Prev. Nutr. Food Sci. 2023;28(2):170-177
https://doi.org/10.3746/pnf.2023.28.2.170
ISSN 2287-8602

# Inhibitory Efficacy of Cycloartenyl Ferulate against α-Glucosidase and α-Amylase and Its Increased Concentration in Gamma-Irradiated Rice (Germinated Rice)

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**ABSTRACT:** Cycloartenyl ferulate is a derivative of γ-oryzanol with varied biological activity, including diabetes mellitus treatment. This research focused on improving the cycloartenyl ferulate accumulation in germinated rice by gamma irradiation under saline conditions. Moreover, the inhibitory potential of cycloartenyl ferulate against carbohydrate hydrolysis enzymes (α-glucosidase and α-amylase) was investigated through *in vitro* and *in silico* techniques. The results revealed that cycloartenyl ferulate increased in germinated rice under saline conditions upon gamma irradiation. A suitable condition for stimulating the highest cycloartenyl ferulate concentration (852.20±20.59 μg/g) in germinated rice was obtained from the gamma dose at 100 Gy and under 40 mM salt concentration. The inhibitory potential of cycloartenyl ferulate against α-glucosidase (31.31±1.43%) was higher than against α-amylase (12.72±1.11%). The inhibition mode of cycloartenyl ferulate against α-glucosidase was demonstrated as a mixed-type inhibition. A fluorescence study confirmed that the cycloartenyl ferulate interacted with the α-glucosidase's active site. A docking study revealed that cycloartenyl ferulate bound to seven amino acids of α-glucosidase with a binding energy of −8.8 kcal/mol and a higher binding potential than α-amylase (−8.2 kcal/mol). The results suggested that the gamma irradiation technique under saline conditions is suitable for stimulating γ-oryzanol, especially cycloartenyl ferulate. Furthermore, cycloartenyl ferulate demonstrated its potential as a candidate compound for blood glucose management in diabetes mellitus treatment.

**Keywords:** cycloartenyl ferulate, diabetes mellitus, gamma irradiation, γ-oryzanol, pigmented rice

## INTRODUCTION

Gamma-oryzanol is a component of steryl ferulates and was first purified from rice bran oil. The three compounds, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate, have been identified as major components of γ-oryzanol (Xu and Godber, 1999). γ-Oryzanol exhibited broad biological activity, such as antioxidant activity, antimicrobial activity, reduced plasma lipid and lipoprotein cholesterol concentrations, and activity against diabetes mellitus (DM) (Wilson et al., 2007; Minatel et al., 2016; Masuzaki et al., 2019; Castanho et al., 2019).

Cycloartenyl ferulate is a significant component of γ-oryzanol. It has the highest amount than the other two major components, 24-methylenecycloartanyl ferulate and campesteryl ferulate (Oka et al., 2010). Kim et al. (2013) reported that the average content of campesteryl ferulate identified from seven Korean rice samples was 32.3% (mg/100 g hulled rice) and was higher than other γ-oryzanol components. Cycloartenyl ferulate (28.2%) identified in domestic Japanese rice exhibited a higher content than 24-methylene cycloartenyl ferulate (22.4%) and campesteryl ferulate (17.8%) (Oka et al., 2010). Moreover, cycloartenyl ferulate possesses some biological activities related to human health, including antimicrobial, neuroprotective, antioxidant, free radical-scavenging, NF-κB-inhibitory activities, and attenuates mast cell degranulation (Islam et al., 2009; Oka et al., 2010; Liu et al., 2021a). However, it has not been identified whether campesteryl ferulates exhibit activity against DM, mainly by inhibiting carbohydrate hydrolyzing enzymes (α-glucosidase and α-amylase).

DM is a chronic disorder and consists of two types (type 1 DM and type 2 DM). Type 2 DM is non-insulin dependent, and therapy mainly focuses on decreasing

Received 27 February 2023; Revised 21 March 2023; Accepted 30 March 2023; Published online 30 June 2023
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blood glucose levels (Brunetti and Kalabalik, 2012). Two carbohydrate hydrolytic enzymes (α-glucosidase and α-amylase) are involved in glucose release (Lebovitz, 1997). Thus, inhibiting these enzymes can decrease the blood glucose levels. Commercial drugs, including acarbose, miglitol, and voglibose, have been used as α-glucosidase inhibitors (Sugihara et al., 2014). The candidate inhibitors from natural products, such as plants and fungi, have also been studied. The phenolic diterpenes, including carnosol and hydroxy *p*-quinone carnosic acid, and two triterpene acids (betulinic acid and ursolic acid) exhibited inhibitory potential against α-glucosidase purified from rosemary (Ma et al., 2020). The bioactive compound, 8-hydroxy-6,7-dimethoxy-3-methylisocoumarine, purified from the mycelium of the endophytic fungi *Xylariaceae* sp. QGS01 exhibited inhibitory activities against α-glucosidase with a half-maximal inhibitory concentration ($IC_{50}$) of 41.75 μg/mL (Indrianingsih and Tachibana, 2017). Rice is a plant with bioactive compounds targeted toward DM (Bhuyan et al., 2022).

Rice is a staple food worldwide, especially in Asia. Rice has been used as an ingredient in traditional medicine due to the presence of various bioactive compounds (Bhat and Riar, 2015). Some of the bioactive compounds identified in rice have been used to treat DM (Pereira et al., 2021). The phenolic compounds, including guaiacol, vanillin, and vanillyl alcohol, identified from pigmented rice extracts, exhibited inhibitory potential against α-glucosidase and α-amylase (Sansenya et al., 2021). Moreover, these phenolic rice compounds demonstrated a mixed-type inhibition against α-glucosidase and α-amylase, similar to standard inhibitors (Nanok and Sansenya, 2021). Rice bran bioactive compounds, such as γ-oryzanol, phytic acid, ferulic acid, and γ-aminobutyric acid (GABA), have affected the pathological mechanisms related to diabetes, such as decreasing blood glucose levels, oxidative stress, glucose intolerance, and serum glucose (Pereira et al., 2021). Therefore, the bioactive compounds of rice exhibited biological activity related to human health, especially for controlling DM.

As reported by a previous researcher, several methods stimulate rice macronutrients and bioactive compounds. The phenolic compounds of rice (*Oryza sativa* L.), including vanillic acid, *p*-hydroxybenzoic acid, vanillin, ferulic acid, *p*-coumaric acid, and benzoic acid, increased under drought stress conditions (Quan et al., 2016). Some macronutrients, such as protein content, increased under medium and high salt conditions (Yao et al., 2022). Moreover, reduced solar intensities affected increased GABA in rice grains (Mo et al., 2015).

Gamma irradiation improved the bioactive compounds in rice. It can induce plant mutations by generating reactive oxygen species (ROS) and DNA damage, producing plant mutations (Qi et al., 2015). The volatile compounds related to the aroma intensities of rice (2-acetyl-1-pyrroline) and GABA content were affected under the gamma irradiation technique (Sansenya et al., 2017). Moreover, the γ-oryzanol content of gamma-irradiated rice increased under salt conditions (Chinvongamorn and Sansenya, 2020). This study investigated the effects of gamma irradiation on the change in cycloartenyl ferulate in Thai pigmented rice under salt conditions, and the inhibitory potential against diabetes-related enzymes (α-glucosidase and α-amylase) of cycloartenyl ferulate through *in vitro* and *in silico* study was investigated.

## MATERIALS AND METHODS

### Chemical reagents and plant material

Cycloartenyl ferulate with a purity of ≥98% was obtained from Wuhan Chem Norm Biotech, *p*-nitrophenyl-α-glucopyranoside (4-*p*NPG, purity ≥99%), α-glucosidase from *Saccharomyces cerevisiae*, and α-amylase from *Aspergillus oryzae* were obtained from Sigma-Aldrich Co.. Thai pigmented rice seeds were obtained from Phetchabun province, Thailand.

### Gamma irradiation and plant growth condition

The rice seeds were sterilized with 0.01 M NaClO for 30 min and rinsed with deionized water. The sterilized rice seeds were placed in a hot air oven at 40°C until the moisture content was less than 13%. The rice seeds were gamma irradiated at the Gamma Irradiation Center and the Nuclear Technology Research Center, Faculty of Science Kasetsart University, Thailand. The rice seeds were exposed to a gamma dose of 0 (control), 5, 40, 100, 500, and 1,000 Gy; the gamma-ray source was $^{137}Cs$.

The control rice seeds (0 Gy) and gamma-irradiated rice seeds (5～1,000 Gy) were soaked in deionized water (without NaCl concentration) and with salt solution (20 mM and 40 mM NaCl) for 24 h. The soaked rice seeds were germinated on the germinating paper moistened at 37°C. The germination experiments were sprayed with distilled water (250 mL/d) and salt solution [20 mM (250 mL/d) and 40 mM NaCl (250 mL/d)]. The rice seedlings were harvested for three days, and the rice samples were kept at −20°C until further experimentation.

### Rice seed extraction

About 0.20 g of ascorbic acid was mixed with the seed samples, which weighed about 1.0 g. The mixture was put into 5 mL of deionized water and left to sit for 30 min at room temperature. The mixture solution was extracted with hexane : isopropanol (ratio of 1:1) for 30 min. Then the extraction was centrifuged at 1,411 *g* for 15 min to discard the rice residues. The supernatant was placed in a hot air oven at 60°C for solvent evaporation. The crude

extract was kept at −20°C until further experimentation.

**Liquid chromatography (LC)-tandem mass spectrometry quantification of cycloartenyl ferulate**

The individual standard stock of cycloartenyl ferulate was prepared in dimethyl sulfoxide (DMSO). One standard mix of 0.1 mg/mL (standard mix 1) was prepared by diluting the standard stock with DMSO. The standard calibration solutions 1.0, 5.0, 10.0, and 20.0 μg/mL were prepared by diluting the standard mix with DMSO. The rice extract samples were dissolved in 4 mL of DMSO and filtered with a 0.22 μm regenerated cellulose membrane filter. Then, 200 μL of the sample was diluted with 200 μL of DMSO, and 10 μL was injected into high-performance LC.

The analysis were performed on the Dionex Ultimate 3000 UHPLC system (Dionex) coupled with electrospray ionization (ESI) tandem mass spectrometer (micrOTOF-Q II, Bruker). The injection volume for all the samples was 10 μL. The separation was achieved using a Zorbax SB-C18 [250 mm×4.6 mm×3.5 μm (Agilent Technologies)] and thermostated at 40°C, with a flow rate of 0.7 mL/min of mobile phase, which included acetonitrile : methanol : isopropanol=25:70:5 and contained 0.1% formic acid. The eluted components were ionized by an ESI source and detected in the mass scanning mode at 50 *m/z* to 1,500 *m/z* at negative ion polarity. The nebulizer gas ($N_2$) was 2 bars. The drying gas was 8 L/min, the dry heater temperature was 180°C, and the capillary voltage was 4.5 kV. The LC-quadrupole time-of-flight data were collected and processed by Compass 1.3 software (Bruker). The target compound, cycloartenyl ferulate, was identified at a retention time of 19.7 min and ions of 601.4 *m/z*. The calibration curves were constructed from the peak areas of different standard concentrations (from 5～20 μg/mL), and the concentration of targeted compounds were calculated based on the equation for linear regression obtained from the calibration curves.

**Enzymatic reaction**

The enzymatic inhibitory potential of cycloartenyl ferulate against α-glucosidase was identified by a modified Archanachai et al. (2021) method. For α-glucosidase inhibitory activity, the substrate 4-*p*NPG (0.10 mM) was mixed with 0.02 mg/mL of α-glucosidase and 0.50 mM of cycloartenyl ferulate. The mixed reaction was incubated at 37°C for 20 min, and the enzymatic activity was stopped with 0.5 M $Na_2CO_3$. The release of *p*-nitrophenol was measured at 405 nm with a spectrophotometer. For α-amylase inhibitory activity, the substrate amylose (0.01 mg/mL) was mixed with 0.05 mg/mL of α-amylase and 0.50 mM of cycloartenyl ferulate. The enzymatic reaction was incubated at 37°C for 15 min. Enzymes (peroxidase-glucose oxidase assay) determined the released glucose, and the absorbance was measured at 475 nm. The inhibition percentage was calculated using $[(A_b-A_s)/A_b]\times100$ ($A_b$=absorbance without sample; $A_s$=absorbance with sample).

**Kinetic study**

The inhibition type of cycloartenyl ferulate against α-glucosidase was determined using a Lineweaver-Burk plot in a double reciprocal form. The reaction conditions, including the substrate and α-glucosidase concentration, were maintained under the same conditions in the sequence reactions above. The cycloartenyl ferulate concentrations in the inhibition-type reaction were 0.00, 0.30, and 0.50 mM.

**Fluorescence analysis**

The interaction between α-glucosidase and cycloartenyl ferulate was analyzed using an FP 8500 fluorescence spectrophotometer (Jasco Corp.), as described by Liu et al. (2011). The fluorescence intensity of 0.05 mg/mL enzyme solution mixed with different concentrations of cycloartenyl ferulate (0.0～0.5 mM) was measured at 295 nm. The emission wavelength was 300～400 nm, the slit wavelength was 5 nm, and the scanning speed was medium.

**Docking analysis**

The structure of cycloartenyl ferulate was constructed and optimized at the B3LYP/6-311++G(d,p) level of theory using the Gaussian 03W program suite (Gaussian, Inc.). The protein target was obtained from the Protein Data Bank as α-glucosidase (7DCH) and α-amylase (7TAA). The three-dimensional structures of the protein targets were subjected to the AutoDockTools program (Morris et al., 2009) to remove crystal water molecules and complex ligands.

For docking calculations, the grid box of 40×40×40 Å with space points of 1.0 Å was set. The grid box centers of 87.688, 92.314, and 15.051 were applied for α-glucosidase. All docking calculations were performed by the AutoDock Vina program (Trott and Olson, 2010). The Vinadocking results were subjected to the protein-ligand interaction profiler (Adasme et al., 2021) web server for analyzing protein-ligand interactions. Additionally, the visualizations of docking results were generated by the Visual Molecular Dynamics program (Humphrey et al., 1996).

**Statistical analysis**

The results, including inhibition percentages and cycloartenyl ferulate content, were determined in triplicate and reported as the mean±standard deviation. One-way ANOVA was used for statistical analysis, and the differences were considered significant at *P* values less than

0.05.

## RESULTS AND DISCUSSION

### The effects of gamma irradiation on cycloartenyl ferulate contents in germinated rice (Thai pigmented rice) under saline conditions

Gamma-oryzanol accumulation in rice can be affected by stress factors, including soil salinity (Tung and Ng, 2016). Recently, a researcher reported that an increase in γ-oryzanol in gamma-irradiated rice was affected under saline conditions (Chinvongamorn and Sansenya, 2020). We observed that cycloartenyl ferulate accumulated in gamma-irradiated and control rice under saline conditions. The cycloartenyl ferulate content in gamma-irradiated (5～1,000 Gy) and control rice (0 Gy) treated with salt concentrations (0, 20, and 40 mM NaCl) are shown in Table 1. The cycloartenyl ferulate content of gamma-irradiated and control rice untreated with salt concentrations (0 mM) was 403.83±4.24～502.87±7.81 μg/g. The highest cycloartenyl ferulate content was obtained from gamma-irradiated rice treated with 100 Gy (502.87±7.81 μg/g). Moreover, the cycloartenyl ferulate content of gamma-irradiated rice (40～1,000 Gy) was higher than the control rice. The range of cycloartenyl ferulate content between 410.97±14.21～759.27±17.49 μg/g and 425.10 ±15.52～852.20±20.59 μg/g were identified from gamma-irradiated and control rice treated with 20 and 40 mM NaCl, respectively. In saline conditions (20 and 40 mM), the cycloartenyl ferulate content of gamma-irradiated rice (5～1,000 Gy) was higher than the control rice. Moreover, the highest cycloartenyl ferulate was obtained from gamma-irradiated rice treated with 100 Gy for 40 mM (852.20±20.59 μg/g) and 1,000 Gy for 20 mM (759.27± 17.49 μg/g) saline conditions. Furthermore, the trend of cycloartenyl ferulate in saline treatment in gamma-irradiated rice had a higher content than untreated under saline concentrations. Gamma irradiation is a classical technique for generating plant mutations. The gamma rays induce the production of ROS or free radicals and cause plant mutations (Beyaz and Yildiz, 2017). Many researchers reported that gamma rays induced the bioactive compounds in plants and rice (Khalil et al., 2015; Sansenya et al., 2017). The release of ROS by gamma rays either damages or modifies the differentiation process, morphology, physiology, and bioactive component biosynthesis pathway depending on the gamma dose (Ashraf et al., 2003; Amirikhah et al., 2021). The low gamma dose enhances seed germination and the growth of some plant species and rice cultivars (Amirikhah et al., 2021; Archanachai et al., 2021). Various gamma-ray doses have affected the decreased or increased plant-bioactive compounds. A researcher reported that gamma rays in the dose range of 5～10 Gy affected the decrease in tocopherol content of the peanut samples (De Camargo et al., 2012). In comparison, the 15～20 Gy of gamma dose stimulated the bioactive compounds and biological properties of *Stevia rebaudiana* (Bert.) (Khalil et al., 2015). Our results showed that a 5～1,000 Gy gamma dose induced cycloartenyl ferulate in Thai pigmented rice. Previous reports and present studies have demonstrated that the stimulation of bioactive compounds had been affected by various gamma-ray doses, such as 5～1,000 Gy. Moreover, several researchers reported that gamma irradiation still affected the physiological and biochemical profiles of mutant plants' second generation (M2 generation). Hanafy and Akladious (2018) reported that the low dose of gamma rays (100 Gy) increased growth, yield characteristics, and some biochemical compounds in $M_1$ and $M_2$ generations of *Trigonella foenum-graecum* L.. Gamma irradiation also affected the chlorophyll variety of the $M_2$ generation of wheat (*Triticum turgidum* ssp. *durum*) compared to the $M_0$ generation (Ahumada-Flores et al., 2021). Thus, gamma irradiation improved the physiological and biochemical properties of the parental plant and could transfer these traits to other varieties.

The gamma irradiation technique has been used with various abiotic stresses (heat, salinity, drought, flood, and heavy metals) to improve many plant species' physiological and biochemical properties (Katiyar et al., 2022). Salinity is an abiotic stress that affects various plants'

**Table 1.** Cycloartenyl ferulate content in gamma-irradiated rice under saline concentration (unit: μg/g)

| Gamma dose (Gy) | Cycloartenyl ferulate content[1)] | | |
|---|---|---|---|
| | 0 mM salt concentration | 20 mM NaCl | 40 mM NaCl |
| 0 | 403.83±4.24$^{c}$ | 410.97±14.21$^{d}$ | 425.10±15.52$^{e}$ |
| 5 | 410.73±2.62$^{c}$ | 483.70±12.06$^{c}$ | 436.40±16.96$^{d}$ |
| 40 | 424.97±5.36$^{b}$ | 494.27±12.72$^{bc}$ | 577.39±15.17$^{c}$ |
| 100 | 502.87±7.81$^{a}$ | 499.70±10.81$^{bc}$ | 852.20±20.59$^{a}$ |
| 500 | 429.73±6.12$^{b}$ | 518.00±9.20$^{b}$ | 816.23±12.44$^{b}$ |
| 1,000 | 495.27±7.01$^{a}$ | 759.27±17.49$^{a}$ | 847.67±17.10$^{a}$ |

The different letters (a-e) within the column indicated significant differences between the differences in the rice sample ($P$<0.05).
[1)]The cycloartenyl ferulate content was determined from the rice extract.

morphological and biochemical properties (Parihar et al., 2015). Previous researchers have reported that gamma irradiation increased the cycloartenyl ferulate accumulation in plants under salt stress (Katiyar et al., 2022). Our results showed that cycloartenyl ferulate in rice seedlings (three days) treated with salt concentration increased (410.97±14.21 μg/g for 20 mM NaCl and 425.10±15.52 μg/g for 40 mM NaCl) when compared to untreated under salt concentration (403.83±4.24 μg/g for 0 mM NaCl). γ-Oryzanol concentration increased in the rice grains, and germinated rice under increasing salt conditions have been reported by previous researchers (Tung and Ng, 2016; Chinvongamorn and Sansenya, 2020). Thus, γ-oryzanol and its derivatives (cycloartenyl ferulate) in rice (grain, germinated, and seedling) were affected by increased saline conditions. Moreover, the cycloartenyl ferulate of gamma-irradiated rice was affected by increased salt concentration (0～40 mM). The highest concentration was obtained from gamma-irradiated rice (100 Gy) treated with 40 mM NaCl. However, gamma-irradiated rice (5～1,000 Gy) treated with 20 and 40 mM salt concentrations exhibited higher cycloartenyl ferulate accumulation than gamma-irradiated rice with untreated salt concentrations (Table 1). Gamma irradiation enhanced the bioactive compound synthesis of plants under saline conditions.

### The inhibition potential and kinetic mechanism of cycloartenyl ferulate against α-glucosidase and α-amylase

The inhibitory potential of cycloartenyl ferulate against α-glucosidase was 31.31±1.43% and approximately 2.5-fold higher than the inhibition percentage against α-amylase (12.72±1.11%). Therefore, the inhibition mode of α-glucosidase against cycloartenyl ferulate was determined. Lineweaver-Burk plot determined the inhibition type of cycloartenyl ferulate against α-glucosidase and showed mixed-type inhibition (Fig. 1). γ-Oryzanol has been investigated for therapeutic management of DM, such as reducing blood glucose levels (Ghatak and Panchal, 2012). The study of γ-oryzanol and its derivative in diabetes management mainly focused on regulating improved metabolic dysfunctions, such as hyperglycemia, hypercholesterolemia, hypertriglyceridemia, and insulin resistance (Szcześniak et al., 2016). However, the inhibition of carbohydrate hydrolysis enzymes (α-glucosidase and α-amylase) has to be studied for γ-oryzanol, especially cycloartenyl ferulate. Ferulic acid is a part of the cycloartenyl ferulate structure studied for inhibitory activity against α-glucosidase and α-amylase and showed inhibition mode as non-competitive and mixed-type inhibition, respectively (Zheng et al., 2020). This research reported that the inhibition potential of ferulic acid against α-amylase is stronger than α-glucosidase with an $IC_{50}$ of 0.622 and 0.866 mg/mL, respectively. Thus, a previous report and our results demonstrated that cycloartenyl ferulate could be a candidate compound for diabetes-related enzyme inhibition. Moreover, the literature also supported that this cycloartenyl ferulate had a high potential against α-glucosidase and displayed mixed-type inhibition.

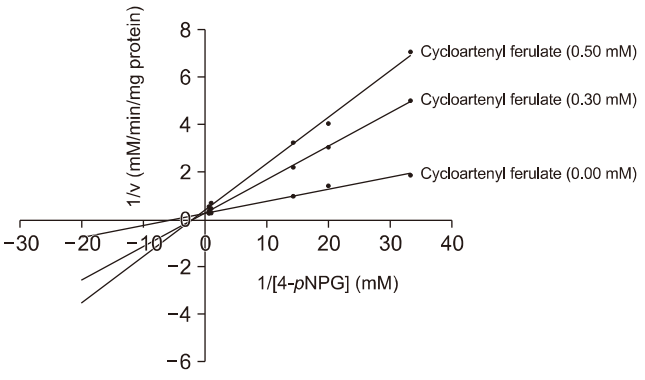


**Fig. 1.** Lineweaver-Burk plot of cycloartenyl ferulate with different concentrations between 0.00, 0.30, and 0.50 mM against α-glucosidase.

### Fluorescence quenching analysis of cycloartenyl ferulate against α-glucosidase

The changes in cycloartenyl ferulate-induced intrinsic tryptophan fluorescence in α-glucosidase enzymes are shown in Fig. 2. When excited at 295 nm, cycloartenyl ferulate exhibited no evident fluorescence emission and contributed negligible fluorescence interference. With the treatment of cycloartenyl ferulate (0～0.5 mM), the fluorescence intensity of α-glucosidase was quenched gradually in a concentration-dependent manner. These results established an interaction between cycloartenyl ferulate and α-glucosidase, which induces a microenvironment variation for TRP, TYR, and PHE residues in α-glucosidase. Possibly, this variation hindered the active center formation and/or the binding of the substrates. The reduction in the fluorescence intensity of α-glucosidase by increasing ferulic acid has been reported by Zheng et al. (2020). Some plant polyphenol sinensetin also showed similar results by increasing inhibitor concentration; a decrease in the fluorescence emission peak of α-glucosidase was observed, which suggested that sinensetin interacted with α-glucosidase (Liu et al., 2021b). Previous studies and our results also supported that increasing the inhibitor concentration can decrease the fluorescence intensity of α-glucosidase. Moreover, all data suggested that cycloartenyl ferulate interacted with α-glucosidase, and specific structural changes altered the internal microenvironment of α-glucosidase.

### Molecular docking analysis

The target ligand, cycloartenyl ferulate, was docked with an AutoDockTools program to understand its binding potential against α-glucosidase and α-amylase. The dock-

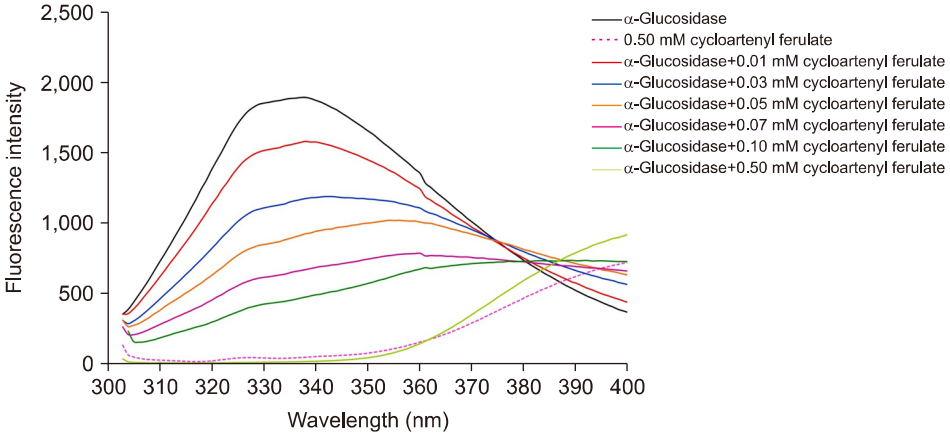


**Fig. 2.** Fluorescence spectra changes of α-glucosidase by cycloartenyl ferulate. The enzyme (0.05 mg/mL) was incubated with specified concentrations of cycloartenyl ferulate (0~0.5 mM) for 30 min at 37°C. The excitation wavelength was 295 nm, and emission spectra were acquired by scanning from 300~400 nm.

**Table 2.** Molecular docking analysis of cycloartenyl ferulate potential binding site with α-glucosidase and α-amylase

| Ligand | Enzyme | Binding affinity (kcal/mol) | Active site residue | Distance (Å) | Interaction type |
|---|---|---|---|---|---|
| Cycloartenyl ferulate | α-Glucosidase | −8.8 | TYR216 | 3.81 | Hydrophobic |
| | | | PHE306 | 3.70 | Hydrophobic |
| | | | VAL346 | 4.00 | Hydrophobic |
| | | | TRP376 | 3.67 | Hydrophobic |
| | | | ASP440 | 3.72 | Hydrophobic |
| | | | THR441 | 3.53 | Hydrophobic |
| | | | LEU447 | 3.93 | Hydrophobic |
| | | | THR441 | 3.08 | Hydrogen bond |
| | α-Amylase | −8.2 | ALA74 | 3.73 | Hydrophobic |
| | | | TYR75 | 3.69 | Hydrophobic |
| | | | TYR82 | 3.84 | Hydrophobic |
| | | | LEU166 | 3.77 | Hydrophobic |

ing results are shown in Table 2. The binding affinity of cycloartenyl ferulate was −8.8 kcal/mol for α-glucosidase and −8.2 kcal/mol for α-amylase, respectively. It has one hydrogen bond and seven hydrophobic interactions with THR441 (hydrogen bonding) and hydrophobic interactions, including TYR216, PHE306, VAL346, TRP376, ASP440, THR441, and LEU447 of α-glucosidase (Fig. 3A). Cycloartenyl ferulate interacted with four hydrophobic interactions, including ALA74, TYR75, TYR82, and LEU166 of α-amylase (Fig. 3B). The docking results indicated that the cycloartenyl ferulate in the active site of α-glucosidase exhibited more binding potential than α-amylase. Moreover, the molecular docking result confirmed the inhibition potential of cycloartenyl ferulate against α-glucosidase and was higher than that against α-amylase. Ferulic acid is a part of the cycloartenyl ferulate structure that docked with α-glucosidase (3A4A) with binding energy of −5.70 kcal/mol. It also docked with α-amylase (5KEZ) and directed binding energy of −5.30 kcal/mol, which was similar to α-glucosidase (Liu et al., 2021b). Our results suggested that the binding affinity of cycloartenyl ferulate exhibited a higher potential than ferulic acid in the active site of α-glucosidase. However, the binding potential of cycloartenyl ferulate and ferulic acid was still lower than the standard inhibitor acarbose with a binding energy of −10.56 kcal/mol against α-glucosidase (3W37) (Abuelizz et al., 2019). Nonetheless, from the docking results of cycloartenyl ferulate and ferulic acid, the amino acids surrounding the ligand might be essential to increase the stabilization of the inhibitor-en-

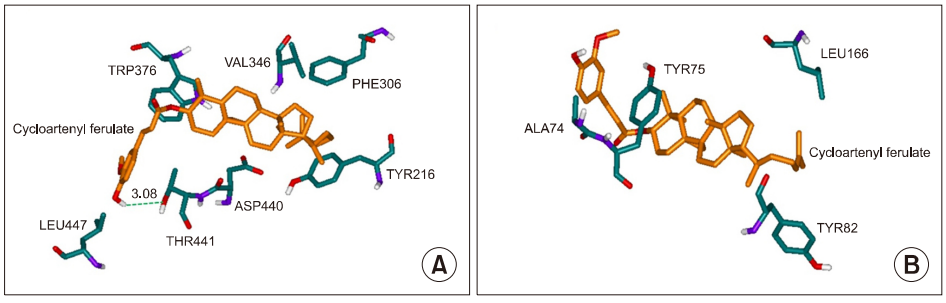


**Fig. 3.** The docking conformation of cycloartenyl ferulate interaction with the active site residues of α-glucosidase (A) and α-amylase (B). The green dotted line indicated the formation of a hydrogen bond between the ligand and amino acid residues.

zyme complex. Furthermore, the binding between cycloartenyl ferulate and the amino acids in the active site of α-glucosidase was confirmed by fluorescence quenching results. Thus, cycloartenyl ferulate could be a potential bioactive compound of a diabetes-related enzyme.

In conclusion, this research investigated the effects of gamma irradiation on cycloartenyl ferulate under saline conditions in Thai pigmented rice (germinated rice). The results showed that 5～1,000 Gy of gamma irradiation increased the amount of cycloartenyl ferulate in germinated rice compared to 0 Gy. Cycloartenyl ferulate concentration was obtained from germinated rice under a gamma dose of 100 Gy. Cycloartenyl ferulate of gamma-irradiated rice under saline concentrations (20 and 40 mM) increased, and the highest concentration was obtained from the gamma dose of 100 Gy under 40 mM salt. The results indicated that the accumulation of cycloartenyl ferulate in germinated rice under saline conditions increased by the gamma irradiation technique and induced its accumulation.

The present study investigated the inhibition potential of cycloartenyl ferulate against α-glucosidase and α-amylase through *in vitro* and *in silico* studies. The inhibition percentage of cycloartenyl ferulate against α-glucosidase was 31.31±1.43% and higher than against α-amylase (12.72±1.11%). Cycloartenyl ferulate displayed a mixed-type inhibition against α-glucosidase as demonstrated by the Lineweaver-Burk plot. Moreover, the decreasing fluorescence intensity of α-glucosidase was affected by increasing cycloartenyl ferulate content. A docking study confirmed that cycloartenyl ferulate stabilized in the active site of α-glucosidase by surrounding with seven binding amino acids (seven hydrophobic interactions and one hydrogen bonding). Moreover, the docking results suggested that cycloartenyl ferulate against α-glucosidase had a higher potential than against α-amylase. The results indicated that cycloartenyl ferulate might be a potential candidate compound for DM treatment.

## FUNDING

This work was financially supported by the Science Research and Innovation Promotion Funding (TSRI). This research block grant was managed under the Rajamangala University of Technology Thanyaburi (RMUTT) (Granted No. FRB65E0628).

## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Concept and design: all authors. Analysis and interpretation: all authors. Data collection: all authors. Writing the article: all authors. Critical revision of the article: all authors. Final approval of the article: all authors. Statistical analysis: SS. Obtained funding: SS. Overall responsibility: SS.

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