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Inhibitory Effects of Muscadine Anthocyanins on α-Glucosidase and Pancreatic Lipase Activities

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ABSTRACT: Inhibitory effects of the Noble muscadine grape extracts and the representative phytochemicals for anthocyanins (i.e., cyanidin and cyanidin-3,5-diglucoside) on two enzymes, that is, α-glucosidase and pancreatic lipase, were investigated regarding their antidiabetic activities. The study demonstrated that the anthocyanin extracts and the selected chemicals obeyed the competitive mode against the enzymes. The methanolic extracts of whole fruit and skin of the muscadine showed inhibitory activities against the α-glucosidase with their IC_{50} values at 1.50 and 2.73 mg/mL, and those against the lipase at 16.90 and 11.15 mg/mL, respectively, which indicated that the muscadine extracts possessed strong antidiabetic activities. Particularly, the ethyl acetate (EtoAc) extract and the butanol (BuOH) extract exhibited much higher inhibitory activities against both enzymes than the $CHCl_3$ and water extracts, while the majority of anthocyanins existed in the BuOH fractions. Moreover, cyanidin exhibited a much stronger antidiabetic activity than cyanidin-3,5-diglucoside, suggesting that anthocyanins may have higher inhibitory activities after being digested. Further chromatographic analysis by high-performance liquid chromatography−mass spectrometry identified five individual anthocyanins, including cyanidin, delphinidin, petunidin, peonidin, and malvidin glycosides.

KEYWORDS: anthocyanins, antidiabetes, α-glucosidase, pancreatic lipase, HPLC-MS

INTRODUCTION

The number of people with diabetes is increasing due to aging and the increasing prevalence of obesity and physical inactivity. Diabetes mellitus, also referred to simply as diabetes, is a kind of metabolic disease in which the body either does not produce enough insulin or does not respond to the produced insulin, resulting in an increase of blood glucose levels and causing serious and irreparable damage to body systems, such as blood vessels and nerves.[1] There are three main types of diabetes: type I diabetes (insulin-dependent diabetes) caused by the body's failure to produce insulin, type II diabetes (noninsulin-dependent diabetes) resulting from insulin resistance, and gestational diabetes, which occurs in about 2−5% of all pregnancies and may develop to be type II or disappear after delivery. Diabetes is considered a chronic killer that threatened at least 171 million people worldwide, or 2.8% of the population, reported in 2000. Type II diabetes is the most common, for example, affecting 90−95% of the U.S. diabetes population.[2]

When there is a lack of enough insulin or the insulin is not used as it should be, glucose (sugar) will accumulate in the blood instead of going into the body's cells, causing the cells to function improperly. One of the therapeutic approaches is to decrease the postprandial hyperglycemia by retarding absorption of glucose via inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase, which is the enzyme for the final step in the digestive process of carbohydrates.[3,4] On the other hand, type I diabetes is caused by progressive destruction of pancreatic insulin-producing β cells, which could be damaged by the accumulated lipids in the pancreas. Therefore, lipase inhibitors have attracted much attention for their antiobesity activities, to reduce the lipid absorption and to protect the pancreas that will enable the β-cells to produce normal levels of insulin.[5]

The consumption of anthocyanins is associated with a variety of health benefits, such as inhibition of carcinogenesis and a tumor cell's invasiveness and proliferation.[6] The polyphenolic structures of anthocyanins also confer antioxidant activities that may reduce the oxidant-induced apoptosis and age-associated oxidative stress. In addition, those bioactive phytochemicals have been demonstrated to possess anti-inflammatory properties[7] and the power to stabilize DNA triple-helical complexes,[8] which are correlated with the prevention of some chronic and degenerative diseases such as diabetes.[9]

This study aimed to investigate the antidiabetic activities of anthocyanins in Noble muscadine through bioassays against α-glucosidase and pancreatic lipase in terms of the accurate dissociation constant (K_i) and IC_{50} values in different solvent extract fractions. Furthermore, specific anthocyanins contributing to the bioactivity were identified by high-performance liquid chromatography with mass spectrometry (HPLC-MS).

MATERIALS AND METHODS

Chemicals and Reagents. α-Glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), lipase from porcine pancreas type II (EC

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3.1.1.3), *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), and 4-methylumbelliferyl oleate (4-MU) were obtained from Sigma-Aldrich (St. Louis, MO). Tris, hydrochloric acid (HCl), formic acid, and HPLC grade methanol, as well as chloroform ($CHCl_3$), ethyl acetate (EtoAC), and butanol of analytical grade were purchased from Fisher Scientific (Suwannee, GA). All standards, that is, cyanidin, cyanidin-3,5-diglucoside, delphinidin-3,5-diglucoside, petunidin-3,5-diglucoside, peonidin-3,5-diglucoside, and malvidin-3,5-diglucoside, were purchased from Chromadex (Irvine, CA).

Sample Collection and Preparation. Red Noble muscadine (*Vitis rotundifloia*) grapes were obtained from Paulk Vineyard (Wray, GA). The skin portion of the muscadine was freshly separated from and compared with the whole fruit. All samples were stored at −20 °C until analysis.

Twenty grams of pregrounded whole fruit or skin part of the muscadine was mixed with 320 mL of methanol (MeOH), 80 mL of distilled water, and 0.1 mL/L acetic acid, followed by 1 h of sonic treatment to extract anthocyanins from the Noble muscadine or its skin.[10] Then, the extract was poured into a 500 mL bottle through a 0.45 μm cellulose acetate filter (Costar Corp., Cambridge, MA) to prepare the original methanolic whole extracts. The extracts were concentrated equivalent to 1 g fresh material/mL. To prepare the other extracts, 10 mL of the methanolic extracts was concentrated to remove the solvent completely with a vacuum rotary evaporator, and then, it was suspended in 100 mL of distilled water. Then, the methanolic extracts dissolved in water were sequentially fractionated by $CHCl_3$, EtoAC, and *n*-butanol (BtOH), using liquid−liquid extraction.[11] The remaining part after three solvent extractions was the H_2O extract. All of the extracts were concentrated by the vacuum rotary evaporator to remove the solvent completely and redissolved in methanol again. The analyzed concentration for all four fractionated extracts (i.e., the $CHCl_3$ extract, EtoAC extract, BtOH extract, and H_2O extract) was accurately weighed equivalent to 2 g fresh material/mL.

Determination of α-Glucosidase Activity. The assay uses *p*NPG as the substrate, which is hydrolyzed by α-glucosidase to release *p*-nitrophenol, a color agent that can be monitored at 405 nm.[12] Briefly, 20 μL of a sample solution was mixed with 70 μL of the enzyme solution (1 U/mL) in 0.1 M phosphate buffer (pH 6.8) and incubated at 37 °C for 6 min under shaking. After incubation, 100 μL of 4 mM ρNPG solution in the above buffer was added to initiate the colorimetric reaction at 37 °C. The released *p*-nitrophenol was monitored at 405 nm every min for a total time of 60 min by a Bio-Tek μQuant 96 micro well plate reader (Bio-Tek Instruments, Inc., Winooski, VT). The highest rate (V) of the initial enzymatic reaction for each sample was measured for the further investigation in this study. All of the samples, for example, the Noble muscadine whole fruit methanolic extract (FME), the fruit $CHCl_3$ extract (FCE), the fruit EtoAc extract (FEE), the fruit BuOH extract (FBE), the fruit water extract (FWE), the Noble skin methanolic extract (SME), the skin $CHCl_3$ extract (SCE), the skin EtoAc extract (SEE), the skin BuOH extract (SBE), and the skin water extract (SWE), were investigated for the α-glucosidase kinetic inhibition. In this study, 1 mg/mL of FME, 2 mg/mL of SME, 0.1 mg/mL of cyanidin, 1 mg/mL of cyanidin-3,5-diglucoside, and the control were selected to react with *p*NPG at different concentrations to determine their inhibitive modes.

Determination of Pancreatic Lipase Activity. The pancreatic lipase activity was measured by using 4-MU oleate as a substrate, as reported by Nakai and his co-workers.[5] An aliquot of 50 μL of the pancreatic lipase solution (2 U/mL) in a 50 mM Tris-HCl (pH 8.0) buffer solution was added into 100 μL of the diluted sample solution and mixed with 50 μL of a 0.5 mM 4-MU solution dissolved in the above buffer in the well of a 96-well microplate to start the enzyme reaction. The plate was immediately placed in the 37 °C preheating FLx800 microplate fluorescence reader (Bio-Tek Instruments, Inc.) to measure the amount of 4-methylumbelliferone released by the lipase every minute for 30 min at an excitation wavelength of 360 nm with a tolerance of ±40 nm and an emission wavelength of 455 nm with a tolerance of ±20 nm. The enzymatic reaction rate for each sample was measured as that as mentioned above. All of the samples were investigated for the pancreatic lipase inhibition, for which 20 mg/mL of FME, 10 mg/mL of SME, 0.04 mg/mL of cyanidin, 0.2 mg/mL of cyanidin-3,5-diglucoside, and the control were studied against the substrate 4-MU oleate at different concentrations to explore the enzymatic kinetic constants and inhibitive mode.

K_i and IC_{50} Values of Effects against α-Glucosidase and Pancreatic Lipase Inhibitory Activities. To determine the V_{max} and K_m constants, the *p*NPG substrate solutions in 1, 2, 3, 4, and 5 mM for the α-glucosidase activity assay and the 4-MU solutions in 0.05, 0.1, 0.25, and 0.5 mM for the pancreatic lipase activity were used to react with the methanol (control) to make the Lineweaver−Burk plot from the Michaelis−Menten equations. The same substrate solutions were also used to react with the SME, the whole FME, the standard cyanidin, and cyanidin-3,5-diglucoside to determine their inhibitive types against the enzymes. Then, a 5 mM concentration of the *p*NPG substrate solutions and a 0.5 mM concentration of the 4-MU solutions were chosen to react with all extracts in series of concentrations, respectively. The K_i values were obtained from the least-squares regression lines that were plotted from the reciprocal of the sample concentration versus the reciprocal of the rate of reactions, for which the formula is listed below:

$$\frac{1}{V} = \frac{K_m}{V_{max}K_i[S]}[I] + \frac{1}{V_{max}}\left(1 + \frac{K_m}{[S]}\right)$$

The IC_{50} value was obtained from:

$$IC_{50} = K_i\left(1 + \frac{[S]}{K_m}\right)$$

where the K_m is the Michaelis constant and the K_i is the dissociation constant. The V_{max} is the maximum rate of the enzymatic reaction. The [S] represents the concentration of substrate, and [I] is the concentration of sample (inhibitor) solution. The IC_{50} value is the concentration of sample (inhibitor) to provide 50% inhibitory activity.

TAC. Two dilutions of the samples were prepared, one with 0.025 M KCl buffer, pH 1.0, and the other with 0.4 M sodium acetate buffer, pH 4.5. Each was diluted by the previously determined dilution factor (DF). The dilutions were allowed to equilibrate for 15 min and measured between 15 min and 1 h after the sample preparation to avoid a longer standing time tending to increase the observed readings. The absorbance of each dilution was measured against a blank cell filled with distilled water at $\lambda_{510\,nm}$ and $\lambda_{700\,nm}$ (for correct haze). The concentration of monomeric anthocyanin pigment in the original sample was expressed in equivalence of cyanidin-3-glucoside and calculated by the following formula:

$$\text{monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

where A is the adjusted absorbance of the diluted samples, MW is the molecular weight of cyanidin-3-glucoside in 449.2, and ε is the molar absorptivity, which equals to 26900 for cyanidin-3-glucoside.[13]

Identification of Individual Anthocyanins by HPLC-MS. The Agilent 1200 series HPLC-MS system was composed of a degasser, binary pump, autosampler, heating exchanger, and UV/vis detector as well as a MS detector, with an Agilent SB-C_{18} column (150 mm × 3.0 mm, particle size of 3.5 μm, Agilent Technologies Inc., St. Clara, CA). Anthocyanins were eluted with a gradient mobile phase formed by 5% (v/v) formic acid aqueous solution (phase A) and methanol (phase B) at a flow rate of 0.8 mL/min. The linear gradient of phase B started from 5% for the first 5 min, increased from 5 to 30% from 5 to 40 min, and up to 40% when the running time was at 50 min. The phase B was

maintained at 40% from 50 to 55 min, and finally decreased from 40 to 5% from 55 to 60 min to end the running. The wavelengths of the UV−visible detector were set at 520 nm. Mass spectra were acquired in a positive ion mode as anthocyanins are subjected to loss of an electron and stable in positive form based on their structures. Ions were scanned from 100 to 800 m/z. Nitrogen was used as the nebulizing gas.[10]

Statistical Analysis. All of the samples were run at least in triplicate. Data analysis was performed in REG and GLM program using SAS software (SAS 9.2) (SAS Institute Inc.). Values were expressed as means ± standard deviations. The differences of means were analyzed with Fisher's least significant difference (LSD) procedure. The difference was considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

α-Glucosidase Inhibitory Activity. α-Glucosidase as a key enzyme involved in sugar metabolism has been often suggested as a good model for studying the effect of nutraceuticals on type II diabetes.[14] However, most of the previous investigations only reported the inhibitory activities of phytochemicals at their specific concentrations, rather than giving deep exploration of the inhibitors' enzymatic mode and their exact IC_{50} values against the α-glucosidase.[15,16] In this study, the type of the inhibitive mode of the enzyme inhibitors from the muscadine extracts was characterized by the kinetic method, from which the K_i and the IC_{50} value were determined. Figure 1 shows their Lineweaver−Burk plots. The L-B plots deduced inhibitive linear equations for the control as $Y = 3.0373X + 2.0176$ ($R^2 = 0.9995$), for FME as $Y = 25.265X + 2.0265$ ($R^2 = 0.9968$) and for SME as $Y = 9.5872X + 2.0232$ ($R^2 = 0.9919$). Although the mathematical equations for all of the inhibitors and the control differ in slopes, their y-intercepts were nearly the same, indicating their enzymatic inhibition modes belonged to the same competitive type. The same results were drawn from the analyses of cyanidin and cyanidin-3,5-diglucoside. Similar results were reported by Zhang[17] and Adisakwattana,[18] who also demonstrated that the inhibition mode of cyanidin-3-glucoside was competitive, while that of cyanidin-3-galactoside was competitive predominant over noncompetitive.

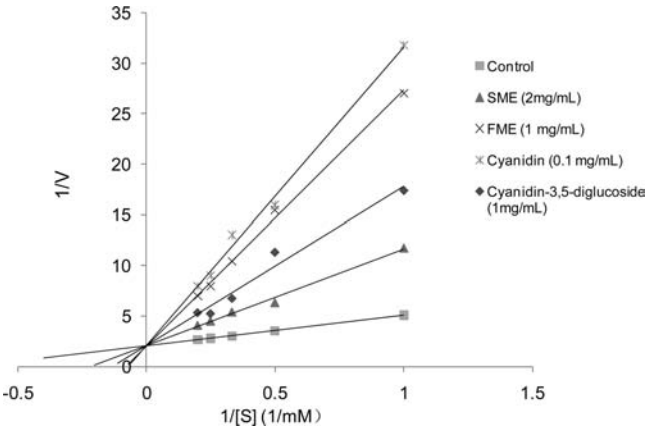

Figure 1. Lineweaver−Burk plots of control, SME, FME, cyanidin, and cyanidin-3,5-diglucoside for the α-glucosidase inhibitory activity.

The K_i and IC_{50} values for all samples were determined based on the competitive inhibition mode and are listed in Table 1. The smaller K_i and IC_{50} values mean the stronger inhibitory activities. The results indicated that even 1.50 mg/mL of FME and 2.73 mg/mL of SME had been enough to inhibit 50% of the α-glucosidase activity, which demonstrated that the Noble muscadine is really a great source of strong natural inhibitor against the α-glucosidase, which is comparable to other reported natural strong inhibitors like oolong tea and green tea extracts.[19] Regarding other solvent extracts, the $CHCl_3$ extracts for both fruit and skin had a weak α-glucosidase inhibitory activity, while the EtoAc extract and BuOH extract exhibited stronger inhibitions. The high inhibitory activities of the BuOH extracts in term of their IC_{50} and K_i values, for instance, 7.19 and 2.66 mg/mL for the FBE based on the fresh whole fruit weight and 2.85 and

Table 1. TAC, K_i, and IC_{50} on α-Glucosidase and Lipase for Different Solvent Extracts from Noble Muscadine Grape Whole Fruit and Skin and Selected Standards[a]

	anti-α-glucosidase		antipancreatic lipase		TAC
samples	K_i (mg/mL)[c]	IC_{50}(mg/mL)[c]	K_i (mg/mL)[c]	IC_{50}(mg/mL)[c]	(mg/100 g FW)[e]
FME	0.56	1.50	8.60	16.90	82.30 ± 1.49 a
FCE	70.09	189.35	95.53	187.67	*b*
FEE	0.76	2.07	42.64	83.78	*b*
FBE	2.66	7.19	19.36	38.04	46.51 ± 0.62 b
FWE	188.37	508.88	56.86	111.71	0.69 ± 0.04 c
SME	1.01	2.73	5.68	11.15	306.48 ± 4.18 d
SCE	634.49	1714.12	105.35	206.98	*b*
SEE	6.54	17.66	15.41	30.27	*b*
SBE	1.05	2.85	15.74	30.93	166.99 ± 0.88 e
SWE	14.88	40.21	21.26	41.77	6.25 ± 0.19 f

	anti-α-glucosidase		antipancreatic lipase	
standards	K_i (mg/mL)[d]	IC_{50}(mg/mL)[d]	K_i (mg/mL)[d]	IC_{50}(mg/mL)[d]
cyanidin	0.01	0.04	0.07	0.17
cyanidin-3,5-diglucoside	1.64	6.01	0.35	0.89

[a] Different letters in the TAC column denote a statistical difference at $P \leq 0.05$. [b] TAC value not detected. [c] mg fresh fruit or skin per mL. [d] mg standard per mL. [e] mg total anthocyanin per 100 g fresh material weight.

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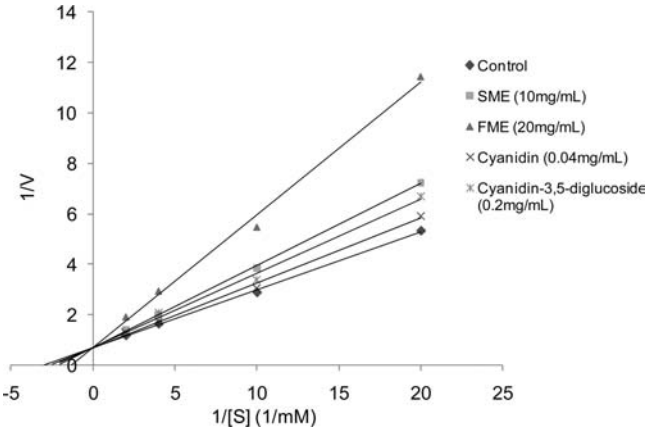

Figure 2. Lineweaver−Burk plots of control, SME, FME, cyanidin, and cyanidin-3,5-diglucoside for the pancreatic lipase inhibitory activity.

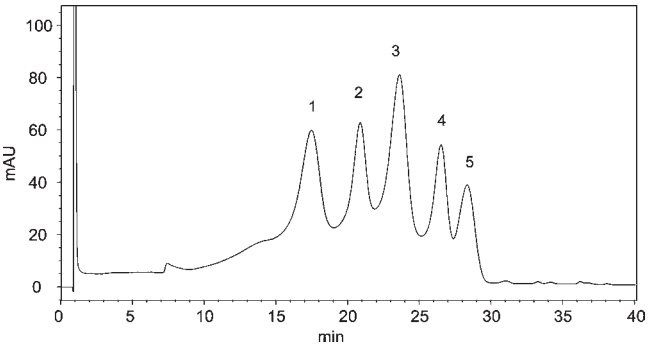

Figure 3. HPLC chromatogram of individual anthocyanins in the Noble SBE fraction (200 mg/mL). Peaks: delphinidin 3-*O*-glucoside-5-*O*-glucoside, $M^+ = 627$ (1), cyanidin 3-*O*-glucoside-5-*O*-glucoside, $M^+ = 611$ (2), petunidin 3-*O*-glucoside-5-*O*-glucoside, $M^+ = 641$ (3), peonidin 3-*O*-glucoside-5-*O*-glucoside, $M^+ = 625$ (4), and malvidin 3-*O*-glucoside-5-*O*-glucoside, $M^+ = 655$ (5).

1.05 mg/mL for the SBE based on the fresh skin weight, were coincident with the high content of anthocyanins in the extracts. According to the total anthocyanin content (TAC), most parts of anthocyanins of the muscadine, both of the whole fruit and skin, were extracted and enriched in the BuOH extracts from the whole methanolic extracts, in which anthocyanins existed as high as 46.51 ± 0.62 and 166.99 ± 0.88 mg/100 g fresh material (Table 1), respectively. This means anthocyanins, especially in the skin of the muscadine, might be a major contributor for the α-glucosidase inhibition. However, in Table 1, the IC_{50} of the skin whole extract against the α-glucosidase was much lower than that of the pure cyanidin-3,5-diglucoside, which was a major anthocyanin in the extract. Therefore, it is also worthy to be mentioned that, besides anthocyanins, other phytochemical polyphenolics in the extracts might also contribute the enzymatic inhibitory activity as shown in the EtoAc extract.

On the basis of the HPLC-MS identification, the cyanidin-3,5-diglucoside was chosen as a standard to be analyzed for the α-glucosidase inhibition. The cyanidin was analyzed in this study as well, because some of the anthocyanins are digested and converted to the corresponding anthocyanidins in small intestinal by β-glucosidase.[20] The results showed that the IC_{50} value of cyanidin (0.04 mg/mL) for the α-glucosidase inhibition was much lower than that of cyanidin-3,5-diglucoside (6.01 mg/mL), which means the cyanidin has a much stronger antidiabetic activity than its glycoside form. It suggests that anthocyanins may exert more α-glucosidase inhibitory activity after being hydrolyzed in intestine.

Pancreatic Lipase Inhibitory Activity. Few reports have been published about the inhibitory activity of nutraceuticals against the pancreatic lipase. Moreno and his co-workers[21] reported that 1 mg/mL of the grape seed extract possessed 80% inhibitory effect on pancreatic lipase. Like studies for the α-glucosidase, most of previous researches only reported the inhibitive activity of the tested samples against the lipase within the range of concentrations instead of the inhibitive mechanism (mode).[5,21,22] Similar to the above-mentioned α-glucosidase inhibitory assay in this study, effects of FME, SME, cyanidin, and cyanindin-3,5-diglucoside were studied for exploring the enzymatic kinetic constants and inhibitive mode. As shown in the L-B plots for the lipase (Figure 2), equations of $Y = 0.2287X + 0.6948$ ($R^2 = 0.9986$), $Y = 0.5266X + 0.6914$ ($R^2 = 0.9940$), $Y = 0.3252X + 0.695$ ($R^2 = 0.9995$), $Y = 0.2565X + 0.6926$ ($R^2 = 0.9955$), and $Y = 0.2949X + 0.6983$ ($R^2 = 0.9931$) represent the enzymatic reaction curves for the control, FME, SME, cyanidin, and cyanidin-3,5-diglucoside, respectively, which have the same *y*-intercept in the *y*-axis. This means that all of the inhibitors (i.e., the extracts, the single anthocyanin, and anthocyanidin) fell into the competitive inhibition mode against the lipase. From the control, V_{max} and K_m values without inhibition are deduced. The V_{max} and K_m in the presence of inhibitors are determined from the respective kinetic curves. K_i and IC_{50} values are calculated taking into account the change in the kinetic constants, as shown in Table 1.

The FME and SME showed the low K_i values at 8.60 and 5.68 mg/mL, and their IC_{50} values as low as 16.90 and 11.15 mg/mL, respectively. This means muscadine is a rich source of the pancreatic lipase inhibitor and possesses a strong lipase inhibitory activity. Regarding the different solvent extract fractions, the IC_{50} values of the FEE and FBE fractions were 83.78 and 38.04 mg/mL, respectively. In contrast, the IC_{50} values of the SEE and SBE were 30.27 and 30.93 mg/mL, respectively, which demonstrated that the skin extracts possessed stronger inhibitory activities against the pancreatic lipase than their counterparts of the whole fruit extracts (i.e., FBE and FEE), as well as the other two solvent extract fractions (i.e., SWE and SCE). The FBE fraction showed a stronger inhibitive activity against the pancreatic lipase than the FEE fraction, which suggested that the anthocyanins in the Noble muscadine might perform most of the inhibitory activity. However, it is also necessary to be mentioned that other phenolics fractionated into the EtoAc extract also contributed to the enzymatic inhibitory activity since the FEE and SEE exhibited high inhibitive activities as well.

Table 1 also lists the K_i values of the cyanidin and cyanidin-3,5-diglucoside for their lipase inhibitory activity, which were 0.07 and 0.35 mg/mL. Meanwhile, their IC_{50} values were 0.17 and 0.89 mg/mL, respectively. On the basis of the results, the anthocyanidin exhibited a much stronger lipase inhibitory activity than its glycosidic anthocyanin. This may also suggest that the anthocyanins in muscadine might be the main bioactive compounds contributing to the lipase inhibitory activity and may exert more pancreatic lipase inhibitory activity after being hydrolyzed in the human's intestine. This result is in agreement with the previous report that anthocyanins are a stronger lipase inhibitor than other natural phenolic compounds.[5] Regarding the

results from both α-glucosidase and lipase inhibitory activities, it is believed that muscadine, a U.S. regional special fruit, can be developed into promising nutraceuticals for human health benefits.

Identification of Individual Anthocyanins. HPLC-MS with positive mode was used for identifying the individual anthocyanins present in the muscadine samples. The peaks were identified primarily based on comparison of the elution order with the published data, our stock standards, the detected molecular weight, and characteristic MS spectra.[10,23] The previous studies suggested that the glycosides linked to the anthocyanidins are likely and primarily to be glucose and in the nonacylated form in muscadine.[24,25] Meanwhile, Wu and Prior[26] reported that two glycosides were likely linked at the 3- and 5-positions of anthcyanidins. According to the HPLC chromatogram of the SBE as shown in Figure 3, peak 1 was identified as delphinidin 3-*O*-glucoside-5-*O*-glucoside with *m*/*z* 627 $[M]^+$ ion, peak 2 was cyanidin 3-*O*-glucoside-5-*O*-glucoside with *m*/*z* 611 $[M]^+$ ion, peak 3 was petunidin 3-*O*-glucoside-5-*O*-glucoside (*m*/*z* 641), peak 4 was peonidin 3-*O*-glucoside-5-*O*-glucoside (*m*/*z* 625), and peak 5 was malvidin 3-*O*-glucoside-5-*O*-glucoside (*m*/*z* 655). Among the six common anthocyanidins in the nature, that is, cyanidin, delphinidin, petunidin, pelargonidin, peonidin, and malvidin, only pelargonidin or its glycosidic derivatives were not found in this study. The revealed anthocyanin composition in muscadine indicated a different chemical profile from other previous reports for grapes.[23,27,28]

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