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Degradation Kinetics of Malvidin-3-glucoside and Malvidin-3,5-diglucoside Exposed to Microwave Treatment

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ABSTRACT: Understanding the factors that contribute to the degradation of bioactive compounds during microwave treatment is meaningful for the practical application of this novel technology. The influence of microwave power, energy density, temperature, pH value, and initial concentration of anthocyanins (Acys) on the degradation behavior of malvidin-3-glucoside (Mv-3-glu) and malvidin-3,5-diglucoside (Mv-3,5-diglu) was investigated in this study. Results showed that the degradation of both Acys was accelerated with the increase of microwave power, energy density, temperature, pH value, and initial concentration of Acys. The degradation process of both Acys followed the first-order kinetics model ($R^2 > 0.94$), whereas the relationship between Acys degradation and energy density fitted to the logistic model well ($R^2 > 0.98$). In addition, Mv-3-glu was more susceptible to the microwave treatment than Mv-3,5-diglu. Compared with heating in a 98 ± 2 °C water bath, both Acys degraded more rapidly under microwave treatment at 100 °C, indicating the occurrence of microwave effect. The results provide a guide for the scientific application of microwave treatment.

KEYWORDS: *microwave, anthocyanins, degradation, kinetics, malvidin-3-glucoside, malvidin-3,5-diglucoside*

INTRODUCTION

Anthocyanins (Acys) are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts.[1] Numerous studies have presented the beneficial health effects of Acys in protecting against DNA cleavage, inhibiting inflammation, anticarcinogenic activities, and preventing cardiovascular and neurodegenerative diseases.[2−5] Therefore, Acys extracts have been applied as important dietary supplements in many countries, and the comprehensive extraction of Acys becomes a favorable utilization pathway for Acys-enriched plants, such as berries and purple corn.

Thermal extraction is the conventional method for the extraction of Acys from purple potatos, grape skins, black carrots, purple carrots, etc.[6] Recently, some new technologies including pulsed electric field (PEF)-assisted treatment, microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE)[7−10] have increased significantly the extraction efficiency and have become alternatives for the extraction of Acys. However, some researchers found that the stability of Acys was influenced by the extraction conditions. Seeram et al.[8] demonstrated that high temperature in combination with high pH caused the degradation of Acys, resulting in the formation three different benzoic acid derivatives in cherry. Zhang et al.[9] found that the Acys degraded significantly after PEF treatment and that the degradation rate constant was 10^3 times higher than that of thermal degradation. Carrera et al.[10] found that significant degradation of Acys occurred during UAE of total Acys from grape skin.

MAE is a novel and green extraction technique that can offer some advantages such as improved efficiency, reduced extraction time, low solvent consumption, low energy input, and high level of automation compared to conventional thermal extraction.[11,12] In our previous study, MAE showed the above-mentioned advantages in extraction of Acys from grape skin with acidified water with citric acid, but we found that Acys degraded significantly under high energy density.[13] Liazid et al.[14] also reported a similar phenomenon and supposed that the degradation of Acys probably occurred due to both thermal and oxidative conditions. However, there is little knowledge on the degradation behavior of Acys during microwave treatment. Malvidin-3-glucoside (Mv-3-glu) and malvidin-3,5-diglucoside (Mv-3,5-diglu) are the main Acys present in grape berries, with proportions ranging from 15 to 45% of total Acys contents in grape berries.[7,15,16] In the present study, the degradation kinetics of Mv-3-glu and Mv-3,5-diglu under different microwave powers, treatment times, temperatures, pH values, and initial concentrations were investigated. The degradation kinetics parameters were deduced and used for the description of the degradation behavior of both Acys during microwave treatment.

MATERIALS AND METHODS

Chemicals. HPLC grade acetonitrile was purchased from Honeywell B&J Technologies Inc. (Ulsan, Korea). Formic acid was purchased from Sigma-Aldrich Technologies Inc. (St. Louis, MO, USA). Citric acid and disodium hydrogen phosphate of reagent grade were purchased from Beijing Chemical Reagents Co. (Beijing, China). Mv-3-glu and Mv-3,5-diglu standards were obtained from Polyphenols (Sandnes, Norway).

The pH 3.5 and 4.5 buffer solutions were made of 0.1 mol/L citric acid and 0.2 mol/L disodium hydrogen phosphate. The pH 1.0 buffer

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solution was made of 1.0 mol/L citric acid and 0.2 mol/L disodium hydrogen phosphate.

The pH 1.5, 3.5, and 4.5 buffer solutions containing 25 and 50 mg/L Mv-3-glu and Mv-3,5-diglu were prepared and used for the further microwave treatment, respectively.

Mircowave and Heat Treatment. A microwave synthesis/extraction reaction workstation (model MAS-II, SINEO Microwave Chemistry Technology Co. Ltd., Shanghai, China; working at frequency of 2450 MHz with the maximum power level of 1000 W) was used in this experiment. The workstation is equipped with a magnetic stirrer (it will run at 1000 rpm rotation during microwave irradiation works) and an infrared temperature measurement system (it has a probe above the solution to detect the solution surface temperature online).

An aliquot (5 mL) of Acys solution was added in a flask and treated under given microwave conditions. The microwave power (300, 500, 700 W), temperature (70, 100 °C), initial condition of Acys (25, 50 mg/L), and pH value of the solution (1.5, 3.5, 4.5) were varied in treatments. Thermal treatment was performed as the control for the microwave treatment at 100 °C. An aliquot (5 mL) of Acys aqueous solution was heated at 98 ± 2 °C in a water bath, and the heating time ranged from 0.5 to 6.0 h. All of the samples were cooled in ice water immediately after the treatment and then filtered through a 0.22 μm filter for further analysis.

Quantification of Mv-3-glu and Mv-3,5-diglu. Mv-3-glu and Mv-3,5-diglu concentrations were detected according to the previously described method with slight modifications.[11] Using a Waters HPLC system (Waters 2996, Milford, MA, USA) equipped with a diode array detector (DAD), Mv-3-glu and Mv-3,5-diglu were separated on an analytical Venusil ASB-C_{18} (250 × 4.6 mm; i.d. = 5.0 μm; Agela Techonologies Inc., Newark, DE, USA) at 30 °C and the flow rate of 0.4 mL/min. Mobile phase was made of 5% aqueous formic acid (A) and acetonitrile (B). Starting isocratically with 10% B, linear gradients were ramped to 12% B at 10 min, 15% B at 20 min, 30% B at 55 min, and finally 10% B from 55 to 60 min. The separation of Mv-3-glu and Mv-3,5-diglu was achieved within 60 min, and the injection volume was 10.0 μL. The calibration graph was produced for both Acys over the concentration range of 2−60 mg/L and showed a linear response with a correlation coefficient of 0.9998.

Kinetic Models. A first-order reaction model has been applied for the description of degradation of Acys from various sources.[6,17,18] The model is expressed as

$$\ln(C_t/C_0) = -k \times t \quad (1)$$

where C_0 is the initial Acys content and C_t is the Acys content after treatment time t (min) at a given condition, k is the rate constant (min^{-1}), and the half-life ($t_{1/2}$) is the time needed for 50% degradation of Acys, which is calculated by the following equation:

$$t_{1/2} = -\ln 0.5 \times k^{-1} \quad (2)$$

A logistic model was found to be suitable to describe the degradation of organic compounds.[19,20] In this study, we used this model to analyze the relationship between energy density and Acys concentration during microwave treatment. The equation expression is

$$\ln(C_t/C_0) = A_1 + (A_2 - A_1)/[1 + (\omega/\omega_0)^p] \quad (3)$$

where C_0 is the initial Acys concentration and C_t is the Acys concentration after treatment time t (min) at a given condition, ω is the given energy density (W/mL), ω_0 is the energy density (W/mL) at the highest degradation rate of Acys, and A_1 and A_2 are constants.

Statistical Analysis. Statistical analysis of the experimental data was performed with ANOVA using Excel 2007 (Microcal Software, Inc., Northampton, MA, USA). Plotting and curve fitting were performed by Origin 8.0 (Origin Lab Co., Northampton, MA, USA). Difference was considered to be statistically significant at the level of $p < 0.05$. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Effect of Microwave Power on the Degradation Kinetics of Mv-3-glu and Mv-3,5-diglu. Hillmann et al. and Zhang et al. found the first-order reaction model was appropriate to simulate the degradation behavior of Acys in grape juice and blackberry juice during thermal treatment (70−90 °C).[21,22] Our results indicated that the degradation of both Acys also followed first-order kinetics under microwave treatment (Figure 1). Moreover, the degradation rate of Acys

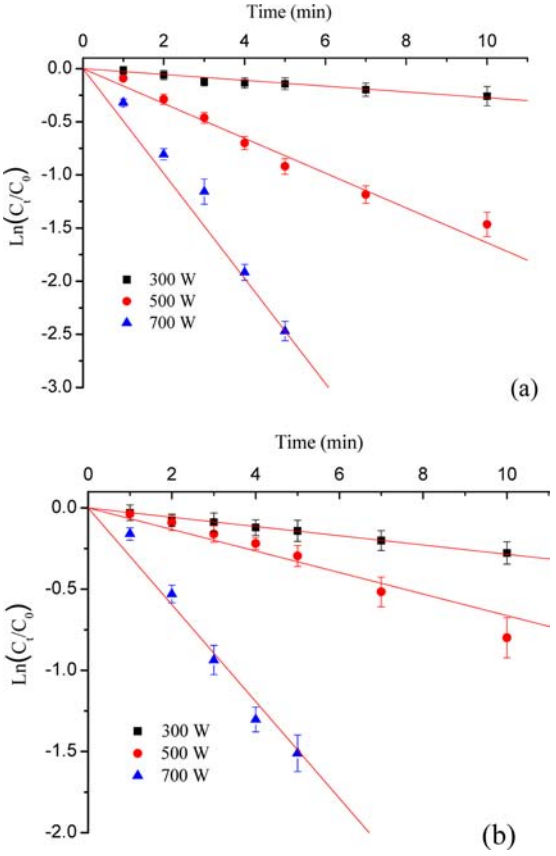

Figure 1. Degradation kinetics analysis of Mv-3-glu (a) and Mv-3,5-diglu (b) exposed to microwave treatment. pH 3.5 and anthocyanin concentration = 25 mg/L.

increased with the increase of the microwave power ($p < 0.05$). The deduced parameters (Table 1) showed that the degradation rate k values of Mv-3-glu and Mv-3,5-diglu increased and $t_{1/2}$ declined with microwave power. Under microwave treatment at 700 W, the k values of Mv-3-glu and Mv-3, 5-diglu were 3−17 and 4−11 times faster than at 500 and 300 W, whereas $t_{1/2}$ values were 66−94 and 78−91% decreased relative to those at 500 and 300 W, respectively.

Compared with the k ($(1.63-4.70) \times 10^{-3}$ min^{-1}) in the degradation of total Acys contents in grape juice[21] and $t_{1/2}$ (9.48−3.54 h) in the degradation of Cy-3-*O*-dioxalyl-glucoside in blackberry juice[22] during heating from 70 to 90 °C for 10 h, the degradation rates of Mv-3-glu and Mv-3,5-diglu under microwave treatment at 700 W are much faster. This means that microwave treatment could accelerate the degradation of Acys.

Furthermore, it could be observed that the degradation of Mv-3-glu was easier than that of Mv-3,5-diglu, indicating the stability of Mv-3,5-diglu is higher than that of Mv-3-glu. This is

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Table 1. Degradation Kinetics Parameters of Mv-3-glu and Mv-3,5-diglu under different Microwave Treatments

monomeric anthocyanin (fixed conditions)	varied conditions	equation	R^2	k (min^{-1})	$t_{1/2}$ (min)
Mv-3-glu	300 W, MT[a]	$y = -2.850 \times 10^{-2}t$	0.9873	2.850×10^{-2}	24.3
(pH 3.5, 25 mg/L)	500 W, MT	$y = -1.639 \times 10^{-1}t$	0.9684	1.639×10^{-1}	4.2
	700 W, MT	$y = -4.937 \times 10^{-1}t$	0.9606	4.937×10^{-1}	1.4
Mv-3,5-diglu	300 W, MT	$y = -2.735 \times 10^{-2}t$	0.9973	2.735×10^{-2}	25.3
(pH 3.5, 25 mg/L)	500 W, MT	$y = -6.629 \times 10^{-2}t$	0.9478	6.629×10^{-2}	10.5
	700 W, MT	$y = -2.981 \times 10^{-1}t$	0.9461	2.981×10^{-1}	2.3
Mv-3-glu	70 °C, MT	$y = -1.299 \times 10^{-2}t$	0.9916	1.299×10^{-2}	53.4
(500 W, pH 3.5, 25 mg/L)	100 °C, MT	$y = -1.475 \times 10^{-1}t$	0.9475	1.475×10^{-1}	4.7
	98 ± 2 °C water bath	$y = -5.221 \times 10^{-3}t$	0.9858	5.221×10^{-3}	120.8
Mv-3,5-diglu	70 °C, MT	$y = -9.782 \times 10^{-3}t$	0.9961	9.782×10^{-3}	70.9
(500 W, pH 3.5, 25 mg/L)	100 °C, MT	$y = -6.212 \times 10^{-2}t$	0.9684	6.212×10^{-2}	11.2
	98 ± 2 °C water bath	$y = -3.841 \times 10^{-3}t$	0.9881	3.841×10^{-3}	211.3
Mv-3-glu	pH 1.5, MT	$y = -2.471 \times 10^{-1}t$	0.9895	2.471×10^{-1}	2.7
(700 W, 25 mg/L)	pH 3.5, MT	$y = -4.483 \times 10^{-1}$t	0.9606	4.483×10^{-1}	1.5
	pH 4.5, MT	$y = -5.474 \times 10^{-1}t$	0.9932	5.474×10^{-1}	1.4
Mv-3,5-diglu	pH 1.5, MT	$y = -2.072 \times 10^{-1}t$	0.9371	2.072×10^{-1}	3.3
(700 W, 25 mg/L)	pH 3.5, MT	$y = -2.942 \times 10^{-1}t$	0.9461	2.942×10^{-1}	2.4
	pH 4.5, MT	$y = -3.584 \times 10^{-1}t$	0.9654	3.584×10^{-1}	1.9
Mv-3-glu	25 mg/L, MT	$y = -4.732 \times 10^{-1}$t	0.9634	4.732×10^{-1}	1.5
(700 W, pH 3.5)	50 mg/L, MT	$y = -5.233 \times 10^{-1}t$	0.9881	5.233×10^{-1}	1.3
Mv-3,5-diglu	25 mg/L, MT	$y = -3.031 \times 10^{-1}$t	0.9605	3.031×10^{-1}	2.2
(700 W, pH 3.5)	50 mg/L, MT	$y = -3.870 \times 10^{-1}t$	0.9878	3.870×10^{-1}	1.8

[a]MT, microwave treatment.

similar to the previous report that the Acys containing disaccharide were more stable than monosaccharidic Acys in blood orange juice.[23] Greater numbers of sugar moieties attached to the phenolic molecule make the structures more complex and, thus, enhance the stability of Acys.[6]

Effect of Energy Density on the Degradation Kinetics of Mv-3-glu and Mv-3,5-diglu. Energy density is defined as the microwave irradiation energy per unit of solvent volume for a given unit of time (W/mL) by Li et al.[13] The changes of Mv-3-glu and Mv-3,5-diglu concentrations with energy density are shown in Figure 2. Their relationship could be fitted well by the logistic function ($R^2 > 0.98$). The concentrations of both Acys declined rapidly when the energy density reached over 60 W/mL ($p < 0.05$). This was in agreement with the result that the yield of Acys extracted from grape skin reached at the maximum under 49.13 W/mL and then declined with the increase of energy density during MAE.[13] This means the choice of suitable energy density is necessary for the protection of certain bioactive compounds from microwave treatment.

To compare the stabilities of Mv-3-glu and Mv-3,5-diglu, the values of energy density were calculated as being 125.18 and 136.50 W/mL respectively, when their degradation rate reached the maximum. This is consistent with Mv-3,5-diglu being more resistant to the microwave treatment than Mv-3-glu.

Effect of Temperature on the Degradation Kinetics of Mv-3-glu and Mv-3,5-diglu. Figure 3 shows that increase of temperature could result in significant degradation of Mv-3-glu and Mv-3,5-diglu ($p < 0.05$). Similarly, the degradation kinetics was well simulated by the first-order reaction model ($R^2 > 0.94$), and the parameters are listed in Table 1. Under treatments at 100 °C the k values of Mv-3-glu and Mv-3,5-diglu were 11- and 6-fold higher than at 70 °C, respectively, and the $t_{1/2}$ values were $^1/_{10}$ and $^1/_5$ less than at 70 °C. On the one hand, this suggested the microwave thermal effect acts on the degradation of Acys, because this observation was similar to the result in the thermal treatment reported by Hillmann et al.[21] Chen[24] proposed both endothermic reactions, the hydrolysis reaction and the ring-opening reaction, on polymethoxy derivatives of 2-phenylbenzopyrylium probably contributed to the degradation of Acys. On the other hand, both Acys degraded under microwave treatment much more rapidly than under thermal treatment (Table 1). For the degradation of Mv-3-glu and Mv-3,5-diglu, their k values under microwave treatments at 100 °C were 257 and 19 times faster than those under heating in a 98 ± 2 °C water bath, and their t values under microwave treatment at 100 °C decreased by 96 and 95% relative to those under heating in a 98 ± 2 °C water bath. The obvious difference between conventional thermal treatments and microwave treatments might be related to microwave effect. It is well-known that microwaves consist of an electric field and a magnetic field, which can penetrate into certain materials and interact with polar components to generate heat. The microwave energy acted directly on the polar molecules by ionic conduction and dipole rotation, so the microwave power acts as a driving force to destroy the structure of Acys molecules.[25] Therefore, Acys degraded more rapidly under microwave treatment than under heating in a water bath at the same temperature.

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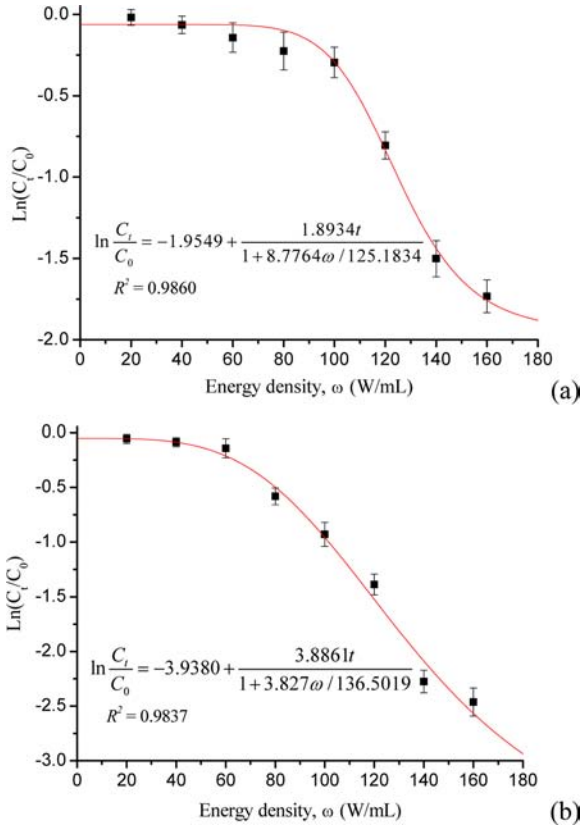

Figure 2. Correlation between microwave energy density and degradation of Mv-3-glu (a) and Mv-3,5-diglu (b). pH 3.5 and anthocyanin concentration = 25 mg/L.

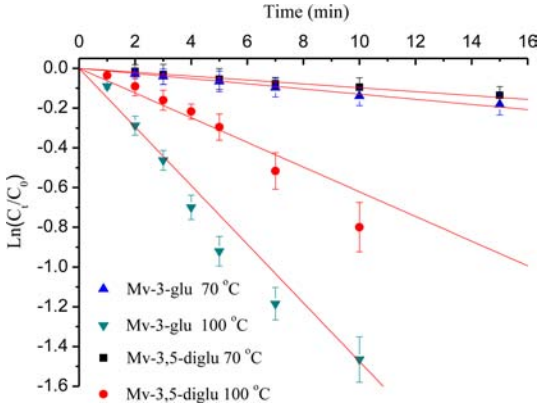

Figure 3. Effect of temperature on the degradation kinetics of Mv-3-glu and Mv-3,5-diglu exposed to microwave. Microwave power = 500 W, pH 3.5, and anthocyanin concentration = 25 mg/L.

Effect of pH on the Degradation Kinetics of Mv-3-glu and Mv-3,5-diglu. The stability of Acys is dependent on the pH of the solution. The results indicated that the degradations of Mv-3-glu and Mv-3,5-diglu were fitted to first-order kinetics ($R^2 > 0.96$), whereas their degradation rates are significantly different under the microwave treatments at different pH levels ($p < 0.05$) (Figure 4). The kinetics parameters (Table 1) showed that the k values increased and $t_{1/2}$ declined when the pH rose from 1.5 to 4.5. For the degradation of Mv-3-glu, the k value at pH 3.5 was about 2 times faster than that at pH 1.5, and the $t_{1/2}$ at pH 3.5 decreased 2 times from that at pH 1.5. A similar effect of pH on the degradation of Mv-3,5-diglu was

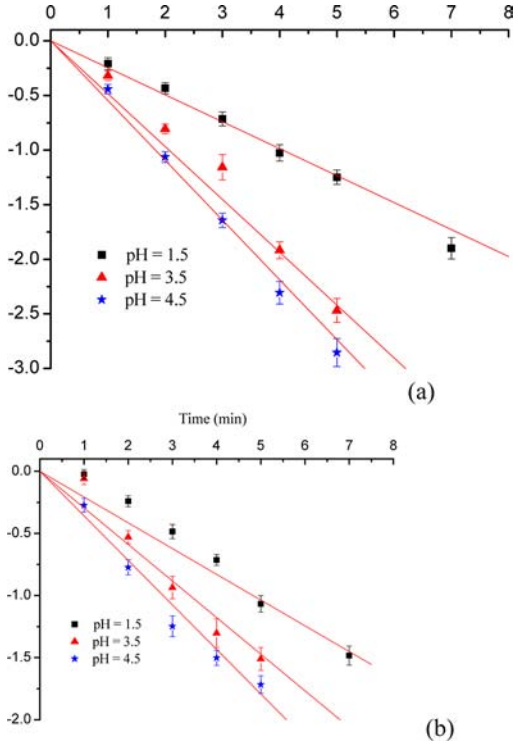

Figure 4. Effect of pH on the degradation kinetics of Mv-3-glu (a) and Mv-3,5-diglu (b) exposed to microwave. Microwave power = 700 W and initial concentration = 25 mg/L.

found. This was in agreement with previous studies.[26,27] Sun et al.[28] studied cyanin-3-sophoroside degradation during thermal treatment and supposed that the different effect on the Acys degradation between pH 1.5 and 3.5 was attributed to the difference in degradation pathways. As the first degradation step, the hydrolysis of the sugar moiety or opening of the heterocyclic ring occurred at pH 1.5 or 3.5, respectively. At a lower pH level, Acys exist primarily in the form of flavylium cations, which have good stability and are harder to open than the heterocyclic ring.[29] Increasing pH values caused rapid loss of the proton, producing quinonoidal base forms, which are more easily degraded by opening of the heterocyclic ring.[30] The influence of pH on the degradation pathways of Acys under microwave treatment should be investigated in the future.

Effect of the Initial Concentration of Acys on the Degradation Kinetics of Mv-3-glu and Mv-3,5-diglu. The initial concentration of Acys influenced significantly the degradation of Acys during microwave treatment ($p < 0.05$) (Figure 5). The degradation rate of Mv-3-glu at 50 mg/L concentration level was twice as fast as that at 25 mg/L, and $t_{1/2}$ showed an 11% decrease relative to that at 25 mg/L (Table 1). Kırca et al.[27] and Hojjatpanah et al.[31] also found that the degradation rate of total Acys increased during the concentration of blood orange juice and black mulberries juice, respectively. Because a high concentration of the substrate could speed the reaction process, the degradation rate of Acys increased at the higher initial concentration level.

In conclusion, the degradation of Mv-3-glu and Mv-3,5-diglu followed first-order kinetics under microwave treatment. The microwave power, energy density, temperature, pH value, and initial concentration of Acys have a significant influence on the degradation of Mv-3-glu and Mv-3,5-diglu. In addition, the stability of Acys was dependent on the structure of Acys. Mv-3-

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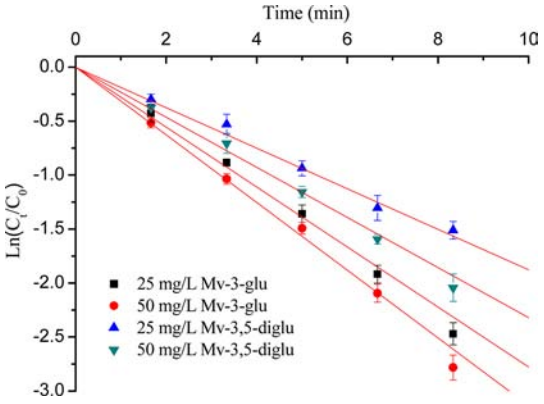

Figure 5. Effect of initial concentration on the degradation kinetics of Mv-3-glu and Mv-3,5-diglu exposed to microwave. Microwave power = 700 W and pH 3.5.

glu was more susceptible to the microwave treatment than Mv-3,5-diglu. This study provides a guide for the application of microwave technology in the extraction of bioactive compounds and sterilization of food. Nevertheless, it is quite likely that the degradation pathway of Acys exposed to microwave is different from that under conventional heating. Therefore, the detailed degradation pathway of Acys should be investigated in the future. Moreover, it should be pointed out that all data were obtained in an aqueous solution model system in this study, and the matrix effect on the degradation of Acys in practical food systems should be considered.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

Acys, anthocyanins; Mv-3-glu, malvidin-3-glucoside; Mv-3,5-diglu, malvidin-3,5-diglucoside; PEF, pulsed electric field; MAE, microwave-assisted extraction; UAE, ultrasound-assisted extraction; HPLC, high-performance liquid chromatography; DAD, diode array detector

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