KMITL Sci. Tech. J. Vol. 7 No. S2 Nov. 2007

FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF KRACHAI-DUM (*Kaempferia parviflora*) WINE

Sukanda Vichitphan[*1,2], Kanit Vichitphan[1,2]
and Prapussara Sirikhansaeng[2,3]

[1]Department of Biotechnology, Khon Kaen University, Khon Kaen, 40002, Thailand
[2]Fermentation Research Center for Value Added Agricultural Products,
Khon Kaen University, Khon Kaen, 40002, Thailand
[3]Graduate School, Khon Kaen University, Khon Kaen, 40002, Thailand

ABSTRACT

Wine fermentation was conducted with three different preparations of Krachai-Dum using tamarind juice as a base composition. In the first preparation, pieces of sliced Krachai-Dum were fermented with tamarind juice. For the second preparation, Krachai-Dum extracts were mixed with tamarind juice and used for fermentation process. The third preparation was performed by adding pieces of sliced Krachai-Dum into fermented tamarind wines during aging process. Three types of Krachai-Dum; unpeeled Krachai-Dum slices, peeled Krachai-Dum slices and Krachai-Dum skin were used separately to prepare Krachai-Dum wine. Tamarind juice without Krachai-Dum was used as control. After fermentation, Krachai-Dum wines were kept at 4 $^{\circ}$C for aging process. Total phenolic compounds, flavonoid contents and antioxidant activity were examined in all samples. Total phenolic compounds ranged from 107.00 ± 9.27 to 306.62 ± 6.21 mgGAE/l. Seven flavonoids were detected in all preparations of Krachai-Dum wine except in the control as follows: 5-hydroxy-7-methoxyflavone, 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, 5-hydroxy-3,7,4′-trimethoxy flavone, 5,7,4′-trimethoxyflavone, 5-hydroxy-3,7-dimethoxyflavone, 3,5,7,3′,4′-penta methoxyflavone and 5,7-dimethoxyflavone. Among these, 3,5,7,3′,4′-pentamethoxyflavone was the major component found in all samples and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone was detected in lowest amount. The total flavonoid contents ranged from 9.59 to 24.41 mg/100ml wine. The wine using Krachai-Dum skin showed higher flavonoid contents than those using peeled or unpeeled pieces. The highest total flavonoid content (24.41 mg/100ml wine) was found in Krachai-Dum wine prepared by adding Krachai-Dum skin to tamarind wine during the aging process of 4 months. *In vitro* antioxidant activity of wine samples before and after 1, 2, 3, 4 months of aging process was examined by DPPH and FRAP methods. Antioxidant activity of wine samples by both methods showed similar results that the activities are higher in Krachai-Dum wine prepared by fermented peeled or unpeeled Krachai-Dum with tamarind juice than those prepared by Krachai-Dum extracts. Phenolic contents and antioxidant activities in all conditions were in the same direction. The results suggested that the phenolic compounds may play an important role in the antioxidant effect in Krachai-Dum wine. However, the total flavonoid content did not correlate with the antioxidant activity of the sample wines.

KEYWORDS: Krachai-Dum wine, *Kaempferia parviflora,* antioxidant, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP)

*Corresponding author: Tel: +66 43 362121 Fax: +66 43 362121
E-mail: sukanda@kku.ac.th

1. INTRODUCTION

Krachai-Dum (*Kaempferia parviflora* Wall. Ex Baker) is a herbaceous plant belonging to the Zingiberaceae family. Since ancient time, it has traditionally been used as a health promoting, stimulating and vitalizing agent [1-2].

Some flavonoids were isolated from the rhizome of *K. parviflora* and its flavonoid constituents have been reported to display various pharmacological effects. Among flavonoids isolated, 5,7,4′-trimethoxyflavone and 5,7,3′,4′-tetramethoxyflavone exhibited antiplasmodial activity against *Plasmodium falciparum* [1]. Moreover, 3,5,7,4′-tetramethoxyflavone and 5,7,4′-trimethoxyflavone possessed antifungal activity against *Candida albicans* and also showed mild antimycobacterial activity [1]. Furthermore, ethanolic extract of Krachai-Dum was found to be active against allergic reaction [3] and had anti-gastric ulcer activity by experimental models in rat [4]. In addition, the ethanol extract and their flavone constituents from *K. parviflora* tincture (called Ya-dong in Thailand) have been shown to inhibit the P-glycoprotein function in a transfected epithelial cell line which may be mainly attributed to 3,5,7,3′4′-pentamethoxyflavone [5]. Recently, Krachai-Dum rhizome extracts also have excellent antioxidant potential, as evidenced by their ability to scavenge free redicals [6].

Krachai-Dum wine has been produced in northeastern part of Thailand. The believing to help relieve impotent symptoms of Krachai-Dum wine could promote its use and have a significant economic impact. Therefore, the identification of the Krachai-Dum wines' health-beneficial chemical components (phenolic compounds, flavonoids) as an antioxidant capability has been determined in this study.

In the present study, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) method have been employed for detection of the antioxidant activity of active compounds commonly present in wines. The spectrophotometric technique employs the 2,2-diphenyl-1-picrylhydrazyl free radical ($DPPH^{\bullet}$) showing a characteristic UV–vis spectrum with a maximum of absorbance close to 517 nm in methanol [7-10]. Antioxidant compounds can donate hydrogen atom to DPPH free radical and DPPH free radical were then stabilized. Color of DPPH solution changed from purple to pale yellow. The exceed of DPPH free radical from the reaction can absorb visible light. Therefore the absorbance can be measured by spectroscopic method at 518 nm [11].

The total antioxidant potential of wines were determined using FRAP assay as a measure of ''antioxidant power'' which measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{2+}-tripyridyltriazine compound from colorless oxidized Fe^{3+} form by the action of electron donating antioxidants [9].

The aim of this study was to investigate the total content of phenolic compounds in different preparations of Krachai-Dum wines and to determine and compare the antioxidant capacity of these samples by two commonly used spectrophotometric methods: DPPH and FRAP assay. In addition, flavonoid components were also determined by HPLC. The correlation of total phenolic compounds, flavonoid contents and antioxidant activities were studied.

2. MATERIALS AND METHODS

2.1 Fermentation process

Black Krachai-Dum rhizomes were obtained from Loei province, Thailand. All fermentation conditions were carried out in diluted (approximately 1:16) tamarind juice to gain 6 g/l total acidity. The juice was adjusted to 22° Brix of total soluble solid by supplementing with sucrose prior to fermentation. Krachai-Dum rhizomes were washed, peeled or unpeeled and sliced into small pieces and used in fermentation juice for wine preparation at 1:30 (w/w) ratio. The

fermentation was carried out at room temperature (~ 27° C) for 20 days using 1×10^6 cell ml^{-1} of *Saccharomyces cerivisiae*, Sweden strain. Samples were racked and kept in refrigerator (~ 4-5° C) for aging process and antioxidant acitivity assays. A total of 9 wine samples used in this study included 8 types of Krachai-Dum wine with different preparations and a control without Krachai-Dum. Krachai-Dum wine fermentation was conducted with three different conditions using tamarind juice as a base composition. In the first condition, pieces of sliced Krachai-Dum were mixed and fermented with tamarind juice. Secondly, Krachai-Dum extracts (extracted with 70° C hot water for 30 min and filtrated) were mixed with tamarind juice and used for fermentation process. The third condition was performed by adding pieces of sliced Krachai-Dum into fermented tamarind wines during aging process. Three types of Krachai-Dum pieces: peeled Krachai-Dum slices, Krachai-Dum skin and unpeeled Krachai-Dum slices were used separately to prepare Krachai-Dum wine. Tamarind juice without Krachai-Dum served as control. All fermentation experiments were performed in duplicates.

2.2 Sample analysis

2.2.1 Flavonoid content determination by HPLC method

Wine samples used for flavonoid content determination were samples of each preparation after 4 months of aging. HPLC analysis was carried out using a Jusco PU-980 Intelligent HPLC system (Jasco, Tokyo, Japan). The flavone derivatives were prepared in a concentration of 30 μM in DMSO and 50 μl was injected onto a reverse-phase TSKgel ODS-80TM column (4.6 × 100 mm, I.D.). The isocratic mobile phase consisted of 0.1% (v/v) trichloroacetic acid and methanol (45:55 % v/v) and flow rate was 1 ml/min. Detection was performed by measuring absorbance at 254 nm using a Jasco UV-970 Intelligent UV/VIS detector [5].

2.2.2 Determination of total phenolic compounds

Total phenolic compounds in samples were determined spectrophotometrically with Folin-Ciocalteu reagent using gallic acid as standard [7]. One milliliter of wine samples in a volumetric flask was diluted with distilled water (46 ml). One milliliter of Folin-Ciocalteu reagent was added and the content in the flask was mixed thoroughly. After 3 min, 3 ml of 2g/100ml Na_2CO_3 was added then the mixture was allowed to stand for 2h with intermitten shaking. The absorbance was measured at 760 nm using spectrophotometer. The concentration of total phenolic compounds was determined with microgram of gallic acid.

2.2.3 Antioxidant activity assay

(1) DPPH method

The 0.1 mmol/l solution of DPPH radical in methanol was prepared and 2 ml of this solution was added to 2ml of water solution containing 100 μl of wine samples. After 30 min of preparation, absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Different concentrations were tested using gallic acid (10-60 mg/l) as a standard for calibration, and expressed as mg gallic acid equivalents per litre of sample (mgGAE/l) [7-8].

(2) FRAP method

The working FRAP reagent was prepared by mixing 10 volumes of 1.0 mol/l acetate buffer, pH 3.6 with 1 volume of 10 mmol/l TPTZ (2,4,6-tripyridyl-S-triazine) in 40 mmol/l hydrochloric acid and with 1 volume of 20 mmol/l ferric chloride. In a reaction tube, 100 μl of sample solution of and 300 μl of deionized water were added into 3 ml of FRAP reagent. Absorbance was measured after 8 min. A standard curve was prepared using different concentrations of $FeSO_4.7H_2O$ (100–1,000 μmol/l). The antioxidant efficiency of the sample solution was calculated with reference to the standard curve given by a Fe^{2+} solution of known concentration. Ferric reducing power of the sample was expressed in mol Fe^{2+}/l. [9].

3. RESULTS AND DISCUSSION

3.1 Flavonoid content determination

The flavonoid content of Krachai-Dum wine samples was shown in Table 1 and HPLC chromatogram was shown in Figure 1. From Table 1, seven flavonoids were detected in all conditions of Krachai-Dum wine excepted in the control. The compounds detected are as follows: 3,5,7,3′,4′-pentamethoxyflavone, 5,7,4′-trimethoxyflavone, 5,7-dimethoxyflavone, 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, 5-hydroxy-7-methoxyflavone, 5-hydroxy-3,7,4′-trimethoxyflavone and 5-hydroxy-3,7-dimethoxyflavone. Among these, 3,5,7,3′,4′-pentamethoxyflavone was the major component found in all samples and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone was detected in the lowest amount in all samples. The total flavonoid contents in these samples ranged from 9.59 to 24.41 mg/100ml wine. The content of total flavonoid after 4 months of aging decrease in the order: C8, C2, C7, C3, C4, C5, C6 and C1. The results showed that conditions using Krachai-Dum skin (C8 and C2) gave higher flavonoid contents than those using peeled or unpeeled pieces. The highest flavonoid contents of 24.41 mg/100 ml were found in C8 (Krachai-Dum wine prepared by the addition of Krachai-Dum skin to tamarind wine during aging process) and the lowest flavonoid contents of 9.59 mg/100 ml wine was found in C1 (Krachai-Dum wine from peeled Krachai-Dum with tamarind juice). These seven flavonoid compounds were not detected in the control. The results reveals that Krachai-Dum skin contains more flavonoid content than the flesh part. The content of flavonoid in the samples also depends on the preparation methodology of Krachai-Dum wine. The total flavonoid content in the wine samples prepared by fermenting Krachai-Dum pieces with tamarind juice was signicantly higher than those prepared by using Krachai-Dum extract.

3.2 Total phenolic compounds determination

The content of phenolic compounds determined by the Folin-Ciocalteu method for the different analyzed wines is shown in Figure 2. Total phenolic compounds ranged from 107.00 ± 9.27 to 306.62 ± 6.21 mgGAE/l. Total phenolic contents after aging for 1 to 4 months of each sample were not significantly different in all samples. However, the phenolic compounds of C7 (peeled Krachai-Dum slices in fermented tamarind wines during aging process) and C8 (Krachai-Dum skin in fermented tamarind wines during aging process) after aging were much higher than those of the same sample before aging. The reason is that C7 and C8 before aging did not contain Krachai-Dum pieces thus the phenolic contents were similar to that of the control. The highest total phenolic content was found in C1 (peeled Krachai-Dum slices in tamarind juice) and C7 whereas the lowest phenolic content was found in Control (tamarind wine without Krachai-Dum). Comparing among the samples containing Krachai-Dum (C1-C8), the lowest phenolic content was found in C8. The results showed that the increase of phenolic contents in C1-C8 derived from Krachai-Dum and Krachai-Dum flesh contained higher total phenolic content than the skin. The content of total phenolics after aging decreased in the order: C1=C7, C3, C8, C4, C6, C2, C5 with the lowest value found in control (107 mgGAE/l).

3.3 Antioxidant activity

From Figures 3 and 4, *in vitro* antioxidant activity of wine samples before and after 1, 2, 3, 4 months of aging process was examined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and ferric reducing antioxidant power (FRAP) method. Antioxidant activity of wine samples by both methods showed similar results that the activities are higher in Krachai-Dum wine prepared by fermented peeled or unpeeled Krachai-Dum with tamarind juice than those prepared by Krachai-Dum extracts. C1 (Peeled Krachai-Dum slices fermented in tamarind juice) and C3 (Unpeeled Krachai-Dum fermented in tamarind juice) showed the similar antioxidant activity. The results showed that the increase of antioxidant activity in C1-C8 derived from Krachai-Dum and Krachai-

Dum flesh contained higher antioxidant activity than the skin. In C7 and C8, antioxidant activities increased when the aging time increased. The average of high reducing power (FRAP) and free radical scavenging activity of samples was almost 2 to 3.5-folds higher than those of the control.

A high positive correlation between phenolic compounds and antioxidant activity was proved [9]. Results in this study indicate that Krachai-Dum wines exhibited high antioxidant power and free radical scavenging activity and ferric reducing antioxidant power were correlated with total phenolic content. The phenolic compounds detected in red wines and white wine from grapes were as high as 1000-4000 and 200-400 mg/l, respectively [12-13]. Considering the content of phenolic compounds of samples and their antioxidant activities, it may be concluded that the antioxidant activity of Krachai-Dum wine is similar to white wine and phenolic compounds may play a very important role in the antioxidant function. In contrast, the flavonoid content does not correlate with antioxidant activity which may be due to the low amount of flavonoids in Krachai-Dum wine and other types of phenolic compounds are responsible for antioxidant activity.

Table 1 Yield and flavonoid content of Krachai-Dum wine samples

Samples/ (%Yield)	Flavonoid content (compounds ± SD (mg/wine 100 ml)							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**Total**
Control (3.75)	ND	ND	ND	ND	ND	ND	ND	ND
C1 (4.20)	5.186±0.0190	2.416±0.0007	1.180±0.0013	0.068±0.0005	0.239±0.0012	0.147±0.0010	0.355±0.0005	9.590
C2 (4.65)	7.739±0.0050	3.334±0.0008	1.708±0.0024	0.099±0.0004	0.405±0.0026	0.184±0.0015	0.466±0.0022	13.940
C3 (4.35)	6.220±0.0030	2.963±0.0004	1.511±0.0007	0.065±0.0011	0.271±0.0018	0.124±0.0004	0.440±0.0008	11.594
C4 (6.55)	6.471±0.0001	2.804±0.0003	1.383±0.0041	0.043±0.0005	0.181±0.0004	0.090±0.0003	0.299±0.0012	11.270
C5 (5.48)	7.970±0.0020	2.390±0.0000	1.664±0.0022	0.053±0.0005	0.211±0.0005	0.114±0.0005	0.295±0.0003	12.697
C6 (6.02)	6.083±0.0007	2.164±0.0006	1.161±0.0003	0.026±0.0014	0.122±0.0014	0.044±0.0005	0.156±0.0016	9.760
C7 (4.15)	7.179±0.0015	3.526±0.0003	1.701±0.0008	0.099±0.0005	0.313±0.0002	0.230±0.0003	0.357±0.0012	13.400
C8 (5.16)	13.843±0.0056	5.608±0.0033	2.870±0.0007	0.195±0.0017	0.637±0.0012	0.382±0.0007	0.866±0.0011	24.410

ND = The compound was not detected.

Compound: A = 3,5,7,3′,4′-pentamethoxyflavone; B = 5,7,4′-trimethoxyflavone; C = 5,7-dimethoxyflavone; D = 5-hydroxy-3,7,3′,4′-tetramethoxyflavone; E = 5-hydroxy-7-methoxyflavone; F = 5-hydroxy-3,7,4′-trimethoxy flavone; G = 5-hydroxy-3,7-dimethoxyflavone. Control = Tamarind juice without Krachai-Dum, C1 = Peeled Krachai-Dum slices fermented in tamarind juice, C2 = Krachai-Dum skin fermented in tamarind juice, C3 = Unpeeled Krachai-Dum fermented in tamarind juice, C4 = Peeled Krachai-Dum extracts in tamarind juice, C5 = Unpeeled Krachai-Dum extracts in tamarind juice, C6 = Krachai-Dum skin extracts in tamarind juice, C7 = Pieces of peeled Krachai-Dum slices in fermented tamarind wines during aging process, C8 = Pieces of Krachai-Dum skin in fermented tamarind wines during aging process.

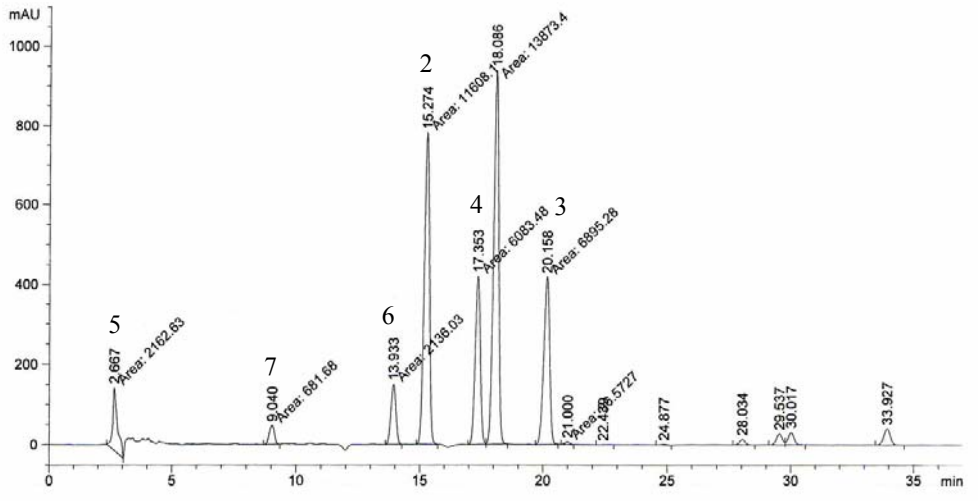

Figure 1 HPLC chromatogram of flavanoids extracted from Krachai-Dum wine (aging for 4 months). Peak identification (compound nos.): 1 = 3,5,7,3′,4′-pentamethoxyflavone; 2 = 5,7,4′-trimethoxyflavone; 3 = 5,7-dimethoxyflavone; 4 = 5-hydroxy-3,7-dimethoxyflavone; 5 = 5-hydroxy-7-methoxyflavone; 6 = 5-hydroxy-3,7,4′-trimethoxyflavone; 7 = 5-hydroxy-3,7,3′,4′-tetra methoxyflavone

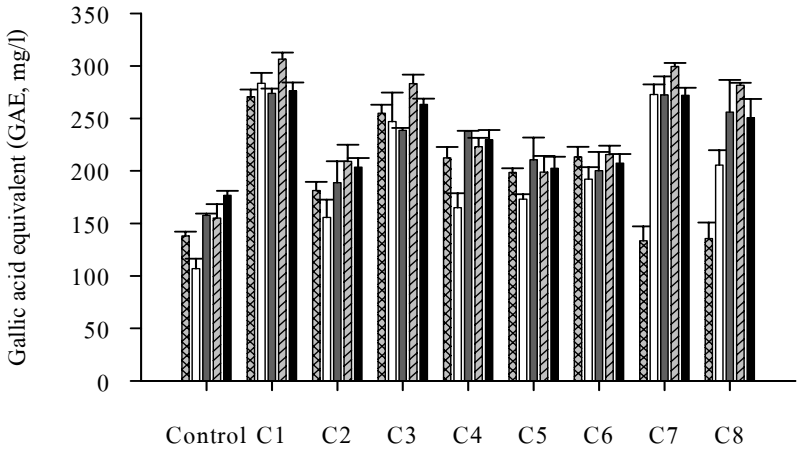

Figure 2 Total phenolic of wines ▨ before aging, □ 1 month aging, ▩ 2 months aging ▨ ; 3 months aging and ■ ; 4 months aging. C1-C8 are the same as described in Table 1.

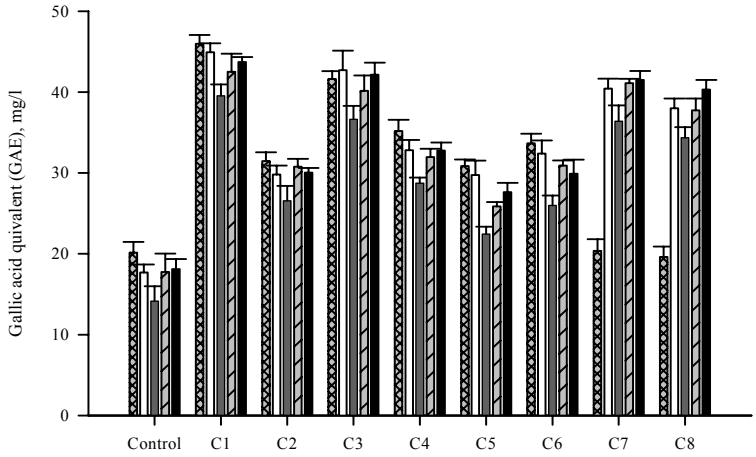

Figure 3 Antioxidant activity of wines by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method expressed as mgGAE/l ; before aging, ; 1 month aging, 2 months aging and ; 3 months aging ; 4 months aging. C1-C8 are the same as described in Table 1.

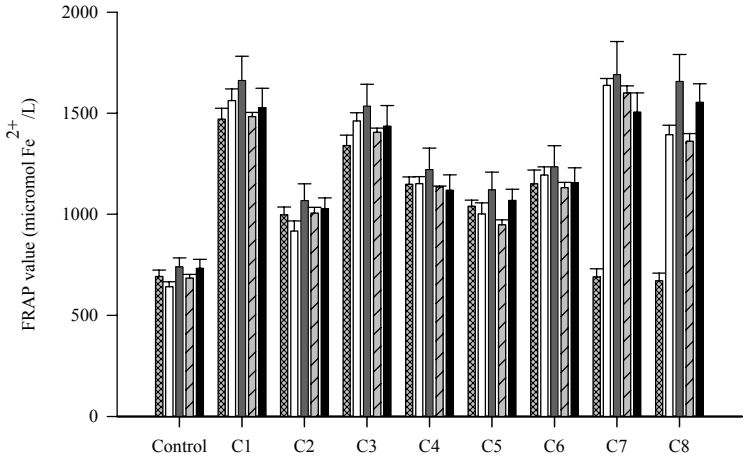

Figure 4 Antioxidant activity of wines by ferric reducing antioxidant power (FRAP) method e xpressed as mgGAE/l; before aging, ; 1 month aging, 2 months aging 3 months aging and ; 4 months aging. C1-C8 are the same as described in Table 1.

4. CONCLUSION

Seven flavonoid compounds: 5-hydroxy-7-methoxyflavone, 5-hydroxy-3,7,3′,4′ tetramethoxyflavone, 5-hydroxy-3,7,4′-trimethoxyflavone, 5,7,4′-trimethoxyflavone, 5-hydroxy-3,7-dimethoxyflavone, 3,5,7,3′,4′-pentamethoxyflavone and 5,7-dimethoxyflavone were detected in Krachai-Dum rhizomes used for fermentation process. Krachai-Dum skin showed higher flavonoid contents

than peeled or unpeeled Krachai-Dum. Antioxidant activity by DPPH and FRAP methods in all conditions were in the same direction. The phenolic compounds may play an important role in the antioxidant effect in Krachai-Dum wine. However, the total flavonoid content did not correlate with the antioxidant activity of the sample wines. Moreover, identification of specific components other than flavonoid should be further investigated.

5. ACKNOWLEDGEMENTS

The authors wish to thank Ms. Khaetthareeya Sutthanut, Faculty of Phamaceutical Science, Khon Kaen University for her kind technical assistance for antioxidant activity assay, Mr. Vinai Sutonthangjai for providing Krachai-Dum rhizomes. This work was partially supported by a grant from Fermentation Research Center for Value Added Agricultural Products (FerVAAP), Khon Kaen University, Graduate School Funding, Khon Kaen University and Center for Research and Development of Herbal Health Products (CRD-HHP) , Khon Kaen University.

REFERENCES

[1] Yenjai, C., Prasanphen, K., Daodee, S., Wongpanich, V. and Kittakoop, P. **2004** Bioactive Flavonoids from *Kaempferia parviflora*, *Fitoterapia*, *75*, 89-92.

[2] Sennil, J., and Trichalee, M. **2003** *Krachai-Dum: Amazing Herb Book 2*. KMP. Siam Media, Bangkok: 42-50. (In Thai)

[3] Tewtrakul, S. and Subhadhirasakul, S. **2007** Anti-allergic Activity of Some Selected Plants in the Zingiberaceae Family, *Journal of Ethnopharmacology*, *109*, 535–538.

[4] Rujjanawate, C., Kanjanapothi, D., Amornlerdpison, D. and Pojanagaroon, S. **2005** Anti gastric Ulcer Effect of *Kaempferia parviflora, Journal of Ethnopharmacology*, *102*, 120-122.

[5] Patanasethanont, D., Nagai, J., Matsuura, C., Fukui, K., Suutthanut, K., Sripanidkulchai, B-O., Yumoto, R. and Takano, M. **2007** Modulation of Function of Multidrug Resistance Associated-proteins by *Kaempferia parviflora* Extracts and Their Components, *European Journal of Pharmacology*, *566*, 67-74.

[6] Pojanagaroon, S., and Rujjanawate, C. **2005** Effect of Internal Color of *Kaempferia parviflora* (Krachai-Dam) Rhizomes as Raw Materials for Wine Processing on Antioxidative Activity. Proceedings of 43rd Kasetsart University Annual Conference, Thailand, 1-4 February.

[7] Que, F., Mao, L., Zhu, C. and Xie, G. **2006** Antioxidant Properties of Chinese Yellow Wine, Its Concentrate and Volatiles, *Lebensmittel-Wissenschaft und Technologie (LWT)*, *39*, 111-117.

[8] Villaño, D., Fernández-Pachón, M.S., Troncoso, A.M. and García-Parrilla, M.C. **2006** Influence of Enological Practices on the Antioxidant Activity of Wines, *Food Chemistry*, *95*, 394-404.

[9] Katalinic´, V., Milos, M., Modun, D., Music´, I. and Boban, M. **2004** Antioxidant Effectiveness of Selected Wines in Comparision with (+)-Catechin, *Food Chemistry*, *86*, 593-600.

[10] Katalinic´, V., Modun, D., Music´, I. and Boban, M. **2005** Gender Differences in Antioxidant Capacity of Rat Tissues Determined by 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and Ferric Reducing Antioxidant Power (FRAP) Assays. *Comparative Biochemistry and Physiology*, *140* (Part C), 47-52.

[11] Milardovic, S., Ivekovic, D. and Grabaric´, B.S. **2005** A Novel Amperometric Method for Antioxidant Activity Determination Using DPPH Free Radical, *Bioelectrochemistry*, *68*,180-185.

[12] Minussi, R. C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastore,G. M., and Duran, N. **2003** Phenolic Compounds and Total Antioxidant Potential of Commercial Wines, *Food Chemistry, 82*, 409-416.

[13] Fernandez-Pachon, M. S., Villaono, D., Garacia-Parrilla, M.C., and Troncoso, A.M. **2004** Anioxidant Activity of Wines and Relation with Their Polyphenolic Composition, *Analytica Chimica Acta, 513*, 113-118