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Research Article

Phytochemicals and Physiological Activities of Sweetpotato (*Ipomoea batatas* L.) Leaves Processed via Full Fermentation

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Abstract

Lee, Y. L., C. L. Huang, T. H. Hung, W. T. Liu, S. Y. Lin, C. H. Chen, Y. H. Ho, Y. F. Lee, and C. E. Tseng. 2025. Phytochemicals and physiological activities of sweetpotato (*Ipomoea batatas* L.) leaves processed via full fermentation. J. Taiwan Agric. Res. 74(1):11–23.

Two cultivars of leafy sweetpotato (*Ipomoea batatas* L.), 'Purple Leaf' (PL) and 'Fancy Leaf' (FL), were mixed (at a ratio of 2 : 1) and processed to produce a full-fermented leaf tea (FFLT). Two harvests of the leafy tissues were collected to study their bioactive phytochemicals and physiological activities. The first harvest was conducted in the winter (collected on 2021/11/16), and the second was in the autumn (2022/09/01). The materials of the former were divided into leaf blade and petiole portions. Extracts with room-temperature distilled water (ddH_2O) were conducted to examine the total phenolics and flavonoids contents, and the reducing power was analyzed to reveal their antioxidative activities. Results showed that the nutritional values of the leaf blade tissues were much higher than those of the petioles. The FFLT samples made from both harvests were prepared with boiling ddH_2O and showed similar reducing power. In contrast, the flavonoid content of the harvest in the autumn was 1.7 times that of the winter, but the latter was slightly higher in the phenolic content than the former. The physiological activity was studied with the FFLT prepared from the second harvest, and its raw material of the leaf tea (RMLT) was also extracted with boiling ddH_2O for comparison. Results showed that both FFLT and RMLT extracts exhibited anti-inflammatory activity by reducing nitric oxide (NO) production; however, the latter increased TNF-α production (a detrimental response), while the former did not. In inhibiting protein glycation, both extracts showed effectiveness, with the FFLT being more effective than the RMLT. These results suggest that the full-fermentation manufacturing process converts certain phytochemicals in sweetpotato leaves to enhance their bioactivity, thereby making the resulting leaf tea a potential food with improved health benefits.

Key words: Leafy sweetpotato, Total phenolics, Total flavonoids, Anti-inflammation, Anti-glycation.

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INTRODUCTION

Sweetpotato, *Ipomoea batatas* L., is a popular summer vegetable in Taiwan and also the sixth most important food crop worldwide. In the tropics, this plant is more tolerant to diseases and pests and to a certain level of moisture (waterlogging) than many other leafy vegetables. In Changhua County (the west side of middle Taiwan), sweetpotato leaves can be harvested 24−26 times annually, with an annual yield higher than other green vegetables. It is noted that sweetpotato has been selected as a primary food source by the U.S. National Aeronautics and Space Administration (NASA) because it can be grown in controlled ecological life-support system. Most varieties of sweetpotato are grown for their roots, which are rich in starch and used as staple food or feeds. Nevertheless, its leaves full of abundant phytochemicals, such as polyphenols and dietary fibers, have drawn researchers' attention. In Taiwan, there are cultivars especially bred for leaves consumption as the leaves are palatable after cooking. Compared to many other commercial vegetables, the sweetpotato tops (composed of leaves and stems) contain higher nutritional components such as vitamin A, ß-carotene, iron, calcium, and zinc. Table 1 lists the nutritional value of sweetpotato leafy tissues (including leaf blades and petioles), published by the Ministry of Health and Welfare of Taiwan, ROC.

Islam (2006, 2014) revealed that the nutritional value of sweetpotato leaves is comparable to that of spinach. The high oxalic acid (a leading cause of stone formation in the human body) may be a problem when consuming spinach, but sweetpotato leaves contain less than one-fifth of that in spinach. The leaves are a good source of antioxidative polyphenolics, composed of caffeic acid and five kinds of caffeoylquinic acid derivatives, and, in this regard, they are better than many other commercial vegetables. Islam (2014) examined 82 kinds of vegetable juices and plant components and found that sweetpotato leaves have an overwhelmingly high antimutagenicity; the biologically active compounds were proven to possess multifaceted functions. The animal experiments revealed that the anti-diabetic compounds in sweetpotato leaves reduce the blood glucose level significantly. Besides, the water extract of sweetpotato leaves (from

Table 1. The minerals and nutrients contents[z] of fresh sweetpotato leafy tissue (including petioles).

Analysis item	Unit	Per 100 g serving	*SD*[y]	No. of sample
Minerals				
Sodium (Na)	mg	39	27	5
Potassium (K)	mg	401	82	5
Calcium (Ca)	mg	105	47	5
Magnesium (Mg)	mg	35	17	5
Iron (Fe)	mg	2.5	0.9	5
Zinc (Zn)	mg	0.5	0.1	5
Phosphorus (P)	mg	44	10	5
Copper (Cu)	mg	0.003		1
Total vitamin A	I.U.[x]	5,960		
Retinol equivalent (RE)	μg	596		
α-Carotene	μg	105	148	2
β-Carotene	μg	3,523	2,818	5

[z] Online data of the Ministry of Health and Welfare, Taiwan, ROC.
[y] Standard deviation.
[x] International unit.

the variety 'Simon1') possesses antibacterial activity against many food poisoning bacteria, including *Staphylococcus aureus*, *Bacillus cereus* and lethal *Escherichia coli* O157. The main components in the antibacterial extract are polysaccharides (Islam 2014).

The phytochemicals in the sweetpotato leaves have been identified as phenolics, anthocyanins, and flavonoids (Luo *et al*. 2021; Jia *et al*. 2022) that displayed remarkable anti-oxidant and hypoglycemic activities, both related to anti-aging and anti-diabetes properties. In Taiwan, four cultivars ('TNG10', 'TNG57', 'TNG66', and 'YSP') leaves have been revealed to possess remarkable antioxidative activity, especially in the 'TNG10' and 'TNG57', which couldprotect cells from H_2O_2-induced cytotoxicity (Liao *et al*. 2011). Currently, aging and diabetic complications such as retinopathy, neuropathy, and nephropathy are correlated to the formation of advanced glycation end products (AGEs) (Sano *et al*. 2022), and the inhibition of AGEs formation could prevent these related disorders. To our knowledge, only the roots of sweetpotato have been studied to show a reduced protein glycation level in diabetic rats (Akhtar *et al*. 2018), and the leaf tissue has not yet been studied.

Owing to the high yield of this vegetable in almost all seasons in Taiwan, food processing is a plausible way to endow sweetpotato leaves with a new character on the food market. Black tea made from *Camellia sinica* is a popular drink on the beverage market for a long time. Its fermentation process creates oxidized polyphenolic compounds, such as theaflavins and thearubigins, which are responsible for the color, taste, and aroma of black tea and are beneficial to human health (Jolvis Pou 2016). In this study, we collected two cultivars of leafy sweetpotato, 'Purple Leaf' (PL, Fig. 1A) and 'Fancy Leaf' (FL, Fig. 1B) and processed their mixed-raw materials to produce tea (Fig. 1C) with a black-tea-similar fully fermentation procedure (Zeng & Hong 2020). Experiments were employed to compare the total phenolics, flavonoids, and reducing power. The bioactivities of anti-inflammation and anti-glycation were analyzed. The results showed that the fully-fermented sweetpotato leaf tea might possess these physiological activities and it is worth studying in the future.

MATERIAL AND METHODS

Plant materials

Two leafy sweetpotato cultivars, 'Purple Leaf' (PL, Fig. 1A) and 'Fancy Leaf' (FL, Fig. 1B), were grown at the bank of the Zhuoshui River in Changhua County (GPS 23.824110, 120.444800), Taiwan. Plant materials were collected twice, the first in the winter (on 2021/11/16) and the second in the fall (on 2022/09/01). The winter's raw materials were divided into leaf blade and petiole parts to give an overview on the bioactive phytochemicals. The sweetpotato leaf tea samples were prepared from these two harvests with a full fermentation procedure (Invention Patent TWI680720B, Republic of China. Zeng & Hong 2020). The mixtures of the leaves (without petiole) of PL and FL at a ratio of 2 : 1 were used. The leaf tea was prepared by the following steps: (1) washed the leaves thoroughly; (2) allowed the leaves to air-dry at 3℃ to 25℃ for 6 to 12 h; (3) withered the leaves to reduce moisture content by 5% to 18%; (4) roasted the leaves at temperatures between 100℃ and 350℃, with a batch size of 3 to 12 kg, for 2 to 15 min; (5) rolled the leaves; (6) loosened the rolled leaves; and (7) baked the leaves several times until the moisture content was reduced to below 4%, which was conducted at temperatures of 40℃ to 350℃, with each baking cycle reducing weight by 6% to 26%. In the final baking cycle, the leaves were roasted from 220℃ to 350℃ until a charred aroma emerged (Fig. 1C).

Sample preparation for analysis of phenolics, reducing power, and flavonoids

In the fresh plant materials (moisture content ~ 80%), five leaves with similar appearance (color and size) were selected. Before extraction, the leaf blades and petioles were divided, and ground in liquid nitrogen with

Fig. 1. The tested leafy sweetpotato cultivars included (A) 'Purple Leaf' (PL), (B) 'Fancy Leaf' (FL), and (C) full-fermented leaf tea sample (FFLT) processed at a ratio of 2 : 1 of the leaves PL and FL cultivars.

mortar and pestle to fine powder. An aliquot of room-temperature ddH_2O at a ratio of 20 : 1 (ddH_2O : sample weight) was added and thoroughly mixed to extract the soluble fraction. The sample of fully-fermented leaf tea (FFLT, without grinding) was added to boiling ddH_2O at a ratio of 20 : 1 (ddH_2O : sample weight) and incubated for 20 min for extraction. The extracts were centrifuged at 13,800× *g* for 20 min, and each respective supernatant was collected and filtered through a 0.22-μm PTFE filter (Jet Biofiltration Co., Guangzhou, China) before analyses.

Total phenolics

The method of Singleton & Rossi (1965)

was applied for the determination of total phenolics. A 15-μL extract was mixed well with 240 μL ddH_2O and 15-μL Folin-Ciocalteau phenol reagent (Sigma-Aldrich Co., Buchs, Switzerland) before 30-μL 35% $Na_2CO_{3(aq)}$ (Merck Co., Darmstadt, Germany) was added to the mixture. The mixture was vigorously mixed with a Vortex Mixer before being centrifuged at 16,200× *g* for 2 min. A 200-μL aliquot of the supernatant was measured for the absorbance at 750 nm. Each sample was measured with triplicates. Gallic acid (GA, Acros Organics, China) solution was prepared with ddH_2O as a standard at concentrations of 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, 0.4, and 0.5 mg mL^{-1}.

Reducing power

The method of Oyaizu (1986) was applied to determine the reducing power. A 100-μL sample was added to 100-μL of 0.2 M sodium phosphate buffer (pH 6.6, Merck Co., Darmstadt, Germany) and 100-μL of 1% potassium ferricyanide (Merck Co., Darmstadt, Germany), mixed thoroughly, and incubated at 50℃ for 20 min. The mixture was then cooled down in ice bath and added 100-μL of 10% trichloroacetic acid (Merck Co., Darmstadt, Germany) to stop the reaction. One hundred μL of the mixture was added with 100-μL ddH_2O and 20-μL of 0.1% ferric chloride (Merck Co., Darmstadt, Germany) solution. The mixture was thoroughly mixed, and the absorbance at 700 nm was measured for each sample in triplicate. Trolox standards (0.08, 0.2, 0.4, 0.6 and 0.8 mM) were prepared in methanol to establish a standard curve.

Total flavonoids

Determining total flavonoids followed the method provided by Meda *et al*. (2005). Fifty μL sample was added with 100-μL 2% aluminum trichloride (Alfa Aesar, Ward Hill, MA, USA) prepared in methanol, and the mixture was preserved at room temperature for 10 min before monitoring the absorbance at 415 nm, with each sample in triplicate. The absorbance values of each sample were then subtracted with their absorbance at 415 nm (The 2% aluminum trichloride solution was replaced with methanol solvent.). Quercetin standards (2, 4, 6, 8, 10, 20, 30, 40, and 50 mg L^{-1}) were prepared in methanol to establish a standard curve.

Sample preparation for analysis of anti-inflammation and anti-glycation assays

The sweetpotato leaves of the second harvest (collected on 2022/09/01) were used for the bioactivity assays, inhibition of inflammation and protein glycation. The FFLT sample was prepared as mentioned above. The RMLT (raw material of FFLT) was also prepared to investigate the influences exerted by the full-fermentation process. Ten fresh leaves of each PL and FL cultivars with similar appearance were selected separately. They were ground independently in liquid nitrogen with mortar and pestle to fine powder. An aliquot of boiling ddH_2O at a ratio of 20 : 1 (ddH_2O : sample weight) was added, thoroughly mixed, and incubated for 20 min to extract the soluble fraction. The mixture of PL and FL extracts with 2 : 1 ratio was mixed to serve as the RMLT sample. The extracts were centrifuged at 13,800× *g* for 20 min, and each respective supernatant was collected and filtered through a 0.22-μm PTFE filter (Jet Biofiltration Co., Guangzhou, China) before analyses. To quantify the sample amounts used in the bioactivity assay, the extract's dry weight was applied. Briefly, each 2.00-mL aliquot of the extract was dried in an oven set at 75℃, and the resulting dry weight was used to calculate the liquid weight needed to achieve a dry weight of 100−1,000 μg, individually.

Anti-inflammation assays

Murine macrophage cell line RAW264.7 was purchased from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan, R. O. C.). The cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium, Hyclone, New York, USA.) supplemented with 10% fetal bovine serum (Fetal Bovine Serum, FBS, Hyclone, New York, USA) and incubated at 37℃ in an incubator with 5% CO_2. For anti-inflamma-

tion activity assay, the RAW264.7 cells were seeded into 48-well plates at a density of 8 × 10^4 cells per well and cultured for 24 h. These cells were treated with 100 μg dry wt. of the extract or 50 μM hydrocortisone (HC, Sigma-Aldrich Co., Burlington, MA, USA) and 1 μg mL^{-1} of lipopolysaccharide (LPS, Sigma-Aldrich Co., Burlington, MA, USA). After 24 h, each well's supernatant was collected to detect the produced amounts of nitric oxide (NO) and tumor necrosis factor-α (TNF-α). The FFLT sample was tested for NO production analysis at the following dosages: 100, 500, and 1,000 μg (dry wt.). The NO production was determined by Griess Reagent (modified) (Sigma-Aldrich Co., Burlington, MA, USA). Concentrations of TNF-α in supernatants were measured with enzyme-linked immunosorbent assay (ELISA) kits (BioLegend Com., San Diego, CA, USA) according to the manufacturer's instructions.

Anti-glycation assays

The method used was modified by Sano *et al*. (2022). Bovine serum albumin (BSA) 4 mg mL^{-1} was incubated with 200 mM glucose with or without 100 μg extract (dry wt.) in 0.1 M sodium phosphate buffer (pH 7.4) for 7 d at 37℃. After incubation, the level of produced glycated BSA was measured with a glycated BSA-specific ELISA. The horseradish peroxidase (HRP) anti-carboxymethyl lysine antibody (ab27686) was purchased from ABCAM Co. (Cambridge, UK) and the enzyme-linked immunosorbent assays were conducted with the provided instructional protocol. The glycation degree (%) = At/Ac × 100, where At is the level of glycated BSA under co-incubation with the tested sample, and Ac is the level of glycated BSA with the incubation mixture without the tested sample as the control. The analysis was conducted in triplicate.

RESULTS

Total phenolics

The total phenolics of the two leafy sweetpotato cultivars PL and FL samples are shown in Fig. 2. The fresh materials, divided into leaf

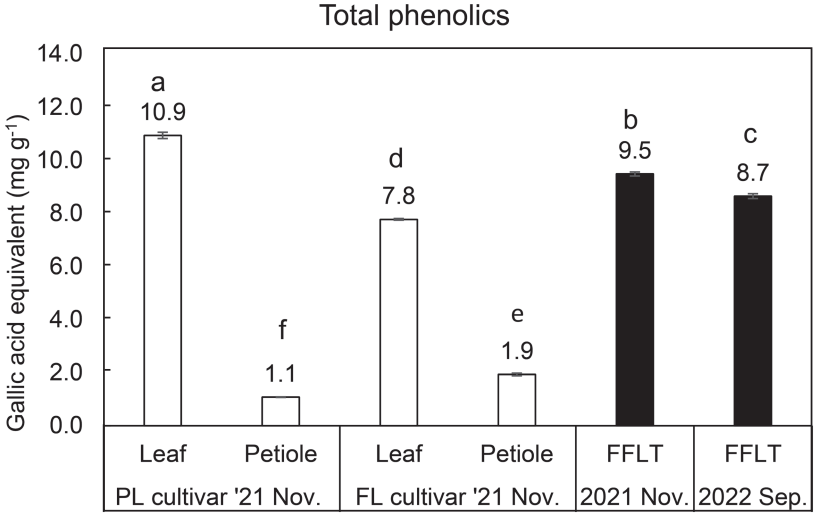

Fig. 2. Total phenolics of samples were extracted with RT (room temperature) ddH_2O (white columns) from fresh materials harvested on 2021/11/16, and with boiling water (black columns) from FFLT samples manufactured in 2021 November and 2022 September, respectively. PL, 'Purple Leaf' cultivar; FL, 'Fancy Leaf' cultivar; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

blades and petioles (collected on 2021/11/16), were extracted with RT (room temperature) ddH_2O. The leaves of PL and FL bore phenolic contents 10.9 ± 0.12 and 7.8 ± 0.03 mg gallic acid equivalent g^{-1} tissue, respectively, much higher than their petioles 1.1 ± 0.01 and 1.9 ± 0.05 mg gallic acid equivalent g^{-1} tissue, respectively. The FFLT (full-fermented leaf tea) manufactured from the two harvests, the first in 2021 November and the second in 2022 September, prepared with boiling ddH_2O, the total phenolics were 9.5 ± 0.07 and 8.7 ± 0.08 mg gallic acid equivalent g^{-1}, respectively. It is noted that the total phenolics of the tea sample in winter was slightly higher than that in autumn, with a statistically significant difference.

Reducing power

The reducing power of the extracts is shown in Fig. 3, showing a similar tendency to their phenolic contents. The leaf blades of PL 36.6 ± 0.38 and FL 23.1 ± 0.20 mM Trolox equivalent g^{-1} tissue, were much higher than their petioles 4.6 ± 0.07 and 6.2 ± 0.02 mM Trolox equivalent g^{-1} tissue, respectively. The extracts of FFLT manufactured in the winter (2021 November) 37.6 ± 1.25 and in the autumn (2022 September) 38.8 ± 1.96 mM Trolox equivalent g^{-1}; they were similar, without statistically significant difference.

Total flavonoids

The total flavonoids of the extracts are shown in Fig. 4. The blades of PL 2.30 ± 0.03 and of FL 0.93 ± 0.04 quercetin equivalent mg g^{-1} tissue were much higher than their petioles 0.11 ± 0.004 and 0.17 ± 0.008 mg quercetin equivalent g^{-1}, respectively. The extracts of FFLT manufactured in the winter (2021 November) were 1.05 ± 0.05 and in the autumn (2022 September) 1.80 ± 0.10 mg quercetin equivalent g^{-1}; the latter 1.7 times higher than the former, with a statistically significant difference.

Anti-inflammatory activity

The anti-inflammatory activity of the FFLT and the RMLT (raw material of leaf tea) extracted by boiling ddH_2O were determined with the productions of NO and TNF-α of the RAW264.7 cells (Fig. 5). The inflammatory response was tested by mixing lipopolysac-

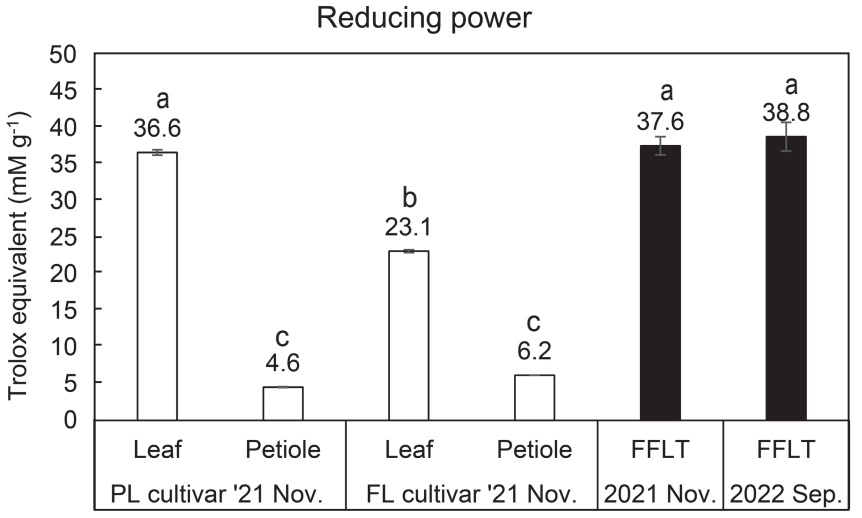

Fig. 3. Reducing powers of samples were extracted with RT (room temperature) ddH_2O (white columns) from fresh materials harvested on 2021/11/16, and with boiling water (black columns) from FFLT samples manufactured in 2021 November and 2022 September, respectively. PL, 'Purple Leaf' cultivar; FL, 'Fancy Leaf' cultivar; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

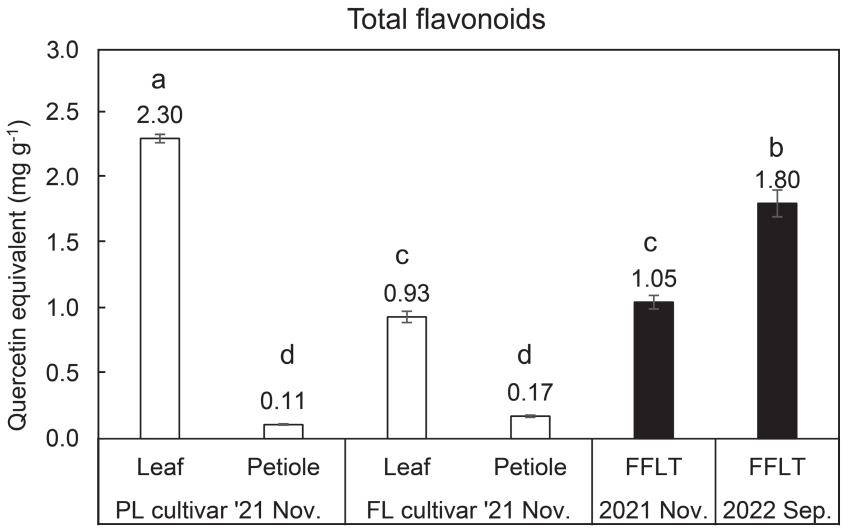

Fig. 4. Total flavonoids of samples were extracted with RT (room temperature) ddH_2O (white columns) from fresh materials harvested on 2021/11/16, and with boiling water (black columns) from FFLT samples manufactured in 2021 November and 2022 September, respectively. PL, 'Purple Leaf' cultivar; FL, 'Fancy Leaf' cultivar; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

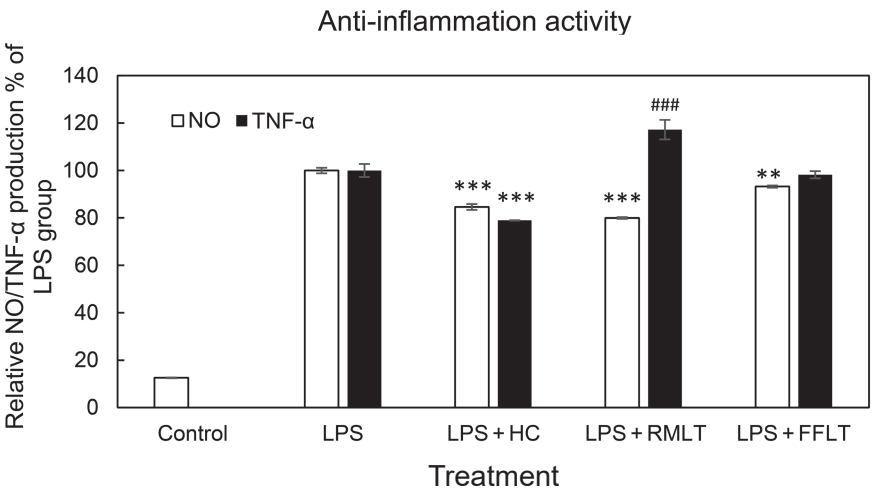

Fig. 5. Bioactivity assay of anti-inflammation with inhibition of nitric oxide (NO) and TNF-α productions. The extract of RMLT and FFLT was applied with 100 μg (dry wt.), white columns representing the NO production and black columns the TNF-α production of RAW264.7 cells, respectively. Lipopolysaccharide is abbreviated to LPS; HC, hydrocortisone; RMLT, raw material of leaf tea; FFLT, full-fermented leaf tea. The analyses were conducted with triplication and the standard deviations shown as error bars ($^{**}P < 0.01$, $^{***/\#\#\#}P < 0.001$ vs. LPS based one-way analysis of variance (ANOVA); * lower, $^{\#}$ higher).

charide (LPS), the inflammation inducer, and the extracts simultaneously, whereas the anti-inflammatory drug hydrocortisone (HC) was used as a positive control. Both FFLT and RMLT effectively reduced the NO production (93.2 ± 0.5% and 80.0% ± 0.4%, respectively,

white columns in Fig. 5) with statistically significant differences. However, they did not inhibit the TNF-α production, especially RMLT significantly enhancing the TNF-α production (122% ± 4%, black columns in Fig. 5). Nevertheless, FFLT showed no change on the TNF-α production (102% ± 2%, without detrimental effect). Different doses of the FFLT extract were further tested with NO production assay. Results (Fig. 6) indicated that the NO production amounts were reduced to 73%, 71%, and 64% in correspondence to FFLT doses of 100, 500, and 1,000 μg, respectively. Therefore, the FFLT extract exhibited a dose-dependent efficacy on the inhibition of NO production.

Anti-glycation of BSA

The anti-glycation of BSA was conducted with the FFLT and RMLT extracts and the results are shown in Fig. 7. Both extracts effectively reduced the glycation degree, and the FFLT showed a higher inhibition activity with an 80% ± 3% glycation degree of the control, better than that of the RMLT (91% ± 3% glycation of the control) with statistically significant difference ($P < 0.05$).

DISCUSSION

Phytochemicals/nutritional value of fresh sweetpotato leaves

In this study the phytochemicals in the fresh leaf and petiole tissues from 2 sweetpotato cultivars, PL and FL, were compared, and the results showed that the phenolics and flavonoids, and reducing powers, were remarkably higher in the leaves than those in the petioles (Figs. 2−4). Jia *et al*. (2022) compared four different aerial parts of leafy sweetpotato and showed the same results. The four parts were buds, leaves, petioles, and stems. Among them, the buds and

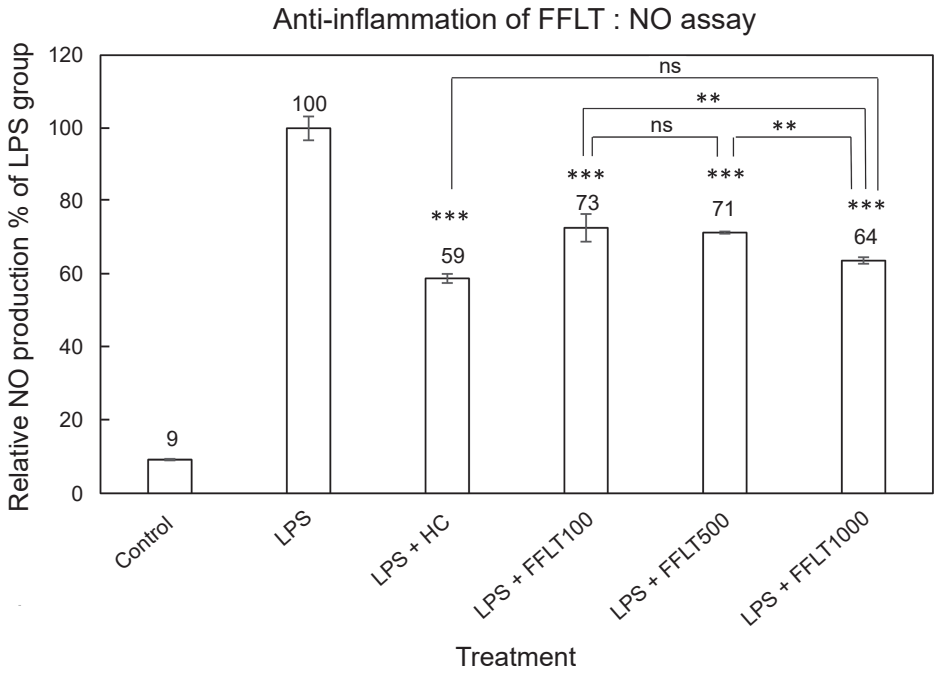

Fig. 6. Anti-inflammation activity of the FFLT sample, with boiling ddH_2O extraction, was determined by nitric oxide (NO) production of RAW264.7 cells. Lipopolysaccharide is abbreviated to LPS; HC, hydrocortisone; FFLT, full-fermented leaf tea; 100, 500, 1,000 were representations of "μg" dry wt. extracts of them, respectively. The analyses were conducted with triplication and the standard deviations shown as error bars ($^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS based on one-way analysis of variance (ANOVA); with pair comparison, ns is no significant difference).

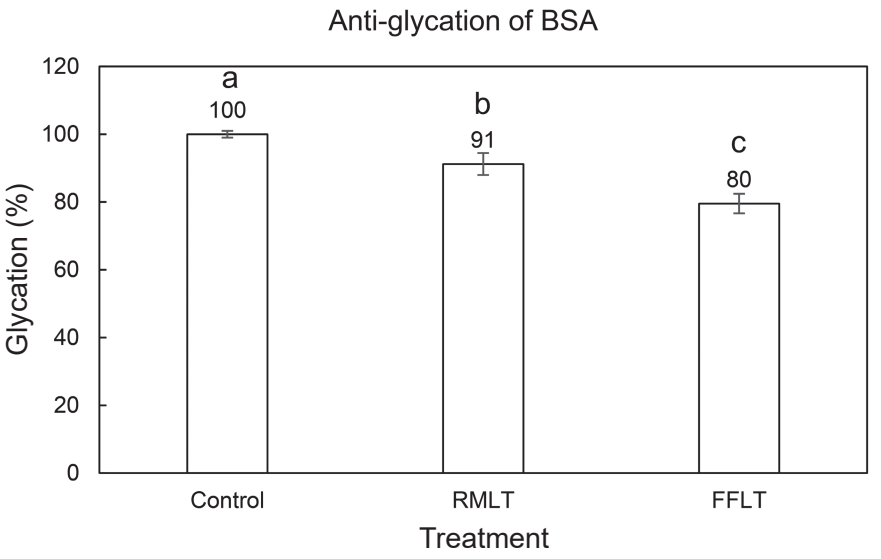

Fig. 7. Anti-glycation of BSA. The extract of RMLT and FFLT was applied with 100 μg (dry wt.). Glycation degree (%) = At/Ac × 100, where At is the level of glycated BSA under co-incubation with tested sample, and Ac the level of glycated BSA with the incubation mixture without the tested sample as the control. RMLT, raw material of leaf tea; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

leaves had higher levels of nutritional value, including total phenolics, total anthocyanins, and antioxidative activities. Luo *et al*. (2021) analyzed the phenolics and flavonoids contents of the sweetpotato leaf with ultra-high-performance liquid chromatography and identified 13 individual phenolic acids and 10 individual flavonoids; the former consisted mainly of 8 isomeric caffeoylquinic acids and the latter mainly chrysin, rutin, hyperoside, and pectolinarigenin. All these phytochemicals are strong antioxidative agents in natural plants.

The FFLT made from the two harvests, raw materials collected in 2021 November and in 2022 September, exhibited similar reducing power (Fig. 3). However, the flavonoid content of the latter (the autumn collection) was 1.7 times of those in the former (the winter collection) (Fig. 4), although the former slightly higher in the phenolic content than the latter (Fig. 2). Kobayashi *et al*. (2019) revealed the seasonal variation of the sweetpotato foliage in the yield and polyphenol content with 3 consecutive years in a greenhouse in Japan. They found that the polyphenol content was negatively related to the yield as well as the air temperature. The polyphenol content was higher in the harvests of May and November, and lower during June to August (in the summer, with a higher yield comparatively). It is consistent with our results that the total phenolics in the winter FFLT were slightly higher than those in the autumn (Fig. 2). Nevertheless, their reducing powers were alike (Fig. 3), their total flavonoids contents were opposite, and the autumn FFLT was significantly higher (Fig. 4). The seasonal variation of plant materials is related to the environment and plant physiological conditions. It is worth studying the difference and realizing suitable seasons of the sweetpotato leaves for manufacturing.

Physiological activity- anti-inflammation and anti-glycation activities of FFIT

Anti-inflammation activity was determined by the murine macrophage cell line RAW264.7.

The production of NO or TNF-α represents the cells undergoing an inflammatory response. The results showed both FFLT and RMLT extracts exhibiting anti-inflammation activity for they reduced NO production rates (white columns in Fig. 5); however, the RMLT increased the TNF-α production (black columns in Fig. 5), and the FFLT did not. Additionally, the FFLT exhibited a dose-dependent effect in the NO assay (Fig. 6). This indicates that the manufacturing process of the FFLT altered the bioactive components of sweetpotato leaves, enhancing their potency as an anti-inflammatory agent.

Wu & Yen (2005) studied different stages of protein glycation. Several flavonoids (luteolin, quercetin, and rutin) were identified to inhibit HbA1C formation ($P < 0.01$), with more effectiveness than aminoguanidine (AG, 10 mM, a well-known inhibitor of AGEs). Luteolin and rutin showed significantly high inhibitory capacity in the middle stage on methylglyoxal-medicated protein modification, with the IC50's at 66.1 and 71.8 μM, respectively. In the final stage of glycation, luteolin was found to be a potent inhibitor that could effectively suppress both the formation of AGEs and the subsequent cross-linking of proteins. Additionally, Sano *et al*. (2022) reported that lignin, the major ingredient of a corn silk water extract, was an active component that could inhibit the glycation of BSA. It is plausible that the dominant bioactive compounds in the sweetpotato leaves were chlorogenic acid derivatives, which are lignin precursors. The FFLT was more potent than the RMLT in inhibiting protein glycation (Fig. 7). In this regard, it is suggested that the active components of sweetpotato leaves were transformed through the full fermentation process to exert enhanced anti-glycation activity.

Full fermentation process of sweetpotato leaves converts a certain level of phytochemicals

Similar to the fully fermented black tea made from *C. sinica*, the fermentation of the sweetpotato leaves create a different character for the beverage with brownish color, light sweet taste, and aromatic flavor. The manufacturing procedure of the FFLT (Zeng & Hong 2020) was modified from the traditional black tea production method. Black tea was originated from unfermented green tea. Catechins account for 90% of the phenolic fraction in green tea, whereas only about 15% of catechins remain unoxidized in black tea (Skotnicka *et al*. 2011). Theaflavins and thearubigins belong to the oxytheotannins. Seven different brands of black tea (marketed in England) showed a similar combination of polyphenols dominated by thearubigins (75−82% of total phenolics) (Rechner *et al*. 2002). This indicates the fermentation procedure oxidized the catechins before being aggregated into thearubigins (Skotnicka *et al*. 2011). Similarly, the manufacturing process of the FFLT might result in the production of considerable bioactive components through oxidation and aggregation reactions of the phytochemicals.

Therefore, it is suggested that the full-fermentation manufacturing process transforms the bioactive components of sweetpotato leaves, resulting in more potent anti-inflammatory and anti-glycation activities. This transformation enhances the potential of the resulting leaf tea as a health-promoting product, making it a promising option with advanced benefits. In the future, it is worthwhile to investigate the active components in the FFLT and to study the seasonal variation of phytochemicals in sweetpotato leaves for further applications.

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全發酵甘藷葉茶之植化素與生理活性分析

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摘要

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採用 2 種葉用甘藷品種「紫葉」(‘Purple Leaf’; PL)、「彩葉」(‘Fancy Leaf’; FL) 取其葉片 (2 : 1 比例) 混合製成一種全發酵葉茶 (full-fermented leaf tea; FFLT)。兩批試驗原料分別採集於 2021 年冬天 (11 月 16 日) 與 2022 年秋天 (9 月 1 日)。第一批冬天材料將樣品區分為葉片與葉柄，以室溫之蒸餾水萃取其植化素，進行總酚與黃酮類化合物含量分析，並分析其還原力以探討其抗氧化能力，結果顯示葉片營養成分高於葉柄。比較此二批原料製成的葉茶，以沸騰熱蒸餾水萃取其植化素，結果顯示其還原力相近，然而第二批秋天採收製成的葉茶中，黃酮類化合物含量為冬天 (第一批原料) 葉茶的 1.7 倍，但是總酚含量則是後者略高。葉茶生理活性的研究，是使用第二批採收的秋天材料，並以葉茶生原料 (raw material of leaf tea; RMLT) 做為比較；同樣以沸騰熱蒸餾水萃取生原料，分析結果顯示 FFLT 與 RMLT 均具有抑制一氧化氮 (nitric oxide; NO) 產生的抗發炎效果，但是後者有增加 TNF-α 產生的發炎反應，而前者則沒有。針對抑制蛋白質醣化的效果，分析顯示兩者均有效，但 FFLT 勝於 RMLT。由這些結果推測，此全發酵製程使甘藷葉中的某些植化素轉變，從而提升其生理活性，使葉茶成為一種具潛力的健康食物。

關鍵詞：葉用甘藷、總酚、黃酮類化合物、抗發炎、抗醣化。

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