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An Analysis on Flavonoids, Phenolics and Organic Acids Contents in Brewed Red Wines of Both Non-Skin Contact and Skin Contact Fermentation Techniques of Mao Luang Ripe Fruits (*Antidesma bunius*) Harvested From Phupan Valley in Northeast Thailand

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Abstract: The experiment was carried out at the Department of Biotechnology, Faculty of Technology, Mahasarakham University, Northeast Thailand during the 2006. The study aimed to determine amounts of flavonoids, phenolics and organic acids in ripe fruits and brewed red wines of both non-skin contact and skin contact winemaking techniques where Mao Luang ripe fruits of both Fapratan and Sangkrow2 cultivars were used. The experiment was laid in a Completely Randomised Design (CRD) with four replications. The results showed that mean values of primary data of fresh Mao Luang ripe fruits on weight of 100 berries (g) and mean values of juice:solids, pH, total soluble solid (TSS, ^{0}brix), total organic acids (TOA, mg L^{-1}), TSS:TOA (%), total flavonoids contents (TFC, mg L^{-1}), total phenolic acids (TPA, mg L^{-1}), total procyanidins contents (TPC, mg L^{-1}) and reducing sugar (g L^{-1}) were 65.62, 3.28, 3.51, 16.50, 49.36, 28.10, 397.90, 76.04, 156.21 and 184.32, respectively. Skin contact Mao Luang red wine gave higher amounts of flavonoids, phenolic acids, anthocyanins of procyanidin B1 and procyanidin B2, organic acids than non-skin contact red wine. The differences were highly significant. Furthermore, ethanol (%) and total acidity (g L^{-1} citric acid) were much higher for skin contact wine than non-skin contact wine but a reverse was found with total soluble solids (^{0}brix), pH where non-skin contact wine gave higher mean values than skin contact wine.

Key words: HPLC-DAD, flavonoid, procyanidin B1, procyanidin B2, phenolic acids, organic acids, red wine, skin contact, non-skin contact

INTRODUCTION

It has been advocated that Mao Luang fruits (*Antidesma bunius*) of several orchard cultivars contained some certain amounts of bioactive substances, e.g., flavonoids, i.e., catechin, procyanidin B1 and procyanidin B2 and also two groups of organic acids, i.e., major and minor groups where these chemical compounds possess its important role as protective agents against fungus and UV irradiation. The chemical compounds also possess some significant effects on human health, particularly the healing of Coronary Heart Disease (CHD) and also the reduction of platelet aggregation, anti-oxidative, anti-carcinogenic and anti-cancer in man (Butkhup and Samappito, 2008; Samappito and Butkhup, 2008). Thus the use of Mao Luang ripe fruits for alcoholic wine makings should provide some important values in man's nutritive values and some medicinal properties. In wine makings, phenolic compounds and organic acids available in ripe fruits of any orchard crops are the essential elements needed to achieve stability, distinctive taste, colour, astringency, bitterness and flavour of any specific wine types (Atanasova *et al*., 2002). Phenolic acid (caffeic acid) had also been recognised as a strong antioxidant against human Low-Density Lipoprotein (LDL) oxidation as reported by Meyer *et al.* (1998). Red wines obviously possess high stability throughout its aging processes due to the availability of bioactive chemical compounds where the chemical compounds could possess positive effects on human health (Kuulasmaa *et al*., 2000; Stoclet *et al*., 2004). However, the technique in making wines has its important features on quality and the amounts of available bioactive substances. It has been stated by Spranger *et al.* (2004) with grape white wines, they stated that the wines being brewed by skin contact fermentation technique (maceration) gave significantly higher amount of phenolic compounds contents than non-skin contact fermentation technique, whilst Hernanz *et al.* (2007) reported that skin contact fermentation aided in an increase in a better flavour of wine than any other fermentation methods. Similarly, the study of Singleton and Trouslade (1983) on skin contact winemaking

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technique also revealed that skin contact technique could contribute more distinctive flavour of wine and it could exert positive health effects, which could be attributable to a moderate wine consumption where the finished wines achieve higher values of pH, potassium and total phenolics levels than non-skin contact brewing technique. The organic acids contents in wines normally include tartaric, malic and lactic acids where these organic acids monitor fermentation processes, balance flavour, taste and colour. Kerem *et al.* (2004) stated that organic acids, malic and lactic acids contained in the skin contact wines could also influence chemical stability, pH and also the quality of wines. Therefore, it may be of tangible value to carry out laboratory works on red winemaking with the use of both non-skin contact and skin contact techniques and then determine the amounts of phenolic acids, organic acids, flavonoids and other chemical compounds in juices of both brewed red wines in order to compare the attained results on the amounts of the chemical compounds of the two red wines since published data on these two different winemaking techniques being made from Mao Luang ripe fruits are limited.

MATERIALS AND METHODS

This laboratory experiment was carried out at the Department of Biotechnology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Northeast Thailand during the 2006. With winemaking techniques, the experiment was laid in a Completely Randomised Design (CRD) with four replications. The treatments used include non-skin contact (control) and skin contact winemaking techniques. The study aimed to determine the following parameters on ripe fruits and juices, i.e., (1) weight of mixed 100 berries of Fapratan and Sangkrow2 cultivars, (2) juice:solids, (3) pH, (4) total soluble solid (TSS,^{0}brix), (5) Total Organic Acids (TOA), (6) TSS:TOA (%), (7) total flavonoids contents (TFC), (8) total phenolic acids (TPA), (9) total procyanidins contents (TPC) and (10) reducing sugar (g L^{-1}). These 10 items were carried out with the use of a mixture of ripe Mao Luang fresh fruits (1:1 by weight) of both Fapratan and Sangkrow2 cultivars and an amount of 100 g of mixed berries was used for an analysis on primary data of No. 1 to 10. The measurements on items No. 3 to 10 were also applied to skin contact and non-skin contact young red wines (fermented altogether for 30 days) apart from the following measurement parameters, i.e., alcohol (% by vol.), density (measured at 20°C), volatile acidity (g L^{-1}), total acidity (g L^{-1}) and total SO_2 (mg L^{-1}). The measurements on flavonoids, phenolic acids, anthocyanins, organic acids, ethanol, TSS (^{0} brix), pH and total acidity (citric acid) were also carried out at days 1, 7, 15 and 30 after fermentation processes for both non-skin contact and skin contact Mao Luang red wines. The mean values of data attained with fresh ripe fruits of Mao Luang in Table 2 were not used for statistical calculations but the rest of other items of those non-skin contact and skin contact wines were used for statistical calculations (analysis of variance).

For this laboratory analysis, some required reagents and chemicals of High Performance Liquid Chromatography (HPLC) grade (Tedia Company, USA) such as methanol, acetonitrile and phosphoric acid were used. Other chemicals include gallic acid, (+)-catechin, (-)-epicatechin, rutin, procyanidin B1, caffeic acid, procyanidin B2, vanillic acid, myricetin, ellagic acid, resveratol, ferrulic acid, lutelin, quercetin, naringenin and kaempferol standards (Sigma Compant, USA) were also used. Deionized water was prepared with the use of a Milli-Qwater Purification System (Millipore, MA, USA). Standard stock solutions of phenolic compounds and organic acids were prepared in methanol and deionized water at concentrations of 0.50 and 50 g L^{-1}, respectively. All solution samples were filtered through with the use of 0.45 μm membrane filters (Millipore) and they were used for direct injections with the use of laboratory equipment namely a Reverse Phase-High Performance Liquid Chromatography (RP-HPLC).

Mao Luang ripe fruits of Fapratan and Sangkrow2 cultivars were harvested in the 2006 (October) from dipterocarp forest of Phupan Valley, Sakhon Nakhon Province, Northeast Thailand. Right after the harvest, an amount of approximately 120 kg of the Mao Luang ripe fruits of both cultivars were carefully taken into the laboratory at Mahasarakham University for fruit juices analysis and wine making processes. An amount of 50 g of fully ripe berries of each Mao Luang cultivar was separately taken out and then thoroughly mixed together for fresh juice analysis and then another 50 kg of ripe fruits of each cultivar were carefully mixed together by hand where laboratory sterilized gloves were used. The mixed amount of ripe fruits (100 kg) was divided into two batches, i.e., 50 kg were used for making skin contact wine and another 50 kg for non-skin contact wine. For non-skin contact winemaking, 50 kg of ripe fruits of both cultivars were mechanically pressed for juices alone, whilst another 50 kg of Mao Luang ripe fruits were used for skin contact winemaking. They were thoroughly crushed to attain juices and then the whole lot included skins and seeds were used for winemaking (Spranger *et al.*, 2004). Eight stainless steel tanks, each has a volume of 100 L, were used for the two wine brewing techniques. Each of them was allocated into their respective tanks and then each

brewing materials of Mao Luang fruits of each tank was added with cane sugar to attain a value of 20° brix with the use of a digital refractometer (Atago-Palette PR 101, Atago Co. Ltd., Itabashi-Ku, Tokyo, Japan) and then added with sulphur dioxide (metabisulphite) at a rate of 40 mg L^{-1} and racked for 24 h before included with a commercial yeast of *Saccharomyces cerevisiae* and finally the 8 tanks of wines were kept for brewing at 25°C for 30 days. At the end of the brewing process of alcoholic fermentation, the wines were racked then an amount of 50 mg L^{-1} of sulphur dioxide was added again to each fermentation tank.

The four replicated tanks of each type of wines were used for further analysis on phenolic compounds, organic acids, ethanol, TSS (^{0}Brix), pH and total acidity (g L^{-1} citric acid). The analysis was carried out after brewing days of 1, 7, 15 and 30 with the use of the method described by Kerem *et al.* (2004). An equipment of High Performance Liquid Chromatography (HPLC) consisted of Shimadzu (Shimadzu Cooperation Analytical and Measuring Instruments Division Kyoto, Japan), LC-20AD Series pumping system, SIL-10AD Series Auto Injector System and SPD-M20A Series Diode Array Detector (DAD) was used to record on line UV spectra of each sample being injected. The column used was an Apollo C_{18} (Alltech) (ϕ 4.0×10 mm, 5 μm) protected with guard column Inertsil ODS-3 (ϕ 4.0×10 mm, 5 μm). Each sample of 20 μL was analysed with HPLC system. The mobile phase on phenolics determinations was carried out with the use of acetonitrile-deionized water (97.8:2, v/v) containing 0.2% phosphoric acid (solvent A) and acetonitrile-deionized water (2:97.8, v/v) containing 0.2% phosphoric acid (solvent B) at a flow rate of 0.6 mL min^{-1} with column temperature of 40° C. The UV-Vis spectra were recorded within a range of 190-400 nm where detection was recorded at 254 nm. The linear gradient started with 20% solvent B, 50% solvent B for 30 min, 60% solvent B for 35 min, 20% solvent B for 40 min with isocratic elution up to 55 min. Quantification was carried out by an integration of the peak value using an external standard method. The mobile phase for organic acids determinations was carried out with the use of a phosphate buffer of 25 mM (pH 2.5). Elution process was carried out with the use of an isocratic elution of the solvent at a flow rate of 0.8 mL min^{-1} with a column temperature of 40°C. The UV-Vis spectra being used ranged from 190 to 400 nm where the detection was made at 210 nm. Component results were recorded by comparing their retention times against those of authentic standards under their respective analysis conditions, whilst quantification was carried out by the integration of the peak readings using an external standard method. The obtained results were statistically analysed using SPSS Computer Program, version 11.0.1 (SPSS, 2001).

RESULTS

Calibration curves of phenolics and organic acids standards: The results of a chromatogram on calibration curves of 16 phenolics compounds showed that myricetin compound ranked the highest and the lowest value was found with gallic acid, whilst the rest gave different standard curves (Fig. 1). Similarly, a diagram of standard calibration curves of 8 organic acids showed that malic acid ranked the highest and the lowest values were found with both citric and benzoic acids, whilst the rest gave different standard curves (Fig. 2). The results derived from HPLC analysis on retention time (min) of phenolics ranged from 4.623 to 32.720 for gallic acid and Kaempferol, respectively (Table 1). Whilst with organic acids, it ranged from 3.532 to 13.010 min. for oxalic and benzoic acids, respectively. Other values on linear range, calibration

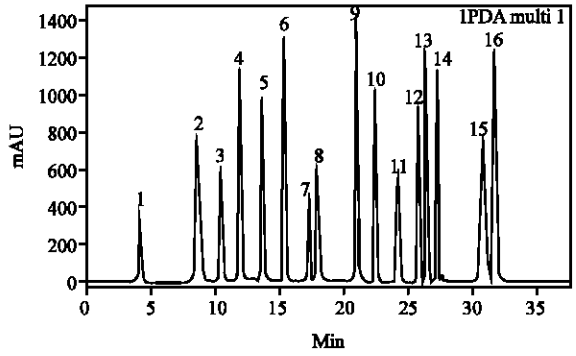

Fig. 1: RP-HPLC chromatogram of sixteen chemical standards being used, i.e., (1) gallic acid, (2) (+)-catechin; (3) (-)-epicatechin, (4) rutin, (5) procyanidin B1, (6) caffeic acid, (7) procyanidin B2, (8) vamillic acid, (9) myricetin, (10) ellagic acid, (11) resveratol, (12) ferrulic acid, (13) lutelin, (14) quercetin, (15) naringenin and (16) kaempferol

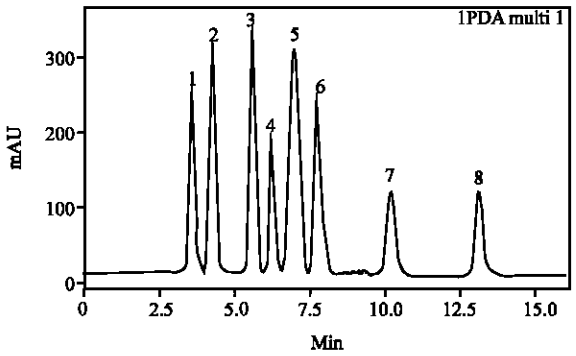

Fig. 2: RP-HPLC chromatogram of eight organic acids standards being used: (1) oxalic acid, (2) tartaric acid, (3) malic acid, (4) ascorbic acid, (5) lactic acid, (6) acetic acid, (7) citric acid and (8) benzoic acid

Table 1: RP-HPLC calibration curves of phenolic acids and organic acids standards with respect to retention time, linear range, correlation coefficients, detection limits and repeatability percentages

Compounds	Retention time (min)	Linear range (mgL^{-1})	Calibration curve	Correlation coefficient (R^2)	Detection limit (mgL^{-1})	Repeatability (%)
Phenolics						
Gallic acid	4.623	0.25-100	$Y = 21977X+54085$	0.9986	0.25	4.07
(+)-Catechin	8.973	0.01-100	$Y = 8089.0X-4882.7$	0.9998	0.50	2.50
(-)-Epicatechin	10.203	0.50-100	$Y = 5949.9X-568.19$	0.9996	1.00	2.48
Rutin	12.621	0.01-100	$Y = 52454X-6272.2$	0.9993	0.05	1.09
Procyanidin B1	14.139	1.00-100	$Y = 2131.6X-1005.7$	0.9999	10.00	2.15
Caffeic acid	15.846	0.25-100	$Y = 27953X-11508$	0.9996	0.25	4.10
Procyanidin B2	16.897	1.00-100	$Y = 2195.3X-989.91$	0.9967	10.00	1.74
Vanillic acid	17.250	50.0-500	$Y = 113529X-2\times10^6$	0.9995	50.00	1.82
Myricetin	21.593	0.01-100	$Y = 62581X-32510$	0.9989	0.05	3.10
Ellagic acid	23.024	0.05-100	$Y = 108328X-3203.7$	0.9999	0.05	3.35
Resveratol	24.506	0.05-100	$Y = 30173X-75411$	0.9984	1.00	1.08
Ferrulic acid	26.013	0.50-100	$Y = 9766.3X+1972.6$	1.0000	0.50	1.27
Lutelin	26.541	1.00-100	$Y = 93464X+4295.8$	0.9999	2.50	1.79
Quercetin	27.104	0.01-100	$Y = 4395.1X-2307$	0.9986	0.50	1.17
Naringenin	31.572	0.01-100	$Y = 2244.5X+2023.7$	0.9995	2.50	1.12
Kaempferol	32.720	0.01-100	$Y = 92563X-19808$	0.9991	0.05	1.47
Organic acids						
Oxalic acid	3.532	50-1000	$Y = 1\times10^7X-1\times10^6$	0.9999	50.00	4.94
Tartaric acid	4.271	1000-10000	$Y = 3\times10^6X-1\times10^6$	0.9998	1000.00	4.78
Malic acid	5.608	1000-25000	$Y = 2\times10^6X-736670$	0.9995	1000.00	3.56
Ascorbic acid	6.289	50-1000	$Y = 330994X-323557$	0.9994	50.00	3.03
Lactic acid	7.091	1000-25000	$Y = 1\times10^6X-116769$	0.9998	1000.00	4.86
Acetic acid	7.708	1000-25000	$Y = 894452X-17646$	0.9999	1000.00	4.27
Citric acid	10.524	1000-25000	$Y = 2\times10^6X-214034$	0.9998	1000.00	3.84
Benzoic acid	13.010	500-25000	$Y = 141364X-110271$	1.0000	500.00	3.65

curves, correlation coefficient, detection limit and repeatability are also included and all of them revealed a high degree of sensitivity where confidential limits were of <5%, thus the results were of a high level of reproducibility and acceptable (Fang *et al*., 2006).

Some laboratory results on ripe fruits of Mao Luang and some analysis data of both brewed red wines: Mean values of fresh weight of 100 berries of ripe fruits of Mao Luang, juice:solids, pH, total soluble solid (TSS, ^{0}Brix), total organic acid (TOA, mg L^{-1}), total soluble solid:total organic acids (TSS:TOA, %), total flavonoids contents (TFC, mg L^{-1}), total phenolic acids (TPA, mg L^{-1}), i.e., gallic, caffeic, vanillic, ellagic and ferrulic acids; Total Procyanidins Contents (TPC), i.e., procyanidin B1 and procyanidin B2 and reducing sugar (g L^{-1}) were 65.62, 3.28, 3.51, 16.50, 49.36, 28.10, 397.90, 76.04, 156.21 and 184.32, respectively (Table 2). With the red wine of non-skin contact wine (with an age of 30 days), the results showed that mean values of pH, total soluble solid (TSS, ^{0}Brix), total organic acid (TOA, mg L^{-1}), total soluble solid:total organic acids (TSS:TOA, %), total flavonoids contents (TFC, mg L^{-1}), total phenolic acids (TPA, mg L^{-1}), i.e., gallic, caffeic, vanillic, ellagic and ferrulic acids; total procyanidins contents (TPC), i.e., procyanidin B1 and procyanidin B2 and reducing sugar (g L^{-1}), alcohol (% vol.), density (at the rate of 20°C), volatile acidity (g L^{-1})[a], total acidity (g L^{-1})[b] and total SO_2 (mg L^{-1}) were 3.42, 7.00, 9.57, 0.73, 66.06, 52.03, 32.67, 2.28,

Table 2: Mean values of weights of 100 berries of Mao Luang ripe fruits, juice:solids, pH, total soluble solid (TSS, ^{0}brix), Total Organic Acids (TOA), Total Soluble Solid: Total Organic Acids (TSS:TOA, %), Total Flavonoids Contents (TFC), Total Phenolic Acids (TPA), Total Procyanidins Contents (TPC), reducing sugar, alcohol, density, volatile acidity, total acidity and total SO_2 of juices of Mao Luang ripe fruits plus two types of brewed red wines (30 days of age), i.e., non-skin contact and skin contact wines

Tested parameters	Mao Luang fruits	Non-skin contact wine	Skin contact wine	Significant levels
Weight of 100 berries (g)	65.62	-	-	-
Juice/solids	3.28	-	-	-
pH	3.51	3.42	3.35	NS
TSS (°Brix)	16.50	7.00	6.60	NS
TOA (mgL^{-1})	49.36	9.57	12.13	***
TSS:TOA (%)	28.10	0.73	0.54	NS
TFC (mgL^{-1})	397.90	66.06	69.34	***
TPA (mgL^{-1})	76.04	52.03	144.11	***
TPC (mgL^{-1})	156.21	32.67	94.69	***
Reducing sugar (gL^{-1})	184.32	2.28	2.31	NS
Alcohol (% vol.)	-	13.84	14.70	NS
Density (20°C)	-	0.9956	0.9923	NS
Volatile acidity (gL^{-1})[a]	-	0.19	0.25	*
Total acidity (gL^{-1})[b]	-	5.63	6.21	NS
Total SO_2 (mgL^{-1})	-	80.14	86.20	NS

TSS: Total Soluble Solid, TOA: Total Organic Acids (oxalic, tartaric, malic, ascorbic, lactic, acetic, citric and benzoic acid). TFC: Total Flavonoids Contents [(+)-catechin, (-)-epicatechin, rutin, myricetin, resveratol, lutelin, quercetin, naringenin and kaempferol], TPA: Total Phenolic Acids (gallic acid, caffeic acid, vanillic acid, ellagic acid and ferrulic acid), TPC: Total Procyanidins Contents (procyanidin B1 and procyanidin B2), [a]Available as acetic acid, [b]Available as tartaric acid, *,**,***Least significant differences at probability, $p<0.05$, <0.01, <0.001, respectively. NS: non Significant. Statistical analysis was carried out between non-skin contact and skin contact wines only

13.84, 0.9956, 0.19, 5.63 and 80.14, respectively. For skin contact red wine (with an age of 30 days), the results showed that mean values of pH, total soluble solid (TSS, ^{0}brix), total organic acid (TOA, mg L^{-1}), total soluble solid:total organic acids (TSS:TOA, %), total flavonoids contents (TFC, mg L^{-1}), total phenolic acids (TPA, mg L^{-1}), i.e., gallic, caffeic, vanillic, ellagic and ferrulic acids; total procyanidins contents (TPC), i.e., procyanidin B1 and procyanidin B2 and reducing sugar (g L^{-1}), alcohol (% by vol.), density (at the rate of 20°C), volatile acidity (g L^{-1})[a], total acidity (g L^{-1})[b] and total SO_2 (mg L^{-1}) were 3.35, 6.60, 12.13, 0.54, 69.34, 144.11, 94.69, 2.31, 14.70, 0.9923, 0.25, 6.21 and 86.20, respectively.

Flavonoids, phenolic acids, anthocyanins, organic acids contents and some other properties of both 30 day brewed red wines: Analyses on flavonoids, phenolic acids, anthocyanins, organic acids contents and some other properties of both 30-day brewed red wines of Mao Luang ripe fruits were carried out. The results showed that mean values of total amounts of flavonoids, i.e., (+)-catechin, (-)-epicatechin, rutin, myricetin, resveratol, lutelin, quercetin, naringenin and kaempferol of skin contact:non-skin contact wines at brewing days of 1, 7, 15 and 30 were: 73.18:40.00; 75.84:29.87; 60.91:46.86; and 69.34:66.06 mg L^{-1}, respectively (Table 3). The differences in each brewing period (days 1-30) between skin contact

Table 3: RP-HPLC mean values of flavonoids, phenolic acids, anthocyanins, organic acids and some other items on ethanol, total soluble solid (TSS, ^{0}brix), pH and total acidity of citric acid of brewed red wines of Skin Contact (SC) and Non-Skin Contact (NSC) being determined at 1, 7, 15 and 30 days after fermentation

Analysed parameters	Days after fermentation							
	SC1	NSC1	SC7	NSC7	SC15	NSC15	SC30	NSC30
Flavonoids (mg L^{-1})								
(+)-Catechin	34.26d	32.97d	40.86c	19.17e	52.42b	35.74d	40.27c	57.40a
(-)-Epicatechin	32.57a	0.50g	30.94b	2.67e	1.14f	7.48d	22.69c	0.68
Rutin	1.61ab	1.40bc	0.26d	1.29bc	1.79ab	0.17d	0.74cd	2.16a
Myricetin[ns]	0.56	0.57	0.60	0.60	0.56	0.71	0.97	0.57
Resveratol	3.41bc	2.93cd	2.52d	2.87d	3.79ab	2.51d	2.51d	3.94a
Lutelin	0.16	-	-	0.09	0.10	-	-	0.39
Quercetin	0.61d	1.31bc	0.66cd	3.18a	1.11cd	0.74cd	1.91b	0.92cd
Naringenin	-	-	-	-	-	-	-	-
Kaempferol	-	0.32	-	-	-	0.22	0.25	-
Total flavonoids	73.18ab	40.00e	75.84a	29.87g	60.91d	46.86e	69.34bc	66.06c
Phenolic acids (mg L^{-1})								
Gallic acid	122.00a	31.51c	115.85b	5.95d	119.01ab	31.35c	123.40a	28.73c
Caffeic acid (NS)	0.76c	3.65b	1.59c	3.52b	1.30c	3.74b	1.17c	4.48a
Vanillic acid (NS)	18.06	18.41	17.94	18.49	18.23	18.67	18.40	18.38
Ellagic acid	0.29	0.24	0.18	0.24	0.26	0.28	0.31	0.26
Fermlic acid	0.39d	0.35d	6.94a	0.17e	2.48b	0.41d	0.83c	0.18e
Total phenolic acids	141.50a	54.16b	142.50a	28.37c	141.28a	54.45b	144.11a	52.03b
Anthocyanins (mg L^{-1})								
Procyanidin B1	21.56c	14.29c	69.65b	64.46b	70.38b	19.04c	78.25a	20.41c
Procyanidin B2	16.21bcd	10.41e	19.18b	13.75cde	38.52a	12.81cde	16.44bc	12.26de
Total procyanidins	37.77e	24.70g	88.83c	78.21d	108.90a	31.85f	94.69b	32.67f
Organic acids (g L^{-1})								
Oxalic acid	0.10	-	-	0.13	0.12	0.11	0.13	0.11
Tataric acid	1.34a	0.50d	0.94b	0.63cd	0.74bcd	0.79bc	0.63cd	0.90b
Malic acid	0.45b	0.37b	1.16a	0.46b	0.51b	0.44b	0.46b	0.45b
Ascorbic acid	1.53bc	1.44bc	8.10a	1.42bc	1.70b	1.18c	1.42bc	1.20c
Lactic acid	0.17	-	0.53	0.65	0.67	0.52	0.64	0.96
Acetic acid	-	0.25	0.10	0.10	-	-	1.03	-
Citric acid	1.48a	0.66b	0.12d	0.41bc	0.38cd	0.57bc	0.41bc	0.53bc
Benzoic acid	4.63d	0.99e	4.55d	7.14a	6.94ab	5.87bc	7.14a	5.42cd
Total organic acids	9.70c	4.21d	15.50a	10.94b	11.06b	9.48c	12.13b	9.57c
Others								
Ethanol (%)	1.23	1.04	9.10	6.72	14.26	10.20	14.70	13.84
TSS (°Brix)	20.02	20.10	16.52	17.31	12.01	14.40	6.60	7.00
pH	3.44	3.46	3.47	3.56	3.50	3.50	3.35	3.42
Total acidity (g L^{-1} citric acid)	4.25	3.18	5.29	4.27	5.98	4.49	6.21	`5.63

Letter(s) within rows represent Least Significant Differences (LSD) of Duncan Multiple Range Test (DMRT) at probability, $p<0.05$. NS: Non Significant, SC1: Skin-Contact wine at day 1 of fermentation; NSC1: Non-Skin Contact wine at day 1 of fermentation; SC7: Skin-Contact wine at day 7 of fermentation; NSC7: Non-Skin Contact wine at day 7 of fermentation; SC15: Skin Contact wine at day 15 of fermentation; NSC15: Non-Skin Contact wine at day 15 of fermentation; SC30: Skin Contact wine at day 30 of fermentation; NSC30: Non-Skin Contact wine at day 30 of fermentation

and non-skin contact wines were large and statistically significant, i.e. in all cases, skin contact wine gave significantly higher amount of flavonoids than non-skin contact wine except at day 30 both became similar but still skin contact wine gave a higher total amount of flavonoids than non-skin contact wine. For phenolic acids, the results showed that mean values of total amounts of phenolic acids at brewing days 1, 7, 15 and 30 of both skin contact and non-skin contact wines were: 141.50:54.16; 142.50:28.37; 141.28: 54.45 and 144.11:52.03 mg L^{-1}, respectively. With amounts of anthocyanins, i.e., procyanidin B1 and procyanidin B2 the results showed that mean values of total amounts of procyanidins at days 1, 7, 15 and 30 of both red wines were 37.77:24.70; 88.83: 78.21; 108.90:31.85 and 94.69:32.67 mg L^{-1}, respectively. For organic acids, the results showed that mean values of total organic acids of both skin contact and non-skin contact red wines at days 1, 7, 15 and 30 were 9.70:4.21; 15.50:10.94; 11.06:9.48 and 12.13:9.57, respectively. With ethanol on days 1, 7, 15 and 30 days after brewing, the results showed that ethanol mean values were 1.23:1.04; 9.10:6.72; 14.26:10.20 and 14.70:13.84% for both skin contact and non-skin contact red wines, respectively. With total soluble solid (^{0} brix), the results at days 1, 7, 15 and 30 after brewing were 20.02:20.10; 16.52:17.31; 12.01:14.40 and 6.60:7.00 for both skin contact and non-skin contact red wines, respectively. There was no statistical significant found between both red wines on brix values. The results on pH values of both red wines tested on days 1, 7, 15 and 30 after brewed were 3.44:3.46; 3.47:3.56; 3.50:3.50 and 3.35:3.42, respectively and mean values of total citric acid values were 4.25:3.18; 5.29:4.27; 5.98:4.49 and 6.21:5.63 g L^{-1}, respectively.

DISCUSSION

With the published data of Butkhup and Samappito (2008) and Samappito and Butkhup (2008) they reported that Mao Luang ripe fruits contain some considerable amounts of flavonoids chemical compounds and different kinds of organic acids where the chemical compounds possess some medicinal properties against some kinds of human illnesses such as gastric intestinal problems of diabetes, dysentery, indigestion, constipation and even Coronary Heart Disease. Thus, the use of ripe fruits of Mao Luang crop for winemaking or for some other beverage drinks should be of tangible value especially for human consumption. Therefore, Mao Luang ripe fruits of both Fapratan and Sangkrow2 cultivars were used for winemaking with the use of two different types of brewing techniques, i.e., skin contact and non-skin contact of winemaking.

The results on calibration curves of phenolic compounds and organic acids revealed that values of correlation coefficients (R^2), detection limits (mg L^{-1}) and repeatability percentages were relatively low where the values indicated a high sensitivity level of accuracy, thus the methods being used were of a good reproducibility and acceptable (Fang *et al*., 2006). With primary data derived from juices of Mao Luang ripe fruits, it was found that mean values of Total Organic Acid (TOA), Total Flavonoids Contents (TFC), Total Phenolic Acids (TPA) and Total Procyanidins Contents (TPC) were comparable to the results reported by Butkhup and Samappito (2008) and Samappito and Butkhup (2008). The result on mean value of ^{0}brix found with Mao Luang juices was relatively high reaching a mean value of 16.50. This mean value indicates a high degree of sweetness taste of the juices. This must be attributable to a high amount of sugar content in fruits of the crop hence it provides a high degree of sweetness taste in juices. The high amount of sugar contents in ripe fruits may favour some advantages on a good taste of the wines where a high palatability table wine could possibly be achieved.

The analysis carried out with both 30 days brewed red wines shown in Table 2 of the results showed that Total Organic Acids (TOA), Total Flavonoids Contents (TFC), Total Phenolic Acids (TPA) and total procyanidins found with skin contact wine were much higher than non-skin contact wine where their significant differences reached an outstanding level of 0.001%. Singleton and Trouslade (1983) reported that skin contact technique being used in winemaking significantly increased high amounts of hydroxycinnamates, gallic acid and flavonoids in the brewed wines. The tremendous differences found between skin contact and non-skin contact wines could be attributable to the differences in brewing techniques where skin contact wine gave much higher amounts of TOA, TFC and TPA than non-skin contact wine. The higher values of TOA, TFC and TPA found with skin contact wine must be due to the fermentation processes of the whole lot of fruits where chemical compounds could have been released from Mao Luang fruits residues unlike the use of juices alone for non-skin contact winemaking, i.e., the wine was brewed without skin and seed of each fruit. Sanchez Palomo *et al*. (2007) with grape white wines reported that an increase in phenolic compounds and organic acid contents found with skin contact wine could have influenced by maceration conditions (time being used in the fermentation process). Furthermore, with this current work volatile acidity of skin contact wine was significantly higher than non-skin contact wine. The reason for this must be attributable to malolactic fermentation as found with Castelao wines

where Spranger *et al.* (2004) with grape white wine reported the findings. Therefore, in most cases, skin contact wine gave more advantages than non-skin contact wine and this method of winemaking should be used whenever red wine of Mao Luang ripe fruits must be brewed. The results agree with the published works reported by a number of workers, e.g., Darias-Martin *et al.* (2000) with Listan Blanco wines, Cabaroglu *et al.* (2002) with white Turkish wines, Selli *et al.* (2006) with Narince and skin contact white wines.

In comparing the analyzed results between skin contact and non-skin contact wines carried out at days 1, 7, 15 and 30 after being brewed, the results showed that in each sampling period of the analyzed samples, in all cases, the mean values of total flavonoids, phenolic acids, anthocyanins of procyanidin B1 and procyanidin B2 and also organic acids of skin contact wine were much higher than non-skin contact wine where the differences were highly significant and persisted throughout the brewing dates of the sampling periods. It seems more likely that the high amounts of anthocyanins could have influenced intensity of red colour of wines where non-skin contact wine was relatively lighter than skin contact wine, i.e. non-skin contact wine possessed smaller amounts of chemical substances of anthocyanins. Thus a darker colour of skin contact wine was found with skin contact wine. Therefore, skin contact winemaking technique should be recommended for winemaking, particularly with the use of Mao Luang ripe fruits. The results of this current work should be useful for Mao Luang crop growers, particularly the growers of Mao Luang plantations in Thailand.

CONCLUSION

It was found that Mao Luang ripe fruits of Fapratan and Sangkrow2 cultivars harvested from dipterocarp forest of Phupan Valley, Sakhon Nakhon Province, Northeast Thailand contained some considerable amounts of flavonoid compounds, phenolic acids, procyanidin B1 and procyanidin B2 and organic acids. Skin contact wine contained much higher amounts of these chemical compounds than non-skin contact wine, thus skin contact winemaking technique was highly recommended for winemaking, particularly when Mao Luang ripe fruits must be used for winemaking.

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