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LC-MS-MS Analysis and the Antioxidant Activity of Flavonoids from Eggplant Skins Grown in Organic and Conventional Environments

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Abstract

Eggplant fruits are known to contain different classes of phenolic phytochemicals (flavonols, phenolic acids, and anthocyanins) that can exert beneficial effects on human health. This study developed methods for the qualitative and quantitative composition analysis of phenolic compounds in the skin of eggplant fruits harvested following conventional and certified organic farming conditions. Eggplant skin was extracted using aqueous methanol prior to phenolic profiling with UHPLC-ESI-MS-MS. Eggplant skin extracts yielded a profile of 16 phenolic acids, 4 anthocyanins, and 11 flavonols, the first report of quercetin-3-diglucoside, myricetin-3-neohesperidoside, myricetin-3-galactoside, kaempferol-3,7-diglucoside, kaempferol-diglucoside and quercetin-3-rhamnoside. Polyphenolic extracts from all sources potently delayed the cupric ion-mediated lag-time for LDL lipid oxidation and protected Apo-B100 proteins against oxidative modification. Organic growing environment positively influences eggplant skin extract phenolic profile but not antioxidant capacity. In conclusion, eggplant skin has a robust profile of phenolic phytochemicals with excellent antioxidant properties.

Keywords

Eggplant Skin, Polyphenols, LDL Antioxidant Activity, Conventional and Organic, HPLC, LC-MS

1. Introduction

Eggplant (*Solanum melongena*) is a member of the nightshade family whose

consumption in the American diet has continuously increased due to ever greater cultural diversity and awareness that consumption of fruits and vegetables provides significant health benefits [1] [2] [3]. Over 80% of the world's eggplants are grown in China, India, Bangladesh, Nepal, and Sri Lanka. Eggplant is grown over 1.7 million hectares world-wide [4]. In the past few decades, there has been significant interest in the potential health beneficial properties of dietary polyphenols. Epidemiological studies have revealed that regular consumption of foods rich in phenolic phytochemicals (fruits, vegetables, whole grain cereal, red wine, green tea, and dietary supplements) is associated with reduced risk of certain types of cancers, cardiovascular, and other neurodegenerative diseases [5] [6] [7]. Eggplant is ranked amongst the top ten vegetables in terms of oxygen radical absorbance capacity due to the fruits phenolic constituents [8], hence eggplant has excellent potential to improve human nutritional health.

The color, size, and shape of eggplant fruits vary with the cultivar type, and differences in phenolic profile have also been observed. Whitaker and Stommel reported phenolic contents in a diverse collection of 115 eggplants representative of the plants in the USDA eggplant collection [3] [9]. Fourteen different phenolic components were identified and grouped into five different classes based upon their chemical properties [3]. Significant differences in total phenolic content and the types of phenolics present were discovered between the eggplants evaluated [3]. Significant different phenolic compound profiles in three different eggplant cultivars have been recently reported, in which the purple-striped eggplant showed the highest diversity of phenolic compounds while the long eggplant exhibited the highest quantity of phenolic compounds [10]. In our previous communications on eggplant, we carried out direct comparison of sonication and pressurized liquid extraction (PLE) procedures on extraction of phenolic acids from eggplant pulp. In addition, we evaluated the influence of different solvents, temperature, hydrolysis conditions, and sample pretreatment on extraction of phenolic acids from eggplant. An optimized method was developed for extraction of phenolic acids from eggplant [11].

Quantity and composition of phytochemicals, e.g. phenolics of fruits and vegetables can be affected by multiple factors such as plant variety and growth conditions (light, soil, temperature, irrigation etc.) [12] [13] [14] [15]. For eggplant, significantly different phenolic compound profiles have been reported in different cultivars and also in different harvest seasons, highlighting the influence of abiotic conditions, predominantly climatic factors, on the production and accumulation of phenolic compounds in eggplant [10]. In another study, reduced levels of phenolic compounds in domesticated eggplant species compared to wild species have been observed, suggesting the effect of artificial selection on the phenolic profile of eggplant [16]. Organic farming as an agricultural method emphasizes the exclusion of plant growth regulators, synthetic pesticides and fertilizers, as well as genetically modified organisms (GMOs). The fast growing organic food market has led to continued interest in understanding the effect of organic farming on food qualities, especially quantities of food nutrients and

health beneficial phytochemicals. Recently, we determined the polyphenols content and antioxidant activity of eggplant pulp samples grown under organic and conventional environmental conditions and found significant different levels of polyphenols in same variety under organic or conventional growth conditions [17] [18].

Different fruit parts, e.g. skin, pulp and seed can have different phytochemical profiles. Eggplant skin serves to protect the fruit from its external environment (*i.e.* insect predation, UV light, potential desiccation, etc.). In contrast, the tissues of eggplant pulp function provide nutrient storage and a chemically stable storage for the development and storage of eggplant seeds. Higher phenolic levels have been observed in the skins of grape, tomato and apple compared to their pulps [19] [20] [21]. The present study is a report on eggplant skin polyphenol composition in two popular hybrid varieties, Blackbell and Millionaire, which were harvested following growth under certified organic and conventional growing environments and their ability to inhibit *ex vivo* LDL oxidation.

2. Materials and Methods

2.1. Eggplant Samples

Blackbell and Millionaire are two popular eggplant hybrids with a dark purple skin and whitish-fleshy pulp that are commonly cultivated and consumed in the United States. They were grown in Hanford sandy loam soil under both under conventional (Alvarez Farm) in Reedley, California and organic (T&D Willey Farm) in Madera, California as described earlier [17]. Eggplant fruits were harvested, placed immediately in ice chest, and sent overnight in refrigeration to the Food Composition Method Development Laboratory in Beltsville, MD. All samples were peeled to isolate the skins (purple outer peel) from the whitish fleshy pulp of the fruits. Samples were stored in a freezer below −60˚C and freeze-dried. The freeze-dried samples were ground in a coffee grinder and the ground samples were stored below −60˚C until extracted and analyzed.

2.2. Chemicals

Phenolic standards 5-caffeoylquinic acid, quercetin-3-glucoside, quercetin-3-rutinoside and quercetin-3-rhamnoside were purchased from Indofine Chemical Company (Hillsborough, NJ, USA). HPLC grade methanol, acetonitrile, formic acid, and water were obtained from Fisher Scientific (Fair Lawn, NJ, USA). The myricetin-3-galactoside standard was isolated and characterized using LC-MS and NMR spectroscopy. Cyanidin-3-glucoside was purchased from Sigma Aldrich (St. Louis, MO, USA). Polyvinylidene difluoride (PVDF) syringe filters with pore size of 0.45 μm purchased from National Scientific Company (Duluth, GA, USA).

2.3. Extraction of Phenolic Acids and Flavonols

Ground freeze dried skins (500 mg) were weighed and placed into a 15 mL plas-

tic tube and 10 mL of 80% aqueous methanol was added. The mixture was vortexed for 2 min and the samples were then left overnight on a shaker. Samples were than centrifuged at 5000 rpm for 15 min and the supernatants were filtered using Whatman filter paper (0.45 micron). The residues were then re-extracted two more times with 10.0 mL 80% aqueous methanol and filtered. All three extracts were combined and concentrated using rotary evaporator at reduced temperature and pressure. After concentration extract was re-dissolved in 1 mL of mobile phase (10% aqueous methanol) and filtered using a PVDF syringe filter. The filtered extract was analyzed by HPLC and LC-MS-MS.

2.4. Extraction of Anthocyanins

Freeze dried skins (100 mg) were extracted using 10 ml mixture of methanol:water:acetic acid (85:15:0.5). Extracts for analysis were prepared in the same way as described for phenolic acids and flavonols. After filtration the extracts were directly injected into HPLC and LC-MS-MS for quantification and identification.

2.5. Analysis of Phenolic Acids and Flavonols

Compounds were separated and identified by a Dionex® HPLC system (PDA-100 detector, AS 50 autosampler and GP50 gradient pump coupled with a PE Sciex API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a turbo ion spray (ESI) source. In brief, separation was performed on a Pursuit XRS C_{18} column (3 μm particle size; 150 mm length × 3.0 mm ID, Varian, Inc., Lake Forest, CA, USA) with a binary solvent system as solvent A consisted of 10% MeOH in H_2O adjusted to pH 3.5 with formic acid and solvent B consisted of 20% H_2O (pH 3.5 with formic acid), 20% MeOH, and 60% acetonitrile. At a flow rate of 0.3 mL·min^{-1}, the following gradient was used: 0 min, 100% A; 10 min 20% A; 20 min, 40% A; 40 min, 0% A; held at 0% A for 15 min. Column equilibration was carried out by flowing 100% Solvent A for 5 min before and after each injection. Effluent from the column was introduced into the triple-quadrupole mass spectrometer under ESI ion source. All experiments were performed in the positive and negative ion mode. The ion spray needle was held at 4200 V in negative ion mode and 5000 V for positive ion mode while the inlet voltage (orifice) was varied to minimize collisional decomposition of molecular ions before entry into the first quadrupole. A molecular ion of phenolic acids and flavonols were identified by simple MS-MS analysis of available standard solutions and extracts. Product ion spectra of these species were acquired by using Q1 to pass the molecular ion of interest. Nitrogen was used to collisionally activate precursor ion decomposition in the second quadrupole, which was offset from the first quadrupole. Subsequently, formed product ions were then detected by scanning the third quadrupole. Nitrogen was used as the drying and nebulizing gas at flow rates of approximately 6000 L/h. For full-scan HPLC-ESI-MS analysis, spectra were scanned in the range of 50 to

1200 m/z. Data acquisition and processing were performed using Analyst software 1.4.2 (Applied Biosystems).

Identifications of 5-caffeoylquinic acid, quercetin-3-glucoside, quercetin-3-galactoside and quercetin-3-rhamnoside were made by comparing retention times, UV spectral patterns, and ESI-MS-MS fragmentation patterns with authentic standards as described in our earlier communication (Singh *et al*., 2009). N-caffeoyl putrescine, 3-caffeoylquinic acid, dihydroxy cinnamoyl amide, N,N-dicaffeoyl spermidine, caffeic acid conjugate, 4-caffeoylquinic acid, 5-cis-caffeoylquinic acid, 3-acetyl-5-caffeoylquinic acid, 3-acetyl-4-caffeoylquinic acid, 3-5-dicaffeoylquinic acid, 4-5-dicaffeoylquinic acid amide were identified by interpreting UV spectra and ESI-MS fragmentation patterns and comparing with published data [3] [17] [22]. Identified phenolics were quantified using an HPLC (Waters, Milford, MA, USA) equipped with a Waters 996 Photodiode Array Detector (PDA). Quantification of all the phenolic acids was based on a standard curve prepared with 5-caffeoylquinic acid.

2.6. Analysis of Anthocyanins

Same aforementioned LC-MS instrument and column were used for anthocyanin analysis. Eggplant skin extract (5 μL) and anthocyanins standards were eluted with a gradient of 5% formic acid in water (solvent A) and 100% methanol (solvent B) at a flow rate of 0.3 mL/min. Following gradient was used: 0 min, 95% A; 2 min, 80% A; 10 min, 80% A; 15 min, 70% A; held at 35 min, 70% A, 35 min, 60% A; held at 50 min, 60% A. Five minutes of equilibration at 95% A was performed before and after each injection. Conditions for mass spectral analysis in the positive ion mode included a capillary voltage of 4200 V, a nebulizing gas of 7.0 psi and a temperature of 350˚C.

2.7. Calibration Curves and Recovery Studies

All standard samples were prepared by adding known amounts of 5-caffeoylquinic acid, M-3-galactoside, Q-3-galactoside, Q-3-rhamnoside and Q-3-rutinoside of six concentrates in 1 ml of methanol. For recovery study, eggplant skin extracts were spiked with known amounts (200 and 500 ng/mL) of 5-caffeoylquinic acid, Q-3-galactoside, Q-3-rhamnoside and M-3-galactoside in triplicate and then extracted and quantified as described above.

2.8. Evaluation of Antioxidant Capacity

The extracts were first solubilized in DMSO, then deionized water and finally in phosphate buffered saline at 5.0, 2.5, 0.5 and 0.25 μg/mL. Venous blood was drawn from each donor and centrifuged (10 minutes × 2000 g). The plasma was then extracted and LDL collected by ultracentrifugation. LDL was dialyzed in PBS three times before use in the antioxidant Lag-time, TBARS and electrophoresis assays (100 μg LDL protein/mL) were determined as described previously and briefly described below [17]. Eggplant antioxidant capacity was determined

by continuously monitoring the kinetics of conjugated diene formation, oxidation was promoted in 100 μg LDL-protein/mL with 3.00 μM Cu^{++} at 37˚C. Changes in A_{234} were monitored and the maximum velocity (Vmax) function of the CD4 software package was used to arbitrarily determine the lag-time. Samples showing no oxidation (no change in absorbance) or not reaching Vmax by 240 min were arbitrarily assigned a 240-min lag-time. Eggplant antioxidant capacity was also with a Beckman-Pergamon agarose lipogel system (Brea, CA), LDL (100 μg protein/mL) in PBS were incubated at 37˚C with 10 μM cupric sulfate in the presence or absence of eggplant skin extracts (2.5 μg/ml) for 95 minutes prior to measurement of electrophoretic mobility (REM).

2.9. Data Analysis

Data are expressed as mean ± standard deviation. ANOVA was used to assess statistical contrast of eggplant cultivar and growing condition on eggplant polyphenol content, multiple comparisons of the individual polyphenolic compounds were carried out using Least Squares Means and a Tukey's adjustment for multiple comparisons. The effect of eggplant extracts on antioxidant activity was analyzed using ANOVA, Least Squares Means and a Dunnett adjustment for multiple comparisons (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

The analyzed eggplant skin extracts contained several different classes of compound, including phenolic acids, anthocyanins and flavonols. Among them, phenolic acids and few flavonols were also identified from eggplant pulp in our prior study [17]. Compared with pulp, eggplant skin is a complex matrix with a more complicated phenolic composition. Eggplant skin extracts were first analyzed using HPLC and LC-MS with different scan modes (product ion, precursor ion and neutral loss scans). Separation of phenolics in skin extracts was achieved with a Pursuit XRS C18 column with binary solvent systems and gradient elution. All of phenolic acids, anthocyanins and some of flavonols were identified based on comparison of retention time and mass spectral data with authentic standards and/or previously published results, and confirmation of the identity of some flavonols required fragmentation pattern interpretation.

3.1. Eggplant Skin Phenolic Acids

Sixteen different phenolic acids were identified in eggplant skin samples, similar with what we have previously reported in eggplant pulp [17]. **Table 1** shows the retention time, UV spectra and mass fragmentation pattern of the identified phenolic acids in eggplant skins. Among them, 5-caffeoylquinic acid (peak-6), 3-5-dicaffeoylquinic acid (peak-13), N-caffeoyl putrescine (peak-2) and 3-acetyl-5-caffeoylquinic acid (peak-12) are the most abundant phenolic acids in analyzed eggplant skins. The concentrations of the four most prevalent phenolic acids were quantified by HPLC-PDA (**Table 2**).

Table 1. Retention times (RT), ultraviolet spectral (λ_{max}), and mass spectral data (molecular ion and the major fragment ions) of the phenolic acids, anthocyanins, and flavonols extracted from the eggplant skin.

Phenolic Acids				
Peak no	RT (min)	λ_{max} (nm)	$[M-H]^-$ and Fragmentation in Electrospray-MS	Structure
1	2.039	234.8, 295.1, 323.7	282, 249, 232, 161, 87	N-caffeoylputrescine derivatives[b]
2	3.002	218.7, 234.8, 294.2, 317.8	249, 232, 161, 87	N-caffeoylputrescine[a,b]
3	13.134	218.4, 236.0, 293.3, 316.6	353, 191, 178.9, 127, 111	3-caffeoylquinic acid[a,b,c]
4	13.923	205.3, 221.2, 287.6, 320.1	470, 375, 355, 334, 191, 179, 135	Dihydroxycinnamoyl amide[a,b]
4a	15.737	205.4, 231.3, 291.6, 327.33	470, 375, 353, 195, 191, 179, 135	Dihydroxycinnamoyl amide[a,b]
5	16.217	217.2, 292.6, 319.4	468, 307, 290, 233, 161	N,N'-dicaffeoylspermidine[a,b]
6	17.545	217.2, 241.9, 297.68, 326.1	353, 191, 178.9, 127, 111	5-caffeoylquinic acid[a,b,c,d,e]
7	19.561	218.4, 243.1, 299.1, 328.5	353, 191, 178.9, 127, 111	Caffeic acid conjugate[a,b]
7a	19.970	218.4, 243.1, 299.1, 328.5	353, 191, 178.9, 127, 111	Caffeic acid conjugate[a,b]
8	20.510	217.8, 242.9, 297.1, 327.1	353, 191, 173.1, 127, 111	4-caffeoylquinic acid[a,b,c]
9	20.905	214.8, 241.9, 318.4	353, 191, 178.9, 127, 111	5-cis-caffeoylquinic acid[a,b]
10	21.485	214.8, 242.6, 298.6, 328.0	395, 353, 191	3-acetyl-5-caffeoylquinic acid[a,b]
11	25.014	218.4, 243.1, 299.1, 328.5	353, 191, 178.9, 127, 111	Caffeic acid conjugate[a,b]
12	26.663	214.8, 242.6, 298.6, 328.0	395, 353, 191	3-acetyl-4-caffeoylquinic acid[a,b]
13	35.380	212.2, 298.6, 326.7	515, 353, 191	3-5-dicaffeoylquinic acid[a,b]
14	36.555	214.8, 242.6, 298.6, 328.0	515, 353, 191	4 -5-dicaffeoylquinic acid[a,b]
Anthocynins				
Peak no	RT (min)	λ_{max} (nm)	$[M]^+$ and Fragmentation in Electrospray-MS	Structure
1	7.21	283/526	772.8, 611.4, 464, 8, 303.3	delphinidin3-rutinoside-5-galactoside[a,b]
2	9.04	283/526	773.5, 611.4, 465.1, 303.3	delphinidin3-rutinoside-5-glucoside[a,b]
3	11.04	283/526	465.2, 303.1	delphinidin-3-glucoside[a,b]
4	12.50	280/526	611.0, 465.0, 303.3	delphinidin-3-rutinoside[a,b]
Flavonols				
Peak no	RT (min)	λ_{max} (nm)	$[M-H]^-$ and fragmentation pattern in ESI-MS/MS	Structure
1	14.14	204, 254, 357	625, 463, 300, 271, 255.1, 243.1, 229.2, 179.2, 151.2	Quercetin-3-diglucoside[a,b]
2	14.93	204, 254, 357	625, 479, 316.1, 270.8, 179.4, 151.1	Myricetin-3-neohesperidoside[a,f]
3	15.31	204, 254, 357	479, 316.1, 270.8, 179.4, 151.1	Myricetin-3-galactosid[a,b,c]
4	16.007	203, 264, 346	609, 284.2, 254.2, 227, 150.8	Kaempferol-3,7-diglucosid[a,e]
5	16.59	203, 264, 346	609, 284.2, 254.2, 227, 150.8	Kaempferol-diglucoside[a,e]
6	18.347	203, 256, 354	609, 300.1, 271.0, 179.1, 134.9	Quercetin-3-rutinoside[a,c]
7	18.59	204, 256, 356	463, 300.2, 271.2, 255.2, 151.1	Quercetin-3-galactoside[a,b,c]
8	19.26	204, 256, 356	463, 300.2, 271.2, 255.2, 151.1	Quercetin-3-glucoside[a,b,c]
9	22.01	203, 264, 346	447, 284.2, 255, 227.1	Kaempferol-3-galactoside[a,d]
10	23.78	203, 264, 346	447, 284.2, 255, 227.1	Kaempferol-3-glucoside[a,d]
11	25.76	203, 264, 347	447, 301, 271.2, 255.2, 151.1	Quercetin-3-rhamnoside[a,b,c]

Phenolic acids: [a]In conjunction with Whitaker and Stommel (2003) [3], [b]Based on LC-MS mass fragmentation, pattern, [c]In conjunction with Ranger *et al.* (2007) [22], [d]In conjunction with Singh *et al.* (2009) [17], [e]Based on comparisons with authentic standards. Anthocyanins: [a]Based on LC-MS mass fragmentation pattern, [b]In conjunction with Wu and Prior (2005) [3]. Flavonols: [a]Based on full scan, neutral loss scan, product ion scan and precursor ion scan, [b]In conjunction with Singh *et al.* (2009) [34], [c]Based on standard, [d]In conjunction with Sanchez-Rabaneda *et al.* (2004), [e]In conjunction with Romani *et al.* (2006) [33], [f]In conjunction with Kazuma *et al.* (2003) [32].

Table 2. Concentrations (μg/g) of the major phenolic compounds extracted from eggplant skin. Each sample was extracted and assayed by HPLC three times (mean ± stdev). Significance ($p < 0.001$) within cultivar between organic and conventional growing condition indicated by (*).

Sample	N-caffeoylputrescine	5-caffeoylquinic acid	3-acetyl-5-caffeoylquinic acid	3-5-dicaffeoylquinic acid	Total
Blackbell Conventional	854 ± 34	4001 ± 4	20 ± 1	1291 ± 5	6168 ± 34
Blackbell Organic	2350 ± 9*	8539 ± 14*	133 ± 5*	1282 ± 3	12,305 ± 15*
Millionaire Conventional	1524 ± 12*	10,519 ± 14	189 ± 11	3316 ± 11	15,547 ± 10
Millionaire Organic	203 ± 4	15,913 ± 26*	665 ± 8*	5060 ± 7*	21,841 ± 16*

Millionaire contains more 5-caffeoylquinic acid and 3-acetyl-5-caffeoylquinic acid, and total phenolic acids under both conventional and organic environments than Blackbell. However, N-caffeoyl putrescine in Millionaire was significantly higher than its organic counterpart. Eggplants cultivated under organic growing conditions are potentially exposed to a larger number of environmental stressors such as insects, soil nutrient quality, relative to conventionally grown eggplants [17] [23]. Therefore, it is possible that organic grown eggplants produce more phenolic acids in reacting to the heavier environmental stresses experienced as part of the organic growing condition. As phenolic acids e.g. chlorogenic acid, caffeic acid and ferulic acid have been shown to possess various health beneficial activities [24] [25] [26] the organic growth environment could potentially improve eggplant skin health promoting potential.

Blackbell eggplant skin extracts contained about 10 times more 3-5-dicaffeoylquinic acid than million in pulps [17] and higher 3-5-dicaffeoylquinic acid levels in Millionaire skin extracts than those of Blackbell. As skin represents the part of the plant with the greatest exposure to environmental stress and the plant most likely to alter its phenolic expression to prevent injury from environmental stress, these results indicate that eggplant skin and pulp have different phenolic distribution/composition patterns resulted from plant tissue stress exposure. Similar results have been observed in other fruits/vegetables such as tomato, grape, apple, pomegranate and mango [19] [20] [21] [27] [28].

3.2. Eggplant Skin Anthocyanins and Flavonol Glycosides

The four eggplant skin anthocyanins characterized by LC-MS were delphinidin 3-rutinoside-5-galactoside, delphinidin 3-rutinoside-5-glucoside, delphinidin-3-glucoside and delphinidin-3-rutinoside (**Table 1**). Their identification was based on comparison of retention time and LC-MS fragmentation pattern with previously published data [29]. In consistent with Wu and Prior's report on whole eggplant (2005) [29], we observed delphinidin-3-rutinoside as the predominant anthocyanin in eggplant skin. Although nasunin (Delphini-

din-3-(p-coumaroyl rutinoside)-5-glucoside) has been reported in eggplant by prior studies [30] [31], we did not detect the correspondence peak in LC-MS analysis.

Besides phenolic acids and anthocyanins, we specifically focused on characterization of flavonol glycosides in eggplant skins. Extracts from eggplant pulps exhibited a very limited flavonol profile in our previous study [17]. In contrast, HPLC and LC-MS chromatograms of eggplant skin extracts exhibited a more complicated flavonol profile. **Table 1** shows the retention times, UV spectra and mass fragmentation patterns of the 11 flavonols identified in the eggplant skin extracts.

Peaks-3, 6, 7, 8 and 11 were identified as myricetin-3-galactoside ($[M-H]^-$ = m/z 479, MS-MS fragments of m/z 479 = 316.1; 270.8; 179.4; 151.1), quercetin-3-rutinoside ($[M-H]^-$ = m/z 609, MS-MS fragments of m/z 609 = 300.1; 271.0; 179.1; 134.9), quercetin-3-galactoside, quercetin-3-glucoside ($[M-H]^-$ = m/z 463, MS-MS fragments of m/z 463 = 300.2; 271.2; 255.2; 151.1) and quercetin-3-rhamnoside ($[M-H]^-$ = m/z 447, MS-MS fragments of m/z 447 = 301.0; 271.2; 255.2; 151.1) respectively based on match of retention time and LC-MS fragmentation pattern with authentic standards.

Peak-1 at RT of 14.14 min showed the molecular ion peak of 625 (m/z) with UV spectra similar to Q-3-glucoside (Peak-7). Product ion scan of this peak showed the fragment ion at m/z 463 ($[Q\text{-}3\text{-}glucoside]^-$), 300 ($[Quercetin\text{-}H]^-$), 271, and 255 correspond to Quercetin-3-diglucoside.

Peak-2 at RT of 14.93 min showed the molecular ion peak at 625 (m/z) and an UV absorbance spectrum similar to peak-3. Product ion scan of this peak showed ions at m/z 479 ($[Myricetin\text{-}rhamnosyl\text{-}H]^-$) and 316 ($[Myricetin\text{-}H]^-$) correspond to myricetin-3-neohesperidoside (**Figure 1(a)**). Precursor ion scan of m/z 317 showed the mass ions at m/z 625.4 and 479.4 confirm its structure of myricetin-3-neohesperidoside [32].

Peak 4 and 5 showed the prominent peaks at m/z 609 and their MS/MS scans showed the fragment ions at m/z 284.2 ($[kaempferol\text{-}H]^-$), 254.2, 227.2 and 150.8. From MS/MS scans we conclude that peak-4 and 5 was kaempferol-3,7-diglucoside (**Figure 1(b)**) and kaempferol-diglucoside respectively [33], identification of the terminal sugar as a diglucose was further confirmed in precursor and neutral loss scans.

The two peaks with RT of 22.01 and 23.78 min (peaks 9 and 10) eluted two compounds with kaempferol-like UV spectra and with $[M-H]^-$ ions at m/z 447 for both. Product ion scan of these two kaempferol derivatives showed the fragments at m/z 284.2, 255 and 227 which are characteristic fragments of the kaempferol aglycone. Based on molecular ion peaks and product ion scans, peaks 8 and 9 were identified as kaempferol-3-galactoside and kaempferol-3-glucoside (**Figure 1(c)**, **Figure 1(d)**) [34].

Compared with our previous report on eggplant pulp, in which only 3 flavonol glycosides (myricetin-3-galactoside, quercetin-3-glucoside and quercetin-3-rhamnoside) were detected [17], the observation in the current study

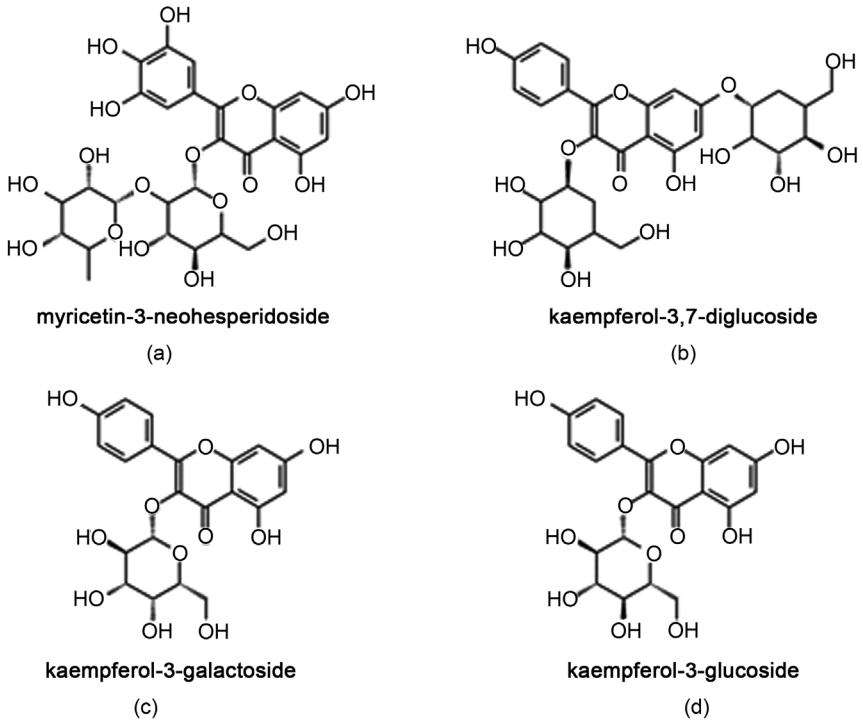

Figure 1. Structures of the four flavonol glycosides-myricetin-3-neohesperidoside (a), was kaempferol-3,7-diglucoside (b), myricetin-3-galactoside (c) and myricetin-3-glucoside (d) identified in eggplant skin samples.

suggests that eggplant skin has a more complex flavonol profile. García-Salas et al. (2004) reported four flavonols glycosides in whole eggplant, including quercetin-3-gentiobioside, kaempferol-3-rutinoside and two kaempferol dihexoside [10]. While we didn't detect kaempferol-3-rutinoside in the current study, the quercetin-3-diglucoside, kaempferol-3,7-diglucoside and another kaempferol-diglucoside reported herein are comparable to the quercetin-3-gentiobioside and two kaempferol dihexoside in the García-Salas *et al.* study. Besides, we also identified other flavonols including myricetin-3-neohesperidoside, myricetin-3-galactoside, and quercetin-3-rhamnoside, which are not reported previously in eggplant. Flavonols are well-known health beneficial phytochemicals [35] [36] [37], illustration of flavonol distribution and composition in fruits and vegetables such as eggplant will add insight to a better understanding of their bioactivities.

3.3. Eggplant Skin Antioxidant Capacity

Eggplant skins extracted in a similar fashion had total phenolic contents of 6168 ± 34, 12305 ± 15, 15547 ± 10, and 21841 ± 16 (μg/g extract dry matter) for Blackbell conventional, Blackbell organic, Millionaire conventional and Millionaire organic, respectively (**Table 2**). Eggplant skin phenolics (5.0 and 2.5 μg extract dry matter/ml) extended the A_{234} lag-time for LDL oxidation by 3.0 μM cupric ions (**Table 3**) and at 5.0 μg/ml the effect was highly correlated with total

phenolic content (R^2 = 0.957) and 5-caffeoylquinic acid (R^2 = 0.9775) (Figure 2). Eggplant skin phenolics did not significantly extend the oxidative lag-time at 0.50 and 0.25 μg extract dry matter/ml (Table 3). Statistically significant differences A_{234} lag-time between the two growing conditions and two cultivars were not detected. Eggplant skin antioxidant effects against 10 μM cupric ions were confirmed in a smaller trial with respect to ability to inhibit changes in relative electrophoretic oxidation at 2.5 μg/mL (Figure 3), with significance for both cultivars and growing conditions versus control, but no significant difference between conditions. In this respect the effect was also highly correlated with extract total phenolic content (R^2 = 0.95, data not shown).

The present study suggests that eggplant skin contains a greater phenolic content and antioxidant capacity relative to extracts prepared using plant tissue from the pulp. Relative to phenolic extracts from eggplant pulp in our prior study [17], eggplant skin extracts were able to significantly inhibit LDL oxidation in the A_{234} assay at twenty-fold lower concentrations while in the presence of a lower level of cupric ion-mediated oxidative stress (10.0 μg extract dry matter/ml

Table 3. Lag-time (A_{234}) for 3.0 μM cupric ion mediated oxidation in the presence of eggplant skin phenolic extracts. Control LDL oxidized in the absence of eggplant had a lag time of 1.2 ± 0.1 hours. Data represented as mean ± Standard deviation; Statistically significant ($P < 0.05$) differences relative to control indicated by (*).

Extract	5.0 μg/ml	2.5 μg/ml	0.50 μg/ml	0.25 μg/ml
Blackbell-Conventional	2.3 ± 0.3*	1.7 ± 0.2*	1.3 ± 0.2	1.3 ± 0.2
Blackbell-Organic	2.6 ± 0.5*	1.7 ± 0.3*	1.3 ± 0.2	1.4 ± 0.1
Millionaire-Conventional	2.7 ± 0.5*	1.7 ± 0.3*	1.4 ± 0.2	1.4 ± 0.3
Millionaire-Organic	3.2 ± 1.1*	2.1 ± 0.4*	1.4 ± 0.2	1.5 ± 0.2

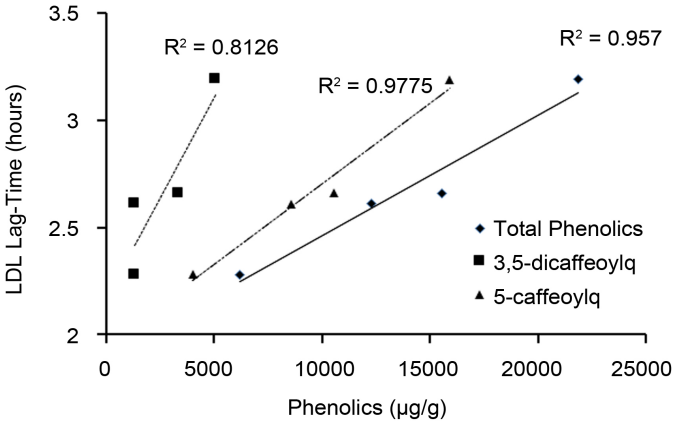

Figure 2. Correlation between ability of eggplant skin (Blackbell conventional, Blackbell organic, Millionaire conventional and Millionaire organic) phenolic extracts (5.0 μg/ml) to inhibit 3.0 μM cupric ion mediated oxidation and their contents of total phenolic acids, 3-5-dicaffeoylquinic acid (3,5-dicaffeoylq), and 5-caffeoylquinic acid (5-caffeoyl q).

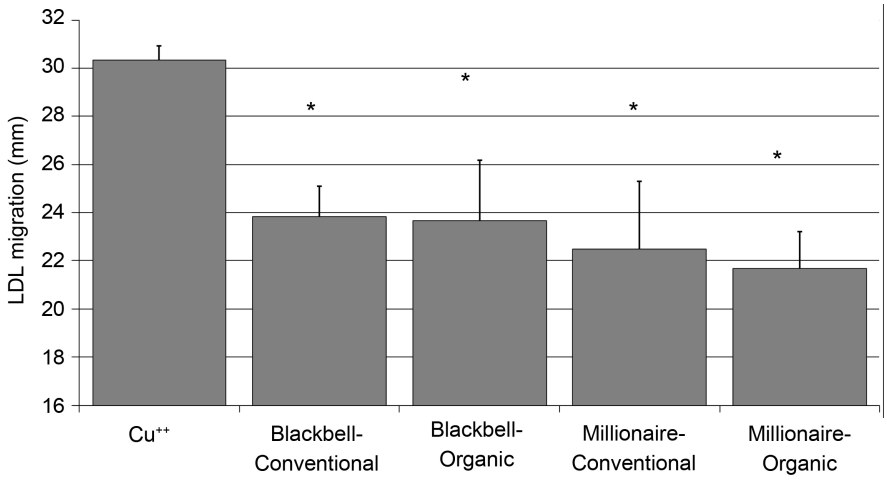

Figure 3. Electrophoretic migration of LDL oxidized by 10 μM Cu^{++} for 100 minutes in the absence and presence of eggplant skin phenolic extracts (2.5 μg/ml). Data expressed as mean ± standard deviations (n = 3). Statistical significance relative to control indicated by (*).

and 10 μM Cu^{++} for eggplant pulp v.s. 0.50 μg/mL and 3 μM Cu^{++} for eggplant skin). Analysis of inhibition of LDL oxidation with the smaller electrophoretic mobility validation trial also suggests antioxidant protection of eggplant skin phenolic extracts. These results indicate that it is important to also consume the eggplant skin together with pump to maximize its human health benefits.

Other investigators have attempted to characterize eggplant antioxidant capacity [30] [38] [39] [40]. While these studies have described eggplant antioxidant capacities, they used chemical assays that are less biologically relevant than the LDL oxidation assay utilized in the current study. Furthermore, these prior studies did not provide precise characterizations of phenolic composition of their extracts. In this respect the present study represents a significant advancement in our understanding of the phenolic profile of eggplant skins as well the ability of these phenolic-rich extracts to inhibit LDL oxidation, a process known to be biologically relevant. Eggplant antioxidant activity is correlated with phenolic content and may be associated with the organic growing conditions.

4. Conclusions

Eggplant skin contains a larger number of individual phenolic compounds than does eggplant pulp and greater amounts of most of the phenolic compounds present in both eggplant skins and pulp. The identification of 5-caffeoylquinic acid, myricetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-glucoside, quercetin-3-galactoside was confirmed by direct comparison with standards' retention time and mass fragment ions. Quercetin-3-diglucoside, myricetin-3-neohesperidoside, kaempferol-3,7-diglucoside, a kaempferol diglucoside derivative, kaempferol-3-galactoside, kaempferol-O-glucoside, quercetin-3-rhamnoside, dephinidin-3-rutinoside-5-galactoside, delphinidin-3-glucoside, delphinidin-3-rutinoside and other phenolics were identified based on their UV absor-

bance spectra and mass fragmentation data. Organic growing conditions were associated with eggplant skin extracts containing greater quantities of phenolic compounds (μg/g extract dry matter) relative to eggplants grown under conventional growing conditions. The ability of eggplant skin extracts to inhibit oxidation of LDL lipids and apo-B100 was positively correlated with phenolic content, but not growing condition. Future studies are needed to characterize how to promote the development of eggplant fruits with higher phenolic content and potentially greater human health benefits. Bioavailability analysis of eggplant skin phenolic acids and the novel flavonols identified in the current study would improve our ability to characterize eggplant skin health promoting properties.

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