.0	590	214

μM for K_i), respectively. 6-Amino-1,3,5-triazine-2,4(1H, 3H)-dione was stimulated by 10 mM proline condition in non-gamma-irradiated rice. Meanwhile, 1-(2-hydroxy-5-methylphenyl)-ethanone was stimulated by gamma irradiation at doses of 500 and 1,000 Gy under 5 and 10 mM proline concentrations. Moreover, the binding affinity of all heterocyclic compounds with α-glucosidase was higher than that of heterocyclic compounds with α-amylase. However, a higher binding affinity and lower K_i were observed in standard inhibitor acarbose than heterocyclic compounds for both α-glucosidase and α-amylase.

Table 4 and Fig. 1 show the results of docking analysis of 6-amino-1,3,5-triazine-2,4(1H,3H)-dione and 1-(2-hydroxy-5-methylphenyl)-ethanone in the active site of α-amylase and α-glucosidase. Two hydrophobic interactions and one hydrogen bond interacted between 6-amino-1,3,5-triazine-2,4(1H,3H)-dione and three amino acids residues (PHE159, PHE178, and GLN279) of α-glucosidase. Meanwhile, three hydrophobic interactions and one hydrogen bond were found between 1-(2-hydroxy-5-methylphenyl)-ethanone and four amino acids of α-glucosidase (TYR72, PHE178, VAL216, and HIS351). An interaction between two hydrophobic interactions and two hydrogen bonds from four amino acid residues (LEU166, LEU173, HIS296, and ASP297) with 6-amino-1,3,5-triazine-2,4(1H,3H)-dione was found in the active site of α-amylase. Moreover, one hydrogen bond, one hydrophobic interaction, and one π-π stacking interaction from two amino acid residues that interacted with 1-(2-hydroxy-5-methylphenyl)-ethanone were found in the active site of α-amylase.

Most natural compounds, especially polyphenol type compounds, that have been studied inhibit digestive enzymes (α-glucosidase and α-amylase) (Aleixandre et al., 2022). Our study also focused on heterocyclic compounds, especially phenolic compounds, against α-glucosidase and α-amylase. Some compounds have a high binding affinity (kcal/mol) against the two digestive en-

Table 4. Molecular docking analysis of 6-amino-1,3,5-triazine-2,4(1H,3H)-dione and 1-(2-hydroxy-5-methylphenyl)-ethanone in the active site of α-amylase and α-glucosidase

Enzymes	Compound name	Residues	Distance ($\times 10^{-10}$ m)	Interaction type
α-Glucosidase	Acarbose	HIS280	3.18	Hydrogen bond
		ALA281	2.94	Hydrogen bond
		PRO312	3.01	Hydrogen bond
		GLU332	3.09	Hydrogen bond
		GLU411	2.99	Hydrogen bond
	6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	PHE159	3.53	Hydrophobic
		PHE178	3.43	Hydrophobic
		GLN279	3.02	Hydrogen bond
	1-(2-Hydroxy-5-methylphenyl)-ethanone	TYR72	3.66	Hydrophobic
		PHE178	3.68	Hydrophobic
		VAL216	3.60	Hydrophobic
		HIS351	3.01	Hydrogen bond
α-Amylase	Acarbose	LEU166	3.61	Hydrophobic
		GLN35	3.13	Hydrogen bond
		ILE152	3.06	Hydrogen bond
		ASP297	3.14	Hydrogen bond
		ARG344	2.94	Hydrogen bond
		ARG344	2.99	Hydrogen bond
	6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	LEU166	3.66	Hydrophobic
		LEU173	3.64	Hydrophobic
		HIS296	2.98	Hydrogen bond
		ASP297	2.91	Hydrogen bond
	1-(2-Hydroxy-5-methylphenyl)-ethanone	TYR82	3.64	Hydrophobic
		TYR82	3.74	π-π stacking (parallel)
		ARG344	3.27	Hydrogen bond

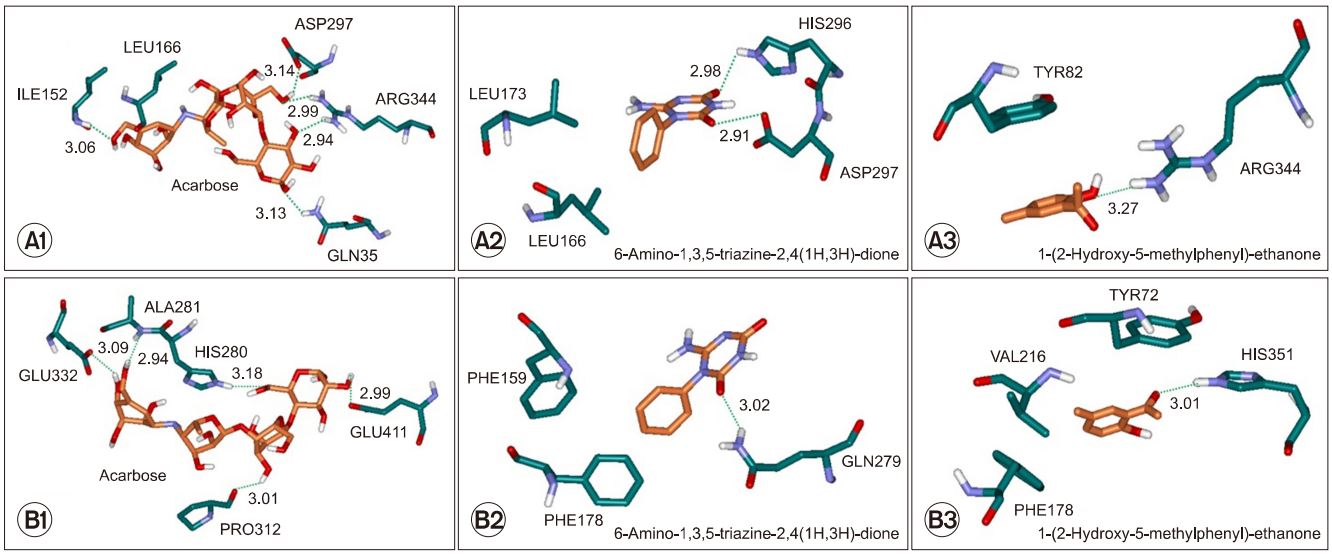

Fig. 1. Binding interaction of acarbose, 6-amino-1,3,5-triazine-2,4(1H,3H)-dione, and 1-(2-hydroxy-5-methylphenyl)-ethanone in the active site pocket of (A) α-amylase and (B) α-glucosidase. A1, A2, and A3 amino acids of α-amylase are surrounded by acarbose, 6-amino-1,3,5-triazine-2,4(1H,3H)-dione, and 1-(2-hydroxy-5-methylphenyl)-ethanone, respectively. B1, B2, and B3 amino acids of α-glucosidase are surrounded by acarbose, 6-amino-1,3,5-triazine-2,4(1H,3H)-dione, and 1-(2-hydroxy-5-methylphenyl)-ethanone, respectively. The green dotted lines indicate hydrogen bonds.

zymes (Table 3). Maltol is a natural product that acts as a food flavor enhancer and has been reported to prevent DPN (Guo et al., 2018). The docking study indicated that maltol has a binding affinity of −5.3 and −4.7 kcal/mol against α-glucosidase and α-amylase, respectively. Ethyl α-D-glucopyranoside, the main compound in Pingguoli pear extract, exhibits antioxidant and hypoglycemic activities (Dai et al., 2022). This compound has a binding affinity of −6.3 and −5.3 kcal/mol against α-glucosidase and α-amylase, respectively. Mohamed et

al. (2022) reported that 4-hydroxy-2-methylacetophenone, the phenolic metabolite identified from *Hibiscus sabdariffa*, has a binding affinity of −4.8 and −4.4 kcal/mol against α-glucosidase and α-amylase, respectively. By contrast, our results showed that the binding affinity of this compound against α-glucosidase and α-amylase was −6.4 and −5.9 kcal/mol, respectively. Moreover, the docking results showed that 2,3-dihydrobenzofuran, which has been reported for the treatment of diabetic retinopathies, has a binding affinity of −6.3 and −5.3 kcal/mol against α-glucosidase and α-amylase, respectively (de Castro Oliveira et al., 2017). 1-(2-Hydroxy-5-methylphenyl)-ethanone with binding affinity of −7.9 and −6.2 kcal/mol and 6-amino-1,3,5-triazine-2,4(1H,3H)-dione with binding affinity of −6.6 and −6.3 kcal/mol showed the highest binding interaction against α-glucosidase and α-amylase, respectively. The docking study revealed that 1-(2-hydroxy-5-methylphenyl)-ethanone has higher binding affinity than 6-amino-1,3,5-triazine-2,4(1H,3H)-dione against α-glucosidase. However, 6-amino-1,3,5-triazine-2,4(1H,3H)-dione has higher binding affinity than 1-(2-hydroxy-5-methylphenyl)-ethanone against α-amylase. Fig. 1 shows that 1-(2-hydroxy-5-methylphenyl)-ethanone and 6-amino-1,3,5-triazine-2,4(1H,3H)-dione have similar positions and are located at the active site of α-glucosidase. However, 1-(2-hydroxy-5-methylphenyl)-ethanone is surrounded by three hydrophobic interactions and one hydrogen, and 6-amino-1,3,5-triazine-2,4(1H,3H)-dione is surrounded by two hydrophobic interactions and one hydrogen, which explains why 1-(2-hydroxy-5-methylphenyl)-ethanone has higher binding interaction than 6-amino-1,3,5-triazine-2,4(1H,3H)-dione against α-glucosidase. The active site of α-amylase 6-amino-1,3,5-triazine-2,4(1H,3H)-dione was located at the −1 subsite and surrounded by two hydrophobic interactions and two hydrogen bonds. Meanwhile, 1-(2-hydroxy-5-methylphenyl)-ethanone was located at the −2 subsite and surrounded by one hydrophobic interaction, one hydrogen bond, and one π-π stacking. This explains why 6-amino-1,3,5-triazine-2,4(1H,3H)-dione has higher binding interaction than 1-(2-hydroxy-5-methylphenyl)-ethanone against α-amylase.

This study evaluated the effects of gamma irradiation under exogenous proline conditions on the growth, 2AP content, and phenolic and flavonoid contents of germinated rice (7 days old). Furthermore, this study determined the changes of bioactive compounds in gamma-irradiated and non-gamma-irradiated rice under proline conditions. In addition, the binding affinity of some heterocyclic compounds, especially phenolic compounds, against digestive enzymes (α-glucosidase and α-amylase) was evaluated through in silico study. The results revealed that rice growth was increased by increasing proline concentration and gamma dose (5 – 100 Gy). However, a gamma dose greater than 500 Gy inhibited rice growth. The highest growth of gamma-irradiated rice was obtained at a gamma dose of 40 Gy under 5 mM proline concentration. The 2AP content of non-gamma-irradiated rice increased when the proline concentration increased. Meanwhile, the 2AP content of gamma-irradiated rice increased when the gamma dose was increased from 5 Gy to 100 Gy without proline concentration. However, the highest 2AP content of gamma-irradiated rice was obtained at a gamma dose of 5 – 100 Gy under 10 mM proline condition. The flavonoid content of rice under proline condition was lower than that without proline condition. However, the phenolic content of rice under 5 and 10 mM proline conditions was higher than that without proline condition. The highest flavonoid and phenolic contents of gamma-irradiated rice were observed at gamma doses of 500 – 1,000 Gy and 1,000 Gy, respectively. In addition, the flavonoid and phenolic contents of gamma-irradiated rice under proline conditions were lower than those without proline condition. Gamma irradiation and proline condition stimulated the synthesis of volatile compounds in gamma-irradiated rice. The docking study showed that some heterocyclic compounds, especially phenolic compounds, inhibited digestive enzymes (α-glucosidase and α-amylase). 1-(2-Hydroxy-5-methylphenyl)-ethanone had the highest binding affinity (−7.9 kcal/mol) against α-glucosidase, whereas 6-amino-1,3,5-triazine-2,4(1H,3H)-dione had the highest binding affinity (−6.3 kcal/mol) against α-amylase. Moreover, the lowest K_i was observed in 6-amino-1,3,5-triazine-2,4(1H,3H)-dione and 1-(2-hydroxy-5-methylphenyl)-ethanone against α-amylase and α-glucosidase, respectively. Thus, the results indicate that gamma irradiation under exogenous proline condition is a suitable method for generating new rice lines containing bioactive compounds related to human health.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept design, project administered, analysis and interpretation, writing the article and critical revision of the article: SS. Analysis and interpretation and statistical analysis: AP, MK, and ST. Obtained funding: SS, ST. Final approval of the article: all authors.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3746/pnf.2024.29.3.354

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