บทบาทของ Glyoxalase-1 ในการยับยั้งปฏิกิริยาไกลเคชั่นของสารสกัดจากมันเทศสีม่วงผ่านแบบจำลองแมลงหวี่

ณัฐกานต์ หวังเกษม[1] ปิยะ เต็มวิริยะนุกูล[2] วรวีร์ อินทชาติ[2] ภรัณยา ธิยะใจ[2] ปราณี พัฒนกุลอนันต์[2] ปรัญรัชต์ ธนวิยุทธ์ภัคดี[2,*]

[1] หลักสูตรวิทยาศาสตรมหาบัณฑิตสาขาวิชาพิษวิทยาและโภชนาการเพื่ออาหารปลอดภัย สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย

[2] สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย

บทคัดย่อ

Glyoxalase-1 (GLO1) เป็นเอนไซม์ที่สำคัญในการทำลายพิษของผลิตภัณฑ์ขั้นสุดท้ายจากปฏิกิริยาไกลเคชั่น (AGEs) ซึ่งเป็นปัจจัยหนึ่งที่ทำให้เกิดการพัฒนาโรคเรื้อรังในระบบเมแทบอลิกของร่างกาย แอนโทไซยานินที่พบในมันเทศสีม่วงมีฤทธิ์ต้านอนุมูลอิสระและต้านการอักเสบที่มีประสิทธิภาพอาจช่วยยับยั้งปฏิกิริยาไกลเคชั่นได้ วัตถุประสงค์ของงานวิจัยเพื่อศึกษาผลของสารสกัดจากมันเทศสีม่วงต่อการยับยั้งปฏิกิริยาไกลเคชั่นผ่านการแสดงออกของยีน GLO1 ในแบบจำลองแมลงหวี่ สกัดสารแอนโทไซยานินจากมันเทศสีม่วงด้วยกรดน้ำส้ม 3% ในเอทานอล 80% ผลจากการวิเคราะห์โปรไฟล์แอนโทไซยานินด้วยวิธีโครมาโทกราฟีของเหลวสมรรถนะสูง พบว่าสารสกัดจากมันเทศสีม่วงมีเพียงไซยานิดินและพีโอนิดินเท่านั้น สารสกัดแสดงฤทธิ์ต้านอนุมูลอิสระในวิธี DPPH, FRAP และ ORAC ที่ระดับ 46.22, 55.39 และ 360.18 μmol TE/g DW ตามลำดับ ระดับของ AGEs และการแสดงออกของยีน GLO1 ถูกวัดโดยใช้ชุดทดสอบ AGEs ELISA kit และเทคนิค qRT-PCR ตามลำดับ ในแบบจำลองแมลงหวี่สารสกัดจากมันเทศสีม่วงที่ความเข้มข้น 2 mg/mL ส่งผลให้การแสดงออกของยีน GLO1 เพิ่มขึ้นอย่างมีนัยสำคัญ ($p < 0.05$) และมีแนวโน้มลดระดับ AGEs การวิจัยนี้ชี้ให้เห็นว่ามันเทศสีม่วงอาจเป็นอาหารที่ช่วยลดการเกิดปฏิกิริยาไกลเคชั่น อย่างไรก็ตามยังคงจำเป็นต้องมีการศึกษาต่อในสัตว์ทดลองและการวิจัยทางคลินิก เพื่อยืนยันการใช้มันเทศสีม่วงเป็นทางเลือกในการพัฒนาอาหารเชิงหน้าที่เพื่อป้องกันและลดภาวะแทรกซ้อนจากโรคเรื้อรังทางเมแทบอลิก

คำสำคัญ: มันเทศสีม่วง แอนโทไซยานิน การต้านไกลเคชั่น Glyoxalase-1 แมลงหวี่

รับบทความ: *4 เมษายน 2568* ***แก้ไข:*** *14 พฤษภาคม 2568* ***ตอบรับ:*** *18 พฤษภาคม 2568*

[*] **ผู้รับผิดชอบบทความ**

ปรัญรัชต์ ธนวิยุทธ์ภัคดี

สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย

อีเมล: pharrunrat.tan@mahidol.ac.th

The Role of Glyoxalase-1 in the Inhibition of Glycation-Reaction of Purple Sweet Potato Extract through the *Drosophila melanogaster* Model

Nattakarn Wangkasem[1] Piya Temviriyanukul[2] Woorawee Inthachat[2] Parunya Thiyajai[2] Pranee Pattanagulanan[2] Pharrunrat Tanaviyutpakdee[2,*]

[1] Graduate student in Master of Science Program in Toxicology and Nutrition for Food Safety, Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand

[2] Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand

Abstract

Glyoxalase-1 (GLO1) is an enzyme that plays an important role in the detoxification of advanced glycation end-products (AGEs), which are byproducts of glycation reactions and contribute to the development of various chronic metabolic disorders. Anthocyanins, found in purple sweet potatoes (PSP), are potent anti-inflammatory and antioxidant compounds that may also prevent glycation reactions. This study aims to investigate the effects of PSP extract on the inhibition of glycation reactions through the expression of the *GLO1* gene in the *Drosophila melanogaster* model. Anthocyanins were extracted from PSP using 3% acetic acid in 80% ethanol. The analysis of anthocyanin profiles using high-performance liquid chromatography revealed that the PSP extract contained only cyanidin and peonidin. The antioxidant activities of PSP were quantified as follows: DPPH, 46.22 μmol TE/g DW; FRAP, 55.39 μmol TE/g DW; and ORAC, 360.18 μmol TE/g DW. The levels of AGEs and *GLO1* gene expression were measured using ELISA kits and qRT-PCR, respectively. In the *D. melanogaster* model, PSP extracts at 2 mg/mL significantly upregulated *GLO1* gene expression ($p < 0.05$) and showed a tendency to reduce AGE levels. This study indicates that PSP could help reduce glycation reactions. However, further research in animal models and clinical studies is needed to confirm the use of PSP as an alternative for the development of functional foods to prevent and reduce complications from chronic metabolic diseases.

Keywords: Purple sweet potato, Anthocyanin, Anti-glycation, Glyoxalase-1, *Drosophila melanogaster*

Received: *4 April 2025,* ***Revised:*** *14 May 2025,* ***Accepted:*** *18 May 2025*

[*]Corresponding author

Pharrunrat Tanaviyutpakdee

Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand

E-mail: pharrunrat.tan@mahidol.ac.th

Introduction

Advanced glycation end products (AGEs) are compounds formed by the Maillard reaction, a non-enzymatic reaction between carbonyl groups of glucose or other reducing sugar and amino groups of protein or lipid. The AGE accumulation of AGEs *in vivo* activates many signaling pathways, producing reactive carbonyl species, oxidative stress molecules, free radicals, and inflammatory responses. Methylglyoxal (MG), the most reactive carbonyl molecule in the human body, can cause dicarbonyl stress and interfere with regular physiological processes. It has been connected to the development of several chronic metabolic diseases, including diabetes, atherosclerosis, and Alzheimer's disease[1, 2]. Reports have shown that patients with diabetes mellitus (DM) exhibit significantly higher blood concentrations of MG compared to non-DM patients[3]. The imbalance of the glyoxalase system, an antiglycation mechanism, may result from elevated accumulation of MG in the body. Glyoxalase-1 (GLO1) is an important glyoxalase enzyme detoxifying the reactive dicarbonyl metabolite MG. It is found in the cytoplasm[4].

Several studies have demonstrated that polyphenol compounds have antiglycation properties both *in vivo* and *in vitro*. These functional substances reduce the progression of diabetes and its associated effects by lowering the synthesis of carbonyl molecules through antiglycation mechanisms[5, 6]. Previous research has found that anthocyanin-rich berry extracts can trap reactive carbonyl species, block glycation processes, and prevent MG-induced amyloid β (Aβ) fibrillation[7]. In addition, cyanidin has been shown to reduce AGE production by enhancing GLO1 activity and glutathione levels[8].

Purple sweet potato (PSP) extract is high in phytochemicals, particularly anthocyanins such as cyanidin and peonidin, which have anti-cancer, anti-diabetic, antioxidant, and anti-inflammatory effects[9]. Indeed, a prior study found that PSPs contain fifteen phenolic compounds, the majority of which are anthocyanins. All ten identified anthocyanins were either cyanidin or peonidin[10]. Thus, PSP extract may suppress glycation reactions by targeting the GLO1 enzyme pathway. Furthermore, researchers discovered that PSP (*Ayamurasaki cultivar*) extracts scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals more effectively than purple cabbage, elderberry, grape peel, and purple corn extracts[11]. However, the inhibition of glycation reactions via the upregulation of the *GLO1* gene by PSP extract has not yet been studied. Therefore, this work aims to evaluate the effects of PSP

extract on the suppression of glycation processes through the modulation of the *GLO1* gene expression in the *Drosophila melanogaster* model.

Materials and Methods

Chemicals

The primary chemicals used in this study included L-carnosine (99% crystalline) and TPTZ (≥99.0% purity) purchased from Sigma-Aldrich (Switzerland); cyanidin chloride (≥95% HPLC), gallic acid (≥98.5% purity), DPPH, Trolox, fluorescein, and AAPH obtained from Sigma-Aldrich (USA); ethanol (99.8% purity, J.T. Baker), acetic acid (glacial, 99.8% purity), Folin-Cioculteu's phenol reagent, and iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 99.0–102% purity) purchased from Merck (Germany). All other chemicals used in the study were analytical grade and obtained from standard commercial suppliers.

Sample preparation

PSPs were obtained from Pathommongkon Market in Nakhon Pathom. The roots were washed thoroughly, steamed for 40 minutes, and allowed to cool to room temperature (25°C). The peels were removed, and the flesh was cut into small pieces. The pieces were freeze-dried, ground into powder using a blender, packed in aluminum foil pouches, and stored at -20 °C until further use.

Sample extractions

1) Sample extraction for antioxidant activity analyses

Purple sweet potato extract (PSPE) samples were prepared according to the previous study[12] with some modifications. One gram of freeze-dried PSP powder was extracted three times with 30 mL of acidified ethanol (3% acetic acid in 80% ethanol) using ultrasonication at 48 °C for 20 minutes. The mixture was centrifuged at 1,500 ×g for 10 minutes and filtered with Whatman No. 541 filter paper. The supernatants were diluted to 100 mL with acidified ethanol (1 g per 100 mL extraction ratio) to a final concentration of 10 mg/mL. PSPE was stored at -20 °C until analysis.

2) Sample extraction for high-performance liquid chromatography (HPLC) analysis

PSPE (5 mL) was evaporated to dryness at 37 °C using a rotary vacuum evaporator. The dried PSPE was dissolved in a methanol solution with 2 N HCl and hydrolyzed for 60 minutes at 100 ± 2 °C. The volume was then adjusted to 5 mL using the same methanol solution and filtered through a 0.2 µm PTFE filter. The filtrate was transferred to a vial for HPLC analysis.

3) Sample extraction for utilization in D. melanogaster culture medium

PSPE (1,000 mL) was evaporated to dryness at 37 °C using a rotary vacuum evaporator. The dried PSPE was dissolved in deionized water and adjusted to a final volume of 100 mL. The extract was stored at its highest concentration at -20 °C until used.

Analysis method of phytochemical contents

1) Anthocyanidin profiles assay

Anthocyanidin concentrations were measured using acid hydrolysis followed by HPLC quantification as previously described with slight modifications[13]. The Agilent 1260 Infinity II system equipped with a photodiode array detector and an Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm) was used. The mobile phases were 0.1% trifluoroacetic acid (TFA) in water (18.2 MΩ·cm), 0.1% TFA in methanol (MeOH), and 0.1% TFA in acetonitrile (ACN). The column temperature was set to 35 °C, with a flow rate of 0.6 mL/min and an injection volume of 10 µL. The standards included delphinidin, cyanidin, peonidin, pelargonidin, and malvidin. The detector was set to scan from 250 to 600 nm, with anthocyanidins were detected at 520 nm.

2) Total anthocyanin contents (TACs) assay

The TACs of PSPE were determined using the pH differential method described in the AOAC Official Method[14]. In a 96-well plate, 50 µL of PSPE was mixed with 200 µL of pH 1.0 buffer (0.025 M potassium chloride) and pH 4.5 buffer (0.4 M sodium acetate). After 20-50 minutes of incubation, the absorbance at 520 and 700 nm was measured using a microplate reader. The TACs were calculated as milligrams of cyanidin-3-glucoside equivalents per 100 grams of fresh and dry weight samples.

3) Total phenolic contents (TPCs) assay

The Folin-Ciocalteu test was used to analyze TPCs in PSPE, as described in a recent study[15]. PSPE (25 µL) was mixed with 10% Folin-Ciocultue reagent (50 µL) and incubated at 25 °C for 5 min. Incubate 7.5% sodium carbonate (200 µL) in the dark at 25 °C for 2 hours. The absorbance at 765 nm was measured with a microplate reader. Gallic acid (10 to 200 µg/mL) was used as the standard. The TPCs were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of fresh or dry weight samples.

Analysis method of antioxidant activities

1) DPPH-radical scavenging activity (DPPH) assay

The DPPH-radical scavenging activity in PSPE was measured using the previous study's method[15]. The PSPE (22 µL) was combined with 200 µL of a 150 µM DPPH free radical solution in 95%

ethanol and incubated in the dark at 25 °C for 30 minutes. The absorbance was then measured at 520 nm with a microplate reader. Trolox (0.01-0.64 mM) was used as the standard.

2) Ferric reducing antioxidant power (FRAP) assay

The FRAP activity was measured in PSPE using the method from the previous study[15] with minor modifications as follows. The PSPE (20 µL) was diluted 5X with deionized (DI) water, then added 150 µL of FRAP reagent (acetate buffer pH 3.50 (10): TPTZ (1): $FeCl_3 \cdot 6H_2O$ (1)) and incubated in the dark at 25 °C for 8 min. The absorbance was measured at 595 nm at 37 °C using a microplate reader. Trolox (7.81 to 500.00 µM) was used as the standard.

3) Oxygen radical absorbance capacity (ORAC) assay

The ORAC activity in PSPE was assessed using the method described in the previous study[15]. The PSPE (25 µL) was diluted 100X with ORAC buffer working solution, added with 150 µL of fluorescein reagent, and incubated in the dark at 37 °C for 30 min. Next, 25 µL of AAPH solution was added. The fluorescence mixture was measured using a fluorescent microplate reader with kinetic excitation at 485 nm and emission at 528 nm. Trolox (3.12 to 100.00 µM) was used as the standard.

Antioxidant activities, including DPPH, FRAP, and ORAC assays, were reported in micromoles of Trolox equivalents (µmol TE) per gram of fresh and dry weight samples.

In vivo anti-glycation capacity assay

1) Animal model and culture condition

Canton-S (number 64349) is a wild-type strain of *D. melanogaster* from Bloomington *Drosophila* Stock Center, USA. It was maintained at a 12-hour light/dark cycle, 25 ± 1 °C, and 65% humidity. The *Drosophila* study was approved by the Institute of Nutrition, Mahidol University-Institute Animal Care and Use Committee (INMU-IACUC) (COA. No. INMU-IACUC 2023/04).

2) D. melanogaster culture media

Standard (yeast-glucose-agar) *Drosophila* medium was prepared according to the previous study[16], with minor modifications, as follows. In short, 1000 mL of medium contained corn flour (125 g), sugar (100 g), yeast (30 g), agar (14 g), water (1000 mL), propionic acid (5 mL), and methyl 4-hydroxybenzoate (nipagin) in 95% ethanol (10 mL). The medium was sterilized and stored at 4 °C until used. This medium was used to sustain the *Drosophila* stock, collect larvae, and set up the experiment.

3) Survival test and experimental medium

The survival test medium included three groups comprising six treatments: the control group (distilled water, DI), experimental group (PSPE at 1, 2, and 4 mg/mL), and the positive control group (carnosine 40 µM and cyanidin 5.88 µM). The medium was prepared by combining 3 g of Formula 4-24 Instant Drosophila medium with 10 mL of each treatment solution. The same medium was used in the experiment, based on the survival test results. PSPE concentrations were determined using data from a previous study[16]. Pure cyanidin at 5.88 µM (positive control) corresponded to a PSPE concentration of 2 mg/mL. The cyanidin concentrations in the experimental treatments were 2.94 µM for 1 mg/mL PSPE and 11.76 µM for 4 mg/mL PSPE.

4) Survival test of D. melanogaster

The *D. melanogaster* survival test was conducted to evaluate the effects of PSPE and positive control substances on the flies' growth and survival rate. The test employed the larvae survival method[17], in which third-instar larvae were maintained in a survival test medium consisting of three groups (six treatments) until they reached the adult stage. A survival rate of at least 80% was used as a screening-level endpoint to determine appropriate concentrations for subsequent experiments. Each treatment used 100 larvae and was conducted in triplicate. The results indicated that all treatments achieved a growth rate exceeding 80%, suggesting that the tested substances did not adversely affect the survival of *D. melanogaster*.

5) Experimental procedure for AGEs and GLO1 gene assay

One-day-old adult male and female *D. melanogaster* were maintained in an experimental medium of three groups (six treatments) for 30 days. The flies were transferred to fresh medium every three days. Afterward, flies from each experimental group were collected and stored at -80 °C. Subsequently, the stored *Drosophila* were analyzed for AGEs levels and *GLO1* gene expression using AGEs ELISA kit and qRT-PCR assay, respectively. The experiment used 100 flies per treatment and was conducted in triplicate.

Analysis method of outcome In vivo anti-glycation capacity

1) Confirmation and identification of AGEs formation by ELISA Kit

Each treatment involved the placement of five flies into labeled, empty 1.5 mL Eppendorf tubes. The tubes were placed in an ice bath to extract protein, and 50 µL of T-PER (Tissue Protein Extraction Reagent) was added before homogenization.

The homogenates were collected on ice and centrifuged at 2490 ×g at 4 °C for 15 minutes. The supernatants were collected into empty Eppendorf tubes and stored on ice until use. The protein concentration was then evaluated using a bicinchoninic acid (BCA) protein assay kit, with all samples adjusted to a final protein concentration of 1 mg/mL. Furthermore, AGEs were quantified using an AGEs ELISA kit (Advanced Glycation End Products Assay Kit, ab238539, Abcam, UK). The concentration of AGEs was determined using an AGE-BSA standard calibration curve.

2) Confirmation and identification of GLO1 gene expression by quantitative real-time reverse-transcription PCR (qRT-PCR)

Total mRNA from flies was extracted using TRIzol reagent. A Nanodrop spectrophotometer was used to quantify total RNA and its purity. Complementary DNA (cDNA) was synthesized from mRNA using the Reverse Transcription Kit (ReverTra Ace^{TM} qPCR RT Master Mix with gDNA Remover, FSQ-301, TOYOBO CO., LTD, Osaka, Japan), and all samples were adjusted to a final cDNA concentration of 200 ng/mL. Furthermore, qRT-PCR was carried out using primer pairs (**DmGLO1 self**: Primer sequence 5′-3′ / Forward = TCCAGCAAACCATGTACCGC / Reverse = CCAGTTTCACCAGCAAGGTC). The gene expression of alphaTub-84B was used as an internal control. (**alphaTub84B self:** Primer sequence 5′-3′/Forward = CCGCTATTGCCACCATCAAG / Reverse = AAATCACCTCCAGGCACCAC).

Statistical Analysis

All data are depicted as the mean ± standard deviation. The concentrations of anthocyanidin in PSPE were calculated using a linear regression equation derived from respective standard curves. AGEs levels among various concentrations of PSPE and the control group were compared using a one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. The *GLO1* gene expression between the PSPE and control groups was compared using an independent *t*-test. Statistical analyses were performed using SPSS (PASW Statistics 18 software). A p-value < 0.05 was considered statistically significant.

Results and Discussion

Phytochemicals content of PSPE

The phytochemical contents of PSPE, including anthocyanidin profiles, total anthocyanin content (TACs), and total phenolic content (TPCs), are shown in Table 1. The analysis revealed that PSPE contained only peonidin and cyanidin (Table 1). Nevertheless, the presence of additional anthocyanins is likely, and further in-depth profiling is recommended for complete characterization. The TACs of PSPE were measured at 194.49 mg CGE/100 g DW (Table 1), which is comparable to the findings of Kurnianingsih *et al*. (2021), who reported 150 mg CGE/100 g DW[18]**.** The TPCs in PSPE were determined to be 541.11 mg and 1668.03 GAE/100 g FW and DW, respectively (Table 1), aligning with previous research that identified the purple-flesh sweet potato variety as having the highest TPC among ten varieties (492.89 mg GAE/100 g FW)[19]. Additionally, Im *et al*. (2021) examined the TPC of five Korean PSP varieties, reporting values ranging from 180 to 737 mg GAE/100 g DW[20]. The phytochemical contents of PSPE in this study are generally consistent with prior research, with minor variations. These differences can be attributed to factors such as PSP variety[21], sample preparation methods (e.g., steaming)[22], and extraction conditions (e.g., solvent type, pH, extraction time, and temperature)[23, 24].

Table 1. Phytochemical contents of Purple Sweet Potato extracts.

Phytochemicals	Purple Sweet Potato extract (PSPE)	
	mg/100 g FW	mg/100 g DW
Anthocyanidin contents		
Peonidin	21.72 ± 1.67	66.96 ± 5.15
Cyanidin	15.02 ± 0.67	46.30 ± 2.08
TACs[1]	63.09 ± 2.72	194.49 ± 8.39
TPCs[2]	541.11 ± 5.97	1668.03 ± 18.42

All data are expressed as Mean ± SD of triplicate experiments (n=3). TACs; total anthocyanin contents, TPCs; total phenolic contents. [1]Expressed as mg cyanidin-3-glucoside equivalents (CGE)/100 g FW or DW. [2]Expressed as mg gallic acid equivalents (GAE) /100 g FW or DW.

Antioxidant activities of PSPE

The antioxidant activities, including DPPH radical scavenging, FRAP, and ORAC assays, are shown in Table 2. The DPPH assay indicated that PSPE had an antioxidant activity of 46.22 µmol TE/g DW (11.57 mg TEAC/g DW.) (Table 2), which is comparable to previous studies on five Korean PSP varieties (11.63–12.23 mmol TE/g DW or 46.47- 48.86 µmol TE/g DW)[20]. The FRAP assay indicated a reducing power of 55.39 µmol TE/g DW (Table 2), aligning with prior findings that the raw deep PSP variety exhibits the highest FRAP values (274.0 mmol FE/g)[25]. The ORAC assay demonstrated that PSPE had a free radical scavenging effect of 116.84 µmol TE/g FW (Table 2), supporting earlier research where purple-flesh varieties consistently showed the highest ORAC values (7.38 mmol TE/100 g FW or 73.8 µmol TE/g FW)[19]. Additionally, previous studies[19, 21] confirm that PSP with higher TPCs exhibits stronger antioxidant properties, suggesting that TPC serves as a key indicator of antioxidant potential in sweet potatoes. Specifically, anthocyanins and anthocyanidins, the major phenolic compounds in PSP, exhibit antioxidant activity through multiple mechanisms: they can donate hydrogen atoms to neutralize free radicals (DPPH assay), chelate transition metal ions (FRAP assay), and scavenge peroxyl radicals (ORAC assay). The antioxidant activities of PSPE demonstrate a similar trend to those reported in earlier studies, with minor differences. These variations can be attributed to differences in sweet potato cultivars, sample preparation methods (including thermal processing), and variations in TPC, all of which significantly influence antioxidant capacity[19, 21, 25].

Table 2. Antioxidant activity of Purple Sweet Potato extracts.

Antioxidant activities	Purple Sweet Potato extract (PSPE)	
	µmol TE/g FW	µmol TE/g DW
DPPH Radical Scavenging Assay	14.99 ± 0.27	46.22 ± 0.82
FRAP Assay	17.97 ± 0.94	55.39 ± 0.94
ORAC Assay	116.84 ± 0.82	360.18 ± 2.52

All data are expressed as Mean ± SD of triplicate experiments (n=3)
µmol TE/g FW: µmol of Trolox equivalents (TE) per gram of fresh weight (FW) samples
µmol TE/g DW: µmol of Trolox equivalents (TE) per gram of dry weight (DW) samples

Anti-glycation capacity

GLO1 is the key enzyme of the glyoxalase system and is essential for the detoxification of AGEs. Elevated expression of GLO1 leads to a reduction in AGE levels[5]. Therefore, the results of the study in AGE levels were compared with those in *GLO1* gene expression.

The effects of PSPE on AGEs in the Drosophila model

The inhibition of AGE formation by PSPE was tested using an AGE ELISA kit, which measures the end products of the glycation process. In Figure 1A, the results indicate that the AGE adduct levels from all treatments show no significant differences compared to the control group or among the groups. The study suggests that the inhibition of AGEs was most effective at 2 mg/mL of PSPE, which was comparable to the inhibition achieved by cyanidin, the positive control. However, at 1 mg/mL and 4 mg/mL of PSPE, the inhibition of AGEs was less pronounced.

According to the study results, at 2 mg/mL of PSPE (containing cyanidin at 5.88 µM), the inhibition of AGEs was nearly equivalent to that observed with pure cyanidin at 5.88 µM, suggesting that this effect may be partly attributed to cyanidin. This observation aligns with prior research by Jang *et al*. (2019) and Xu *et al*. (2015), which noted that cyanidin and other anthocyanins found in PSP possess potent antioxidant properties capable of mitigating oxidative stress, a significant contributor to AGE formation[26, 27]. Furthermore, a study by Suantawee *et al*. (2016) demonstrated that cyanidin effectively combats glycative stress and inhibits the formation of AGEs induced by glucose and MG. The study elucidated that cyanidin inhibits glycation by preventing oxidative DNA damage mediated by lysine/MG, regardless of the presence of copper ions. These results indicate that cyanidin possesses the ability to directly trap MG and additionally reduce the generation of superoxide anions and hydroxyl radicals in the lysine/MG system[28]. However, PSPE at 4 mg/mL tended to increase AGE levels, possibly due to its higher dosage, which could lead to a pro-oxidant effect inducing oxidative stress. This is similar to findings from previous research by Cai *et al*. (2018), which demonstrated that high doses of purple sweet potato anthocyanins (PSPA) could induce liver injury through a pro-oxidant mechanism, as evidenced by elevated malondialdehyde (MDA) levels and reduced glutathione (GSH) levels in models of alcohol-induced liver injury[29].

The effects of PSPE on GLO1 gene expression in the Drosophila model

The expression of the *GLO1* gene in PSPE was evaluated using qRT-PCR. As shown in Figure 1B, the results indicate that the expression of the *GLO1* gene significantly increased in the PSPE 2 mg/mL group *($p < 0.05$)*, the cyanidin group ($p < 0.05$), and the carnosine (positive control) group *($p < 0.001$)* compared with the control group. No significant changes were observed for PSPE at 1 mg/mL or 4 mg/mL.

According to the study results, carnosine, cyanidin, and PSPE at 2 mg/mL upregulate the *GLO1* gene. The gene expression levels in the group treated with PSPE at 2 mg/mL (containing cyanidin at 5.88 µM) and the group treated with pure cyanidin at 5.88 µM were comparable, suggesting that the cyanidin in PSPE may contribute to the increased gene expression. Previous studies indicate that cyanidin elevates glutathione levels and GLO1 activity, thereby reducing oxidative stress and MG-induced apoptosis[8]. Additionally, cyanidin chloride activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway in colon cancer cells, which is induced by TNF-α[30]. Furthermore, Sukprasansap *et al*. (2020) demonstrated that cyanidin-3-O-glucoside (C3G) activates the Nrf2-antioxidant response element (ARE) pathway[31] and Nrf2 regulates the transcription of various detoxification and antioxidant enzyme genes, including *GLO1* and *SOD-2*[32]. Moreover, Nrf2 decreases intracellular MG stress and elevates GLO1 activity[33]. Aragonès *et al*. (2021) explain that under normal conditions, Nrf2 is linked to KEAP1, which directs its degradation via the ubiquitin-proteasome system (UPS). However, when Nrf2 is activated by compounds such as cyanidin, it translocates to the nucleus and binds to the antioxidant response (ARE) in the *GLO1* promoter, increasing *GLO1* expression[34].

However, gene expression in PSPE at 1 and 4 mg/mL was relatively similar, mirroring the levels of AGE shown in Figure 1 (A). The lack of gene upregulation at 1 mg/mL may be due to an insufficient dose. In contrast, at 4 mg/mL, the high dose of PSPE acts as a pro-oxidant, inducing oxidative stress and leading to a reduction in GSH levels[29]. Since GLO1 levels are directly proportional to GSH concentration, GLO1 activity decreases when GSH is depleted[34].

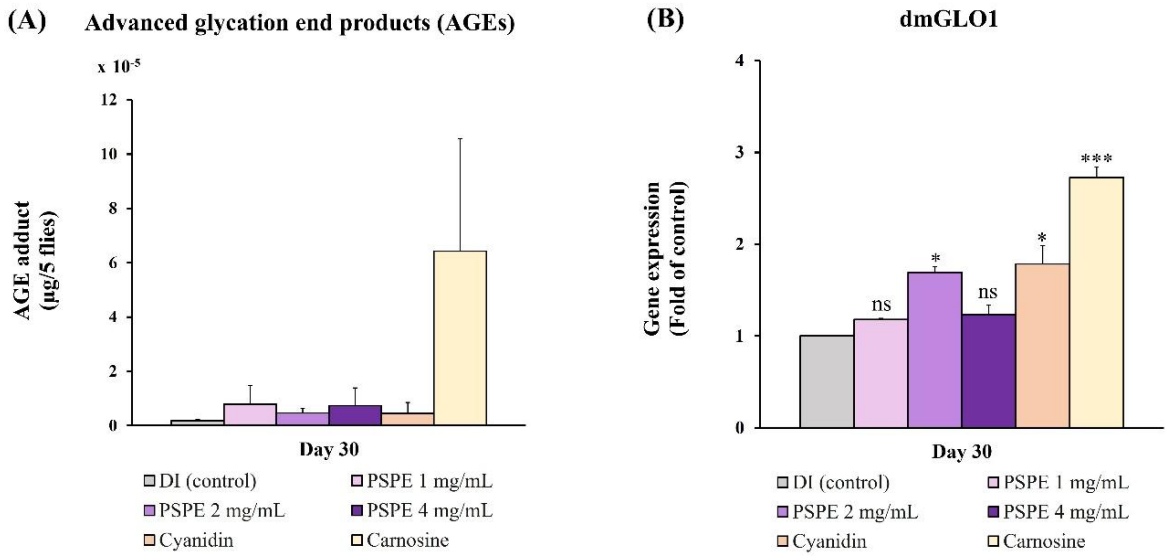


Figure 1. The anti-glycation effects of *Drosophila melanogaster* after 30-day treatment

(A) Effects of PSPE on AGEs: Data are expressed as Mean ± SD of duplicate experiments. AGEs adduct was evaluated, and statistical significance was analyzed by one-way analysis of variance (ANOVA) and Bonferroni multiple comparison test *($p < 0.05$).* **(B)** Effects of PSPE on *GLO1* gene expression: Data are expressed as Mean ± SD of duplicate experiments. The quantitative gene expression was evaluated, and statistical significance was analyzed by *t*-test against the control group. Significant values; *; $p < 0.05$, ***; $p < 0.001$ and ns; non-significant.

Conclusion

In conclusion, this study demonstrated that PSPE at a concentration of 2 mg/mL, containing cyanidin at 5.88 µM, has the potential to inhibit glycation reactions by significantly upregulating the expression of the *GLO1* gene in the *D. melanogaster* model and its effectiveness appears to be dose-dependent. However, the reduction in AGE levels was not statistically significant, possibly due to the low AGE levels detected in this study. Future research should induce higher AGE levels in *Drosophila*, such as through H_2O_2 exposure or a high-sugar diet, to better evaluate the effects of PSPE on glycation inhibition.

Acknowledgments

The authors thank the laboratory facility at the Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand, for providing the laboratory instruments.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Song Q, Liu J, Dong L, *et al*. Novel advances in inhibiting advanced glycation end product formation using natural compounds. *Biomed Pharmacother* 2021; 140: 111750.
2. Gill V, Kumar V, Singh K, *et al*. Advanced glycation end products (AGEs) may be a

striking link between modern diet and health. *Biomolecules* 2019; 9(12): 888.

3. Lapolla A, Flamini R, Dalla Vedova A, *et al.* Glyoxal and methylglyoxal levels in diabetic patients: quantitative determination by a new GC/MS method. *Clin Chem Lab Med* 2003; 41(9): 1166-73.
4. Shinohara M, Thornalley PJ, Giardino I, *et al.* Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J Clin Invest* 1998; 101(5): 1142-7.
5. Yeh WJ, Hsia SM, Lee WH, *et al.* Polyphenols with antiglycation activity and mechanisms of action: a review of recent findings. *J Food Drug Anal* 2017; 25(1): 84-92.
6. Xiao JB, Högger P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. *Curr Med Chem* 2015; 22(1): 23-38.
7. Ma H, Johnson SL, Liu W, *et al.* Evaluation of polyphenol anthocyanin-enriched extracts of blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry for free radical scavenging, reactive carbonyl species trapping, anti-glycation, anti-β-amyloid aggregation, and microglial neuroprotective effects. *Int J Mol Sci* 2018; 19(2): 461.
8. Suantawee T, Thilavech T, Cheng H, *et al.* Cyanidin attenuates methylglyoxal-induced oxidative stress and apoptosis in INS-1 pancreatic β-cells by increasing glyoxalase-1 activity. *Nutrients* 2020; 12(5): 1319.
9. Mohanraj R, Sivasankar S. Sweet potato *(Ipomoea batatas [L.] Lam)*--a valuable medicinal food: a review. *J Med Food* 2014; 17(7): 733-41.
10. Frond AD, Iuhas CI, Stirbu I, *et al.* Phytochemical characterization of five edible purple-reddish vegetables: anthocyanins, flavonoids, and phenolic acid derivatives. *Molecules* 2019; 24(8): 1536.
11. Kano M, Takayanagi T, Harada K, *et al.* Antioxidative activity of anthocyanins from purple sweet potato, *Ipomoera batatas* cultivar *Ayamurasaki*. *Biosci Biotechnol Biochem* 2005; 69(5): 979-88.
12. Wang F, Zhang S, Deng G, *et al.* Extracting total anthocyanin from purple sweet potato using an effective ultrasound-assisted compound enzymatic extraction technology. *Molecules* 2022; 27(14): 4344.
13. Zhang Z, Kou X, Fugal K, *et al.* Comparison of HPLC methods for determination of anthocyanins and anthocyanidins in bilberry extracts. *J Agric Food Chem* 2004; 52(4): 688-91.
14. Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J AOAC Int* 2005; 88(5): 1269-78.
15. Temviriyanukul P, Kittibunchakul S, Trisonthi P, *et al.* Analysis of phytonutrients, anti-mutagenic and chemopreventive effects of tropical fruit extracts. *Foods* 2021; 10(11): 2600.
16. Han Y, Guo Y, Cui SW, *et al.* Purple sweet potato extract extends lifespan by activating autophagy pathway in male *Drosophila melanogaster*. *Exp Gerontol* 2021; 144: 111190.
17. Xiao G. Methods to assay the behavior of *Drosophila melanogaster* for toxicity study. In: Pan X, Zhang B, eds. Environmental toxicology and toxicogenomics: principles,

methods, and applications. New York (NY): Springer US; 2021: 47-54.

18. Kurnianingsih N, Ratnawati R, Nazwar TA, *et al.* Purple sweet potatoes from East Java of Indonesia revealed the macronutrient, anthocyanin compound and antidepressant activity candidate. *Med Arch* 2021; 75(2): 94-100.
19. Sun Y, Pan Z, Yang C, *et al.* Comparative assessment of phenolic profiles, cellular antioxidant and antiproliferative activities in ten varieties of sweet potato (*Ipomoea batatas*) storage roots. *Molecules* 2019; 24(24): 4476.
20. Im YR, Kim I, Lee J. Phenolic composition and antioxidant activity of purple sweet potato (*Ipomoea batatas* (L.) Lam.): varietal comparisons and physical distribution. *Antioxidants (Basel)* 2021; 10(3): 462.
21. Teow CC, Truong V-D, McFeeters RF, *et al.* Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem* 2007; 103(3): 829-38.
22. Tokuşoğlu Ö, Yildirim Z. Effects of cooking methods on the anthocyanin levels and antioxidant activity of a local Turkish sweet potato (*Ipomoea batatas* (L.) Lam) cultivar *Hata*. *TJFC* 2012; 17: 87-90.
23. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010; 15(10): 7313-52.
24. Chen CC, Lin C, Chen MH, *et al*. Stability and quality of anthocyanin in purple sweet potato extracts. *Foods* 2019; 8(9): 393.
25. Tang Y, Cai W, Xu B. Profiles of phenolics, carotenoids and antioxidative capacities of thermal processed white, yellow, orange and purple sweet potatoes grown in Guilin, China. *Food Science and Human Wellness* 2015 ;4(3): 123-32.
26. Xu J, Su X, Lim S, *et al.* Characterisation and stability of anthocyanins in purple-fleshed sweet potato P40. *Food Chem* 2015; 186: 90-6.
27. Jang H-H, Kim H-W, Kim S-Y, *et al. In vitro* and *in vivo* hypoglycemic effects of cyanidin 3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside, an anthocyanin isolated from purple-fleshed sweet potato. *Food Chem* 2019; 272: 688-93.
28. Suantawee T, Cheng H, Adisakwattana S. Protective effect of cyanidin against glucose- and methylglyoxal-induced protein glycation and oxidative DNA damage. *Int J Biol Macromol* 2016; 93: 814-21.
29. Cai Z, Song L, Qian B, *et al.* Understanding the effect of anthocyanins extracted from purple sweet potatoes on alcohol-induced liver injury in mice. *Food Chemistry* 2018; 245: 463-70.
30. Lee DY, Yun SM, Song MY, *et al.* Cyanidin chloride induces apoptosis by inhibiting NF-κB signaling through activation of Nrf2 in colorectal cancer cells. *Antioxidants (Basel)* 2020; 9(4): 285.
31. Sukprasansap M, Chanvorachote P, Tencomnao T. Cyanidin-3-glucoside activates Nrf2-antioxidant response element and protects against glutamate-induced oxidative and endoplasmic reticulum stress in HT22 hippocampal neuronal cells. *BMC complement med ther* 2020; 20(1): 46.
32. Syed NA, Bhatti A, John P. Molecular link between Glo-1 expression and markers of hyperglycemia and oxidative stress in vascular complications of type 2 diabetes mellitus. *Antioxidants (Basel)* 2023; 12(9): 1663.

33. Alfarano M, Pastore D, Fogliano V, *et al.* The effect of sulforaphane on Glyoxalase I expression and activity in peripheral blood mononuclear cells. *Nutrients* 2018; 10(11): 1773.

34. Aragonès G, Rowan S, Francisco SG, *et al.* The glyoxalase system in age-related diseases: nutritional intervention as anti-ageing strategy. *Cells* 2021; 10(8): 1852.