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Comparative active compounds and antioxidant activity between the sweet- and sour-type star fruit (*Averrhoa carambola* L.) *In Vitro*

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ABSTRACT

Background: Star fruit (*Averrhoa carambola L.*) is seasonal and originates from many Southeast Asia countries, including Thailand. Previous evidence claimed that it has various antioxidative compounds such as phenolics, saponins, flavonoid C-glycosides, tannin and L-ascorbic acid. In Thailand, the sweet-type of star fruit (SF) is cultivated and marketed more than the sour-type, but their different antioxidant and active compounds between both types have not been investigated.

Objectives: This study aimed to compare the active compounds and anti-oxidant activity between sweet- and sour-type SF *in vitro*.

Materials and methods: Active compounds such as total phenolic compound, total flavonoids and L-ascorbic acid in extracts were evaluated between sweet- and sour-type SF crude extracts by using Folin-Ciocalteau reagent, aluminum chloride colorimetric assay and high-performance liquid chromatography, respectively. Antioxidant activity on scavenging radicals such as the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) cation and 1,1-diphenyl-2-picrylhydrazyl (DPPH) cation and nitric oxide (NO) was analyzed. Moreover, the protective activity of glutathione (GSH) oxidation from free radicals generated by high voltage (HV)-stimulation in a mixture of plasma micro/nanobubble water; the same as that of protein oxidation in bovine serum albumin (BSA) and malondialdehyde (MDA) from 2,2'-Azobic (2-amidinopropane) dihydrochloride (AAPH), was evaluated *in vitro*.

Results: Sour-type SF extract at 1 gm showed higher total phenolics (1,625±2.3 µg equivalent gallic acid [GAE]), total flavonoid (245±3.6 µg equivalent quercetin), and ascorbic acid (Vit C) (565±4.5 µg) than sweet-type (520±3.5 µg GAE, 187±2.5 µg, and 513±2.6 µg). In addition, sour-type SF showed a lower dose of inhibitory concentration of 50% (IC50) than sweet-type on scavenging DPPH (32.32±2.3 & 58.9±2.4 mg) and NO (23.1 ± 1.1 mg & undetected). However, IC50 on $ABTS^{\bullet+}$ scavenging of sweet-type was lower than that of sour-type (348.8±2.5 & 511.9±2.6 mg). Sweet-type showed protective effects with a dose response at 0.25-1.0 mg of extract, 125-500 µg of protein carbonyl and 62.5-500 µg of lipid peroxidation. However, sour-type at high doses showed pro-oxidant activity on increased GSH oxidation, protein carbonyl and MDA formation.

Conclusion: Sour-type SF showed higher active antioxidants, such as total phenolics, total flavonoids and Vit C as well as radical scavenging of DPPH and NO, than sweet-type SF. However, high concentrations aggravated GSH, protein and lipid oxidation. Whereas, sweet-type SF showed beneficial protective effects.

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Introduction

Many fruits contain various multi-vitamins and polyphenolic compounds, and have antioxidant activity that benefits human health.[1] Star fruit (SF) or Carambola is seasonal with the scientific name of *Averrhoa carambola* Less and has been cultivated in many countries of Southeast Asia, including Thailand. Nowadays, SF has many species or varieties such as Taiwan (big size with a green edge and sweet taste), Malaysia (big size with a sweet taste, and a lot of juice), and Guangdong, China (big size and white with a sweet taste).[2] However, two distinct classes of carambola can be found in Thailand; small size with a sour taste and big size with a sweet taste. They generally have typical characteristics of a five-pointed star-like cross section and green to yellowish skin, and has a very sour-slightly-sweet flavor.[3] Previous reports showed that the chemical constituents of SF are flavonoid C-glycosides, saponins, tannin,[4,5] L-ascorbic acid, (-) epicatechin and gallic acid (GAE).[6,7] In addition, its pharmaceutical values as a traditional medicine are anti- pyretic, appetite stimulation, laxation, diuretics and digestives.[2,7] In 2016, a study of SF juice supplement sour-type folk variety in Chiang Mai province showed L-ascorbic acid (16-17 mg in 100 g of extract) and retinoic acid (0.1-0.2 µg in 100 g of extract).[8] Furthermore, supplementation of fresh ripe sour-type SF juice at 100 g for one month, could increase high density lipoprotein (HDL) and decrease low density lipoprotein (LDL) as well as reduce inflammatory status by decreasing tumor necrosis factor (TNF)-α, interleukin-23 (IL-23) and nitric oxide (NO) levels in aging people. However, other types of star fruit; e.g., bigger size and sweeter taste, are available in Malaysia and India and distributed in many Thai markets. Updated data in 2020 showed that the sweet-type had antioxidant and anti-inflammatory activity in *in vitro* study, active compounds composed of total phenolic, total flavonoids and L-ascorbic acid.[9] In addition, it could improve total antioxidant capacity (TAC) and ascorbic acid (Vit C), and reduced lipid peroxide, as well as TNF-α in the plasma of people suffering from chronic obstructive pulmonary disease (COPD), after taking one-month of a prototype supplement containing sweet-type star fruit and honey.[10] Thus, both types of SF showed antioxidant compounds and effectiveness in people.

Unfortunately, the comparative activity between both types of SF had not been confirmed. Therefore, this study aimed to confirm their active compounds, especially total phenolic compound, total flavonoids, L-ascorbic acid, and scavenging activity on radicals such as organic cation radicals, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) $(ABTS)^{\bullet+}$ and NO. Moreover, the effect of protective activity on glutathione (GSH), protein and lipid peroxide formation from oxidative stress *in vitro* model is very challenging.

Materials and methods

Star fruit preparation

Raw sweet-type SF from the Malaysian variety was cultivated at organic gardens in Pathum Tani province and purchased for this study, whereas, the sour-type SF was purchased from a local farm in Chiang Mai province. Both types were baked in sealed boxes for 2 weeks until ripe (Figure 1), and then cleaned by soaking in clean water five times before blending in a fine homogenizer. The fibers and seeds were removed by filtering with a clean filter cloth and the SF juice was kept in a clean bottle before producing it in dry powder form or crude extract by the freeze-drying technique at the MANOSE RERSEARCH CENTER, Suthep sub-district, Mung district, Chiang Mai, Thailand. The final yield of crude extracts from fresh SF juice (5.0 %/*w:w*) was collected in a dark bottle and refrigerated before future analysis.

Figure 1. *Star fruit; sweet-type (left) and sour-type (right).*

Active compound analysis

Total phenolics

The total content of phenolics in crude extracts of sweet- and sour-types of SF were evaluated by following the Singleton and Rossi's protocol,[11] in which 50 µL of extracts (6.25-25 mg/mL) was mixed with 1.8 mL of diluted Folin-Ciocalteau reagent (10% v/v) (Merck KGaA, Germany), and kept in the dark for 5 min before adding 1,200 µL of (7.5%) sodium carbonate (Merck, Darmstadt). After that, the tubes were incubated for 60 min, and the pellets removed by centrifuging at a short high speed of 10,000 rpm, and the supernatant was read at 765 nm by spectrophotometry (Drawell Scientific, Shanghai). The total phenolic content at 1 gm of crude extract was calculated by comparing with standard GAE (0.008-1.0 mg/mL) (Fluka, Switzerland).

Total flavonoid content

Total flavonoid content in crude extracts of sour- and sweet-types was determined using the aluminum chloride colorimetric assay, adapted from a previous protocol.[12] Crude extracts at 25, 50 and 100 mg/mL, or different dilutions of standard quercetin (0.078-2.5 mg/mL) (Aldrich, Germany) at 500 µL, were added in 100 µL of 10% AlCl3 (Fischer Scientific, UK) solution. Then, 100 µL of sodium acetate solution (1.0 mol/L) (Fischer Scientific, UK) was added to 2.8 mL of deionized water. After 30 min incubation in the dark at room temperature, absorbance was measured by spectrophotometry (Drawell Scientific, Shanghai) at 415 nm. Total flavonoid content of both extracts at 1 gm was expressed as the mg of quercetin (Sigma-Aldrich, Germany).

L-ascorbic acid assay

The protocol for evaluating Vit C content in SF crude extracts from the sour- and sweet-types was performed by high-liquid chromatography (HPLC).[13] Before analysis, each extract at 20 mg was dissolved in 1.0 mL of deionized water, with the pellets being removed by short high-speed centrifugation at 10,000 rpm. Supernatant was filtered through a micro-filter (0.22 µm) before being analyzed in the HPLC system. The

specific peak and concentration of L-ascorbic acid in extracts were identified with a C18 reverse phase column (Eclipse Plus C18: 5 µm, 4.6 x 250 mm; Agilent, USA) under formic acid (0.1% *v/v*) (Sigma-Aldrich, Germany) as a mobile phase (pH 2.5) at a flow rate of 0.8 mL/min. Specific retention time for Vit C peak within 3.90-4.01 min was presented by a diode array detector (DAD) (SPD-MZOA, SHIMADZU, JAPAN) at 244 nm. The concentration of L-ascorbic acid in each extract was compared to standard Vit C (Fisher Scientific, UK).

Antioxidant activity assays

DPPH scavenging assay

Scavenging activity of the sour- and sweet type SF extracts that bleached the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was evaluated as in the previous protocol.[14] $DPPH^{\bullet+}$ was generated by mixing the DPPH (CALBIOCHEM, Darmstadt, Germany) in Ethanol (Merck KGaA, Darmstadt, Germany). Different concentrations of both types of SF extracts were added at 12.5-100 mg to DPPH solution in the dark for 30 min before reading the absorbance with a spectrophotometer (Drawell Scientific, Shanghai) at 515 nm. The percentage of scavenging or inhibitory concentration of 50% (IC_{50}) of sweet- or sour-type SF extract from a global curve fitted the equation in the SigmaPlot progm for Windows (version 11.0).

$ABTS^{\bullet+}$ scavenging assay

Scavenging activity of sour- and sweet-type SF extracts that bleached the 2,2-Azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) cation was evaluated by following the previous protocol.[15] Stock $ABTS^{\bullet+}$ solution was generated by mixing ABTS (CALBIOCHEM, Darmstadt, Germany) solution (14 mmol/L) with 14 mmol/L of potassium persulfate (Merck KGaA, Darmstadt, Germany) in deionized water for 12 h in the dark before diluting in deionized water for starting absorbance of 0.70±0.02 at 734 nm by spectrophotometry (Drawell Scientific, Shanghai). Ten µL of sour- or sweet-type SF extract (100-800 µmg/mL) was added to 990 µL of working $ABTS^{\bullet+}$ solution in a plastic cuvette (size 1.5 mL), and gently alternated inversely 3 times before absorbance was read. The concentration of extracts (mg) at 50 percent of scavenging or reduced $ABTS^{\bullet+}$ between sour- and sweet-type SF was calculated by the global curve fit equation in the SigmaPlot program for Windows (version 11.0).

Nitric oxide (NO) scavenging assay

NO scavenging protocol was adapted from a previous report.[16] NO was generated by dissolving sodium nitroprusside (AnalaR NORMAPUR, VWR, Prolabo, Belgium) in deionized water (10 mmol/L), and kept in light at room temperature for 3 h before evaluation. The reaction mixture (3 mL) containing 2 mL of (10 mmol/L) sodium nitroprusside (SNP), 0.5 ml of saline phosphate buffer containing KH_2PO_4, Na_2HPO_4, NaCl and KCl (Merck, USA) (pH.7.4) and 0.5 mL of standard GAE (Fluka, Switzerland) solution or aqueous sour- or sweet-type SF extracts (6.25-100 mg/ml) was incubated at 25°C for 150 min. A 0.5 mL of the reaction mixture was taken to mix with 1.0 mL of sulfanilamide (Fluka, China) (1% in 2.5% of H_3PO_4, Merck, USA) and allowed to stand for 5 min in the dark at room temperature before a further 1 mL of napthyl ethylene diamine dihydrochloride (0.1% in water) (VWR, Prolabo, Belgium) was finally added. When the mixed solution was allowed to stand for 20 min at 25°C, absorbance at 537 nm was read by spectrophotometry (Drawell Scientific, Shanghai). The concentration of extracts (mg), at 50 percent of scavenging or reduced $ABTS^{\bullet+}$ between the sour- and sweet-type SF, was calculated by the global curve fit equation in the SigmaPlot program for Windows (version 11.0).

Protective activity of star fruit extracts

Glutathione (GSH) oxidation from high-voltage (HV) stimulation

The protective activity of GSH from free radicals was performed as in a previous study by stimulating HV in micro/nano-bubble (mnb) water mixture or using the Plasma-nano bubble technique at the High Voltage Engineering Laboratory, Department of Electrical Engineering, Faculty of Engineering, Rajamangala University of Technology Lanna, Chaing Mai, Thailand (Figure 2).[17] Previous reports demonstrated that discharged plasma in ionized water is able to dissociate water molecules and produce many reactive species such as radicals (hydroxyl radicals, OH°; superoxide radical, O°), hydrogen peroxide (H_2O_2), etc.[18] Then, GSH can be oxidized directly by those radicals in the system.[19] The laboratory-made plasma generator in this model study consisted of an HV power supply and a discharged plasma electrode. Micro/nano-air bubble water was generated in deionized water by a micro-bubble generator (AURA Tec Co., Ltd., model OM4-MDG-045) before preparing stock GSH (Sigma, St. Louis, Co, USA) at 100 mg/mL. One hundred mL of stock GSH solution was prepared in a 150-mL beaker before standing in a plastic box. The HV power supply used a high voltage transformer and direct current (DC) half wave circuit to convert input current at 1.5-2.0 amps, 100 volts and 50 Hz into an HV of up to 6 kVp and 1 Ap of discharged current. The discharged plasma electrodes had a ground electrode placed at the bottom of the beaker, and an anode electrode of tungsten (1.5 mm diameter) was dipped into the solution to produce the electrical plasma discharged in it. The protective effects between sweet- and sour-type SF extract at 0.25-1.0 mg was evaluated at 5-min incubation, designed at the same standard as Vit C (Fischer Scientific, UK) at 0.2 mg/mL, and confirmed in the system. Residual GSH concentration was determined using the 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) protocol.[20] Two hundred µL of mixed solution was taken to mix with 500 µL of DTNB (Sigma-Aldrich, Germany) and 500 µL of phosphate buffer (pH 8.0) solution. After incubating at room temperature for 5 min, a clear yellow supernatant solution was read by spectrophotometry at 412 nm (Drawell Scientific, Shanghai). The percentage of GSH was presented by comparing with non-HV stimulation.

Protein carbonyl formation in AAPH oxidized BSA

Protein oxidation was modified in bovine serum albumin (BSA) (20%) (Plasma Fractionation Center, The Thai Red Cross Society, Thailand) from 2-2' azo-bis-(2-methyl-propionamidine) HCl (AAPH) oxidation as in a previous protocol.[21] A mixture

of 200 µL of BSA (5 mg/mL), 400 µL of AAPH (200 mmol/L), and 100 µL of extract or GAE solutions (125-500 µg/mL) was incubated for 2 hours at room temperature. Protein carbonyl in the mixture was identified from a previous protocol.[22] A protein pellet in 400 µL of mixture was separated after precipitating with tricarboxylic acid (TCA) (10%), washed three times with ethanol-ethyl acetate (1:1, *v/v*) (1 mL) and centrifuged at 3,000 rpm for 3 min. The protein pellet was redissolved in 500 µL of guanidine hydrochloride (6 mol/L) and 500 µL of 2,4-Dinitrophenylhydrazine (DNPH) (10 mmol/L). After incubation for 10 min, absorbance was read by spectrophotometry at 370 nm (Drawell Scientific, Shanghai). The protein carbonyl was calculated by using a molar efficiency of $2.2x10^4$ cm^{-1} M^{-1}.

Figure 2. *High-voltage stimulation in the micro/nano-bubble water system. (Figure was modified with copyright permitted from a previous publication[9]).*

Lipid oxidation in erythrocytes from AAPH oxidation.

The last model of protective effect on lipid peroxidation of SF extracts was studied in healthy whole blood from AAPH oxidation.[23,24] Blood samples (10 mL each) were obtained by venipuncture from elderly healthy volunteers, who were aware of the study design and gave informed consent under the Ethic Human Committee at the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (AMSEC-62FB-001). Blood of 1.0 mL treated with AAPH in the presence or absence of the SF extracts at 62.5-500 µg/mL was incubated for up to 4 hours at 37°C. A negative control that ran together with an equivalent volume of isotonic buffer solution did not change the contents of thobarbituic acid-reactive substances (TBARs) significantly in red blood cells (RBCs) within 6 hours. After incubation for 4 hr and centrifugation at 3,000 rpm for 5 min, malondialdehyde (MDA) in plasma was detected with the reaction of TBARs.[25] A 250 µL of H_3PO_4 (0.4 mol/L) and 250 µL (0.6%) of thobarbituic acid (TBA) were added to 1 mL of reaction mixture before incubating at 95°C for 60 min. After stopping the reaction by cooling in an ice bath, the pink color of the supernatant obtained was read by spectrophotometry (Drawell Scientific, Shanghai) at 532 nm. Tetramethoxypropane was used as standard. The protective effect on MDA formation of extracts was confirmed by standard Vit C.

Statistical analysis

All data were represented with the mean and standard error of mean (SEM). Non-parametric Kruskal-Wallis and Mann-Whitney U tests were used for statistical analysis between standard antioxidants and different doses of extracts.

Results

The results of active compounds

The active compounds are represented in Table 1. Sour-type SF extract at 1 gm showed the higher total phenolics (1,625±2.3 µg of equivalent GAE) and total flavonoids (245±3.6 µg of equivalent quercetin) when compared to sweet-type SF extract (520±3.5 mg GAE & 187±2.5 µg). In addition, the results of Vit C content in both extracts were higher in sour-type (565±4.5 µg/g extract) than in sweet-type (513±2.6 µg/g extract), which confirmed the specific retention time of standard Vit C (Figure 3).

Table 1 Active compounds of star fruit extract (1 gm).

Active compounds	Sweet-type SF	Sour-type SF
Total phenolics (µg GAE)	520±3.5	1,625±2.3*
Total flavonoids (µg QE)	187±2.5	245±3.6*
Vit C (µg)	513±2.6	565±4.5*

Note: ** p<0.05 from Two-Independent-Samples Tests) (Mann-Whitney U test).*

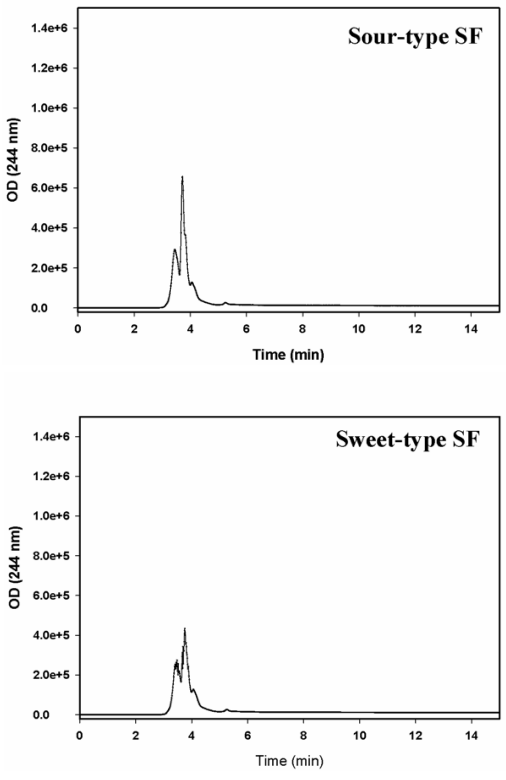

Figure 3. *HPLC peak of Vit C in both sour- and sweet-type SF extracts at 20 mg/mL and standard Vit C at 45 µg/mL.*

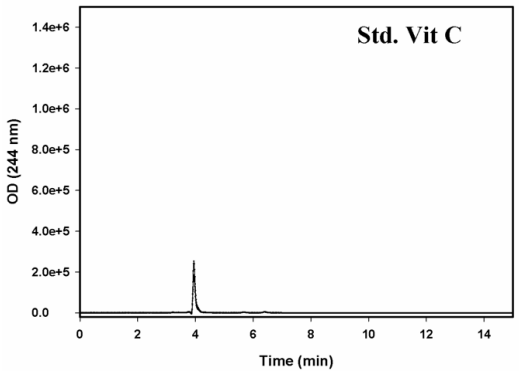

Figure 3. *HPLC peak of Vit C in both sour- and sweet-type SF extracts at 20 mg/mL and standard Vit C at 45 µg/mL.*

Radical scavenging activity

The results of three scavenging models on three radicals: DPPH cation, NO and $ABTS^{\bullet+}$ is presented in Figure 4. Sour-type SF showed higher activity with a lower concentration on scavenging DPPH (32.32±2.3 mg) and NO (23.10±1.1 mg), when compared to sweet-type SF (58.90±2.4 mg and non-detected) (Figure 4.A & C). However, sweet-type SF showed the higher activity on scavenging $ABTS^{\bullet+}$ (348.80±2.5 mg), when compared to sour-type SF (511.90±2.6 mg) (Fig. 4.B).

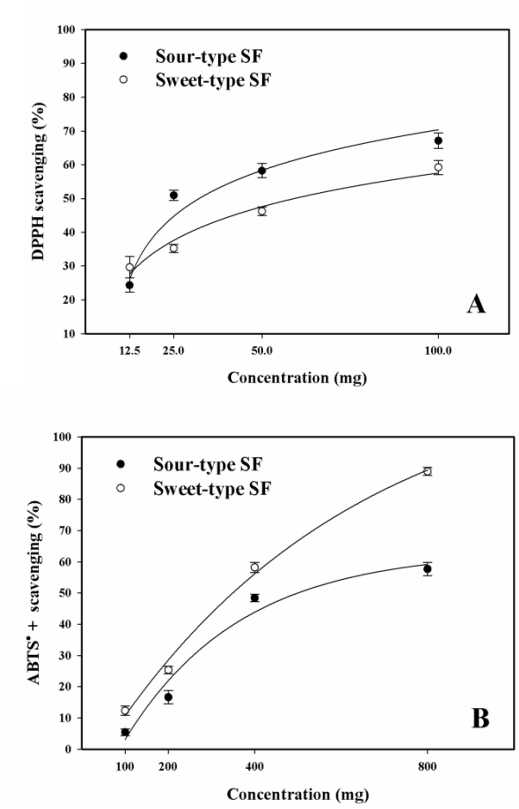

Figure 4. *Radical scavenging activity of sour- and sweet-type SF extracts. A: DPPH, B: ABTS, and C: NO.*

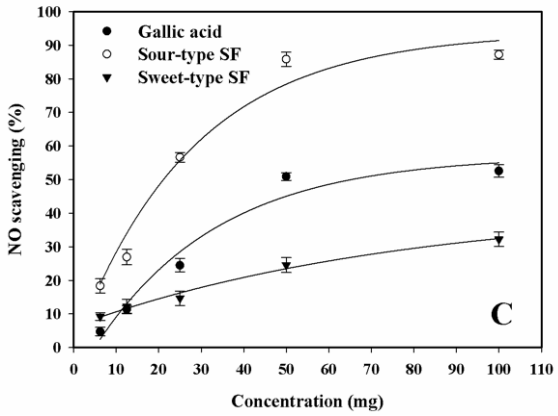

Figure 4. *Radical scavenging activity of sour- and sweet-type SF extracts. A: DPPH, B: ABTS, and C: NO.*

Protective activity of star fruit

The results of protective activity between sour- and sweet-type SF extracts were represented by three models: protective effects on GSH from high-voltage stimulation, protein carbonyl formation from AAPH-oxidized BSA, and protective activity of lipid peroxide formation in AAPH-oxidized whole blood.

In the system of oxidation, the GSH by HV was confirmed as in the previous study.[9] The GSH was oxidized and significantly reduced from 100±0.08 to 20.80±0.04 % after high-voltage stimulation for 5-15 min. Protective effect in the system was confirmed by standard Vit C (0.2 mg) with time-dependence (100±0.2, 94.5±0.16, 80.43±0.21, and 73.11±0.12%). Sweet-type SF extract showed protective effect on GSH oxidation (88.45±2.12, 84.19±2.10, and 77.29±1.5 %) with dose responses from 1.0-0.25 mg. Although, the sour-type SF extract showed protective effect on GSH oxidation from HV, high doses (1.0 and 0.5 mg/mL) showed pro-oxidative effects (79.81±1.28 and 82.92±0.38 %) when compared to lower concentrations (85.42±0.25%) (Figure 5A).

The results showed the protective effect of SF extract on BSA from AAPH oxidation. Protein carbonyl at 4.2±0.08 mmol/g protein was produced in the system after AAPH-oxidation, and significantly reduced to 2.06±0.04, 1.47±0.11, and 1.28 mmol/mg protein when GAE co-incubated with dose-response. The sweet-type SF extracts showed significant reduction of protein carbonyl to 2.41±0.08, 1.4±0.07 and 0.95±0.04 mmol/g protein, with a dose response of 125-500 µg when compared to non-treated AAPH oxidized BSA. Whereas, sour-type SF extract showed slightly inhibitory activity at 125 µg (3.89±0.21 mmol/g protein). However, it presented the pro-oxidative effect by significantly increasing the protein carbonyl content depending on the concentration being at 250 (5.06±0.10 mmol/g protein) and 500 µg (5.5±0.27 mmol/g protein) when compared to non-treated AAPH oxidized BSA (4.22±0.08 mmol/g protein) (Figure 5B).

The last protective model of SF extracts is presented in Figure 5C. The MDA formation in RBCs was increased after oxidation from AAPH (3.94±0.21 µmol/L) without any

treatment (first bar), when compared to that in those not oxidated (1.34±0.11 µmol/L) (data did not shown). The protective effect was confirmed by comparing with standard Vit C at 62.5-500 µg/mL (2.57±0.123 to 0.95 µmol/L). Sweet-type SF extract showed a protective effect on MDA formation with dose responses from 62.5 µg (2.95±0.23 mmol/L), 125 µg (2.81±0.12 mmol/L), 250 µg (2.72±0.15 mmol/L, and 200 µg (2.45 mmol/L). Data showed similarity to the sour-type SF extract at 62.5 µg (2.94±0.21 mmol/L), 125 µg (2.74±0.17 mmol/L) and 250 µg (2.75±0.15 mmol/L), but the pro-oxidative effect from high dose extract at 500 µg showed higher MDA formation (4.52±0.12 µmol/L), when compared to non-treated RBCs from AAPH oxidation and all of them treated with SF extracts (Figure 5C).

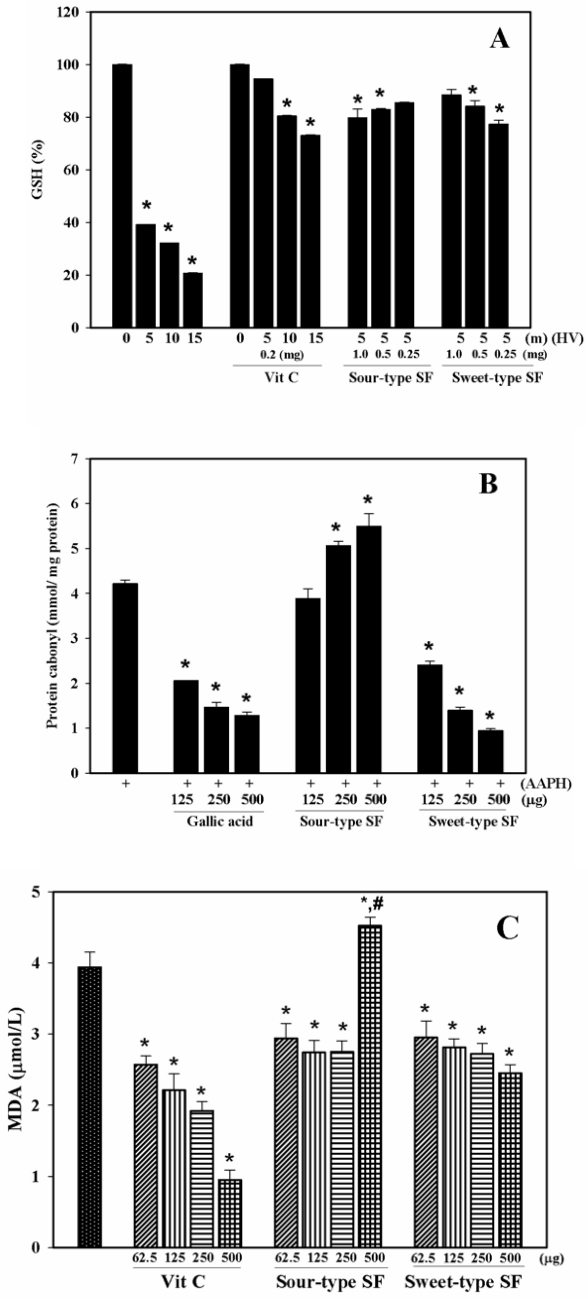

Figure 5. *Protective effects of SF extracts; sour- and sweet-types compared to standard Vit C or GAE, and control (first bar). GSH: glutathione, MDA: malondialdehyde, *,# p<0.05 from Kruskal-Wallis H test.*

Discussion

This study was an updated and a confirmed work of SF distributed in Thailand,[9,10] and it also supports a previous study on elderly people.[8,20] The results in this study represented active compounds such as phenolics and Vit C is the same as in the previous evidence from the data.[4,5,6,7] In particular, the sour-type in Chiang Mai province, Thailand, contained approximately 16-17 mg of L-ascorbic acid in 100 g of extract.[8] Whereas, the yield of L-ascorbic acid in sweet-type SF was lower at approximately 5-6 mg in 100 g of extract.[9] The results in this study also presented more L-ascorbic acid in the sour-type when compared to the sweet-type as well as total phenolics and total flavonoid contents.

Moreover, this study proved the activity of extract on scavenging radicals in different modes; DPPH, $ABTS^{\bullet+}$ and NO, which is all important in the basic knowledge of the antioxidant activity. These three models are based on the different activities of active compounds, be they hydrophilic or lipophilic compound in either type of SF extract. DPPH can be applied slowly to the antioxidant activity of various types of antioxidant compounds, and even with weak antioxidants[26] that are utilized in aqueous and non-polar organic solvents or both hydrophilic and lipophilic antioxidants.[27] Whereas, ABTS cation radicals represented TAC.[28,29] Furthermore, NO scavenging also was shown in elderly people, in which plasma NO was reduced after consumption of SF juice for 4 weeks.[20] NO scavenging of SF extract was evaluated following the previous protocol, which was generated from SNP in deionized water.[12] Thus, hydrolipic compound, such as L-ascorbic acid, was found in both types of SF extract as expected. In addition, the results on NO scavenging was confirmed with standard GAE, which is a versatile scavenger that rapidly deactivates a wide variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[30] $ABTS^{\bullet+}$ is the last model in the scavenging assay, and its scavenging[5] is prepared in deionized water. The results in this study showed that sour-type SF had a lower dose of IC50 or higher scavenging activity of DPPH and NO when compared to sweet-type. However, the IC50 on $ABTS^{\bullet+}$ scavenging of sweet-type had lower and higher activity than in sour-type. Therefore, a higher content of L-ascorbic acid, total phenolics and total flavonoids in sweet-type may not reflect the results because a previous report claimed that total phenolic and flavonoid compounds directly affect antioxidant capacity.[31] Unfortunately, other non-phenol compounds in sweet-type SF have been preferred such as diglucosides, carambolasides and phenylpropanoids; (+)-isolariciresinol 9-*O*-β-D-glucoside, (+)-lyoniresinol 9-*O*-β-D-glucoside, (–)-lyoniresinol 9-*O*-β-D-glucoside and 1-*O*-feruloyl-β-D-glucose, three benzoic acids, protocatechuic acid, and 1-*O*-vanilloyl-β-D-glucose.[32] Therefore, some analytical results should be confirmed in the future. However, the results confirmed that both sweet- and sour-type of SF showed antioxidant compounds, which affect scavenging free radicals and are important in the physiological function of humans.

Moreover, results of the protective effect of SF extract on main antioxidant GSH were confirmed in an *in vitro* model. GSH with HV stimulation was studied previously in

the oxidation model.[9] Surprising results of sour-type SF in that study compared previous evidence of sweet-type having higher concentration and reduced protective activity in the protection of GSH. GSH was oxidized in the system with timely response from 5 to 15 min of stimulation, similar to a previous study.[9] When using standard L-ascorbic acid, the protective effect was presented in comparison to the non-treated system. The results showed that sour-type extract acted with pro-oxidant activity. Previous potentially relevant articles showed that Vit C was used to produce pro-oxidant by free radical formation; H_2O_2 generation.[33] Free radical formation in the micro/nano-bubble water system was recognized as the plasma-nano bubble technique,[17] in which gas bubbles were produced into any liquid.[34] After the electrical current is released in micro/nano water bubbles in a short time, many reactive species such as radicals, hydroxyl radicals, OH°; superoxide radical (O_2-), H_2O_2, etc. are generated,[35,36] which could be rechecked by optical emission spectroscopy (OES).[17] Therefore, those free radicals could be oxidized by GSH in this study.[19] The results of provoked activity on GSH oxidation in sour-type SF may be the high content of Vit C when compared to the sweet-type.

The results of SF sweet-type extract also showed the protective effect on protein and lipid peroxidation with the dose response. In contrast, the sour-type extract showed pro-oxidative effect on protein and lipid oxidation. It is possibly the higher concentration of Vit C that is referred to in previous evidence.[37] Similarly, previous evidence showed that storage of erythrocyte with Vit C increased protein sulhydryls (P-SH) levels and decreased superoxide dismutase (SOD), referring to the modulator of oxidative stress condition.[38] Moreover, a higher flavonoid in sour-type SF possibly may involve the pro-oxidant behavior in this study. Previous evidence reported that flavonoids contain multiple hydroxyl substitutions and important peroxyl radical activity.[39] Thus, the results in this study showed the pro-oxidation activity of SF sour-type and antioxidant activity of sweet-type in *in vitro* models. Thus, the results in this study supported previous studies in which participants who had chronic obstructive pulmonary disease (COPD)[10] stabilized with antioxidant activity in sweet-type SF. However, the benefits of sour-type SF must be considered and need to be studied further.

Conclusion

Sour-type SF showed higher active antioxidant compounds such as total phenolics, total flavonoids and Vit C as well as radical scavenging activity of DPPH and NO than sweet-type. However, its high concentration aggravated GSH, protein and lipid oxidation. On the other hand, sweet-type SF showed higher activity on scavenging ABTs radicals and beneficial protective effects in in vitro models.

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Conflicting interests

The authors report no conflicts of interests in this study.

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