International Scholars Journals

Advances in Food Science and Technology ISSN: 6732-4215 Vol. 12 (4), pp. 001-010, April, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals



*Full Length Research paper*

# Effect of skin contact treatments on the aroma profile and chemical components of mulberry (*Morus alba* Linn.) wines

**Supachai Samappito[1] and Luchai Butkhup[2]***

[1]Department of Biotechnology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand.
[2]Laboratory Equipment Center, Mahasarakham University, Mahasarakham 44000, Thailand.

Accepted 14 December, 2023

**A study was conducted to determine the effect of extended skin contact time on the sensory properties of mulberry wine. Aroma components were analyzed by headspace–solid phase microextraction coupled with gas chromatography–mass spectrometry (HS–SPME/GC–MS). The contents in aroma compounds were related to the skin contact time. Compared with immediate pressing and 6 h skin contact, 12 h skin contact improved the sensory characteristics. Skin contact slightly increased some aroma attributes, including phenylethyl alcohol, 1-decanol, 3-ethoxy-1-propanol, ethyl acetate, isobutyl acetate, ethyl octanoate, isoamyl acetate, ethyl butanoate, ethyl hexanoate, and ethyl butyrate. Attributes such as fruity, solvent, floral and fatty had a significant increase in wines elaborated with skin contact. In the present work, the aromatic profile of mulberry wine was first characterized. With regard to the overall aromatic characteristics and quality, the skin contact wines gave better aroma than the control wine.**

**Key words:** Mulberry, aromatic compound, skin contact.

## INTRODUCTION

Recently, sensory studies based on consumer preferences indicated that flavor of wine was found to be one of the most important attributes considered when purchasing wines (Pozo-Bayon et al., 2001). The volatile composition influences the organoleptic characteristics of wines, particularly the aromatic characteristics (Santos et al., 2004). Wine flavor presents an extremely complex chemical pattern in both qualitative and quantitative terms. Over 1000 volatile compounds have been identified in sherry wines with a wide concentration range
varying between hundreds of $mgL^{-1}$ down to the $\mu gL^{-1}$ or $ngL^{-1}$ level (Munoz et al., 2007) . Moreover, wine aroma is generated by several classes of compounds, such as hydrocarbons, alcohols, terpene alcohols, esters, aldehydes, ketones, acids, ethers, lactones, sulphur and nitrogen compounds (Tao et al., 2008). Aroma production is influenced by several factors: environment (soil, climate), fruit variety, ripeness, fermentation conditions and biological factors (that is, yeast strain and other components of the oenological microflora), winemaking processes and aging (Tesevic et al., 2005). Most of the volatile compounds may play a role in the aromatic profile of each wine type depending on their concentration. In some cases it has been possible to isolate a few key compounds, mostly representing the typical flavor of a wine (Tesevic et al., 2005), while in the majority of wines several compounds seem to cooperate, with specific ratios between them (Guth, 1997). A better understan-ding of the key aroma compounds helps to control quality and may have an impact on the wine technological processes (Bonino et al., 2003).

Skin maceration generally prompts increased concentrations of most aroma components in the final wine, though the end-result is influenced by maceration conditions (time and temperature) and by the fruit variety used (Sanchez Palomo et al., 2007; Selli et al., 2006). During maceration, the concentration of the aroma components sometimes increases and as a result of the skin contact process, the quality of wine may improve, due to the extraction of the aroma compounds from the skin (Selli et al., 2003), but sensory changes are not always produced. Otherwise there is a risk of negative

*Corresponding author: E-mail: Tak_biot2000@yahoo.com.

effects, such herbaceous aroma, bitter flavor or over-strong color (Sanchez Palomo et al., 2007). However, skin contact increases the phenolic compounds of wines and in some cases may cause more astringent and bitter taste (Cabaroglu et al., 1997). For this reason, maceration conditions must be carefully chosen.

Mulberry (*Morus alba* Linn) belongs to the Moraceae family and it is widely grown under varied climatic conditions in the northeast region Thailand, which is the large cultivation area and also the largest producer of mulberry fruit and becoming one of the important mulberry wine producers in Thailand. Mulberry fruits have a special sweet, an exquisite taste and flavor (Doymaz, 2004); they are also used to make excellent wine, which enjoys great popularity in the marketplace.

The aim of this study was to determine the aromatic composition of wine made from mulberry using HS–SPME coupled with GC–MS after 0, 6, 12 and 24 h of skin contact and their effect on the sensory profile of wines.

## MATERIALS AND METHODS

### Reagents and chemicals

All reagents used were of analytical grade. Absolute ethanol was purchased from BDH (VWR International Ltd., Lutterworth, England). The chemical standards were used as internal standards, 2-octanol, was purchased from Sigma–Aldrich (Madrid, Spain). Deionized water was prepared by a Milli-Q Water Purification system (Millipore, MA, USA) . All authentic standards (analytical grade) were purchased from Sigma-Aldrich and Fluka (St. Louis, MO, USA).

### Preparation of wine samples

Healthy mulberry fruits (120 kg) were manually harvested at optimum maturity at the Silk Innovation Center, Mahasarakham University, northeast Thailand, and transported to the Department of Biotechnology, Faculty of Technology, Mahasarakham University. The mulberry fruits had been harvested on October - November, 2008 season at 19 ± 2°Brix. Mulberry must have a titratable acidity, as tartaric acid, of 5.61 $gL^{-1}$, pH 3.50, and reducing sugar 192.80 $gL^{-1}$. After harvest, mulberries were divided into four batches. The first batch was treated in the standard way with minimal skin contact and considered as control. In this way, mulberries were pressed in a horizontal press and 50 $mgL^{-1}$ of sulphur dioxide was added. The juice was then settled at 20°C for 24 h, and then racked. For the skin contact experiment, the mulberries were destemmed and crushed. The second batch was subjected to skin contact for 6 h, and the third batch for 12 h, and the fourth batch for 24 h with addition of 40 $mgkg^{-1}$ of sulphur dioxide, and then pressed in a horizontal press. The juice was settled and racked, as mentioned above. The pasteurized mulberry musts obtained after decanting were inoculated with 10 $gL^{-1}$ of active dry yeasts of *Saccharomyces cerevisiae* strain Ruby. ferm (Chr Hansen, Denmark). Fermentations were conducted using 40-litre stainless -steel tanks kept at 20°C. At the end of the alcoholic fermentation, resulting in red wine with 11 - 12% (v/v) alcohol content after 30 days of fermentation, the red wine was then racked and provided with sulfur dioxide 50 $mgL^{-1}$. After settlement, the finished wine was stored at 4°C in the tank. Wine samples were collected 6 months after winemaking and then subjected to HS–SPME/GC–MS analysis.

### Determination of total phenolic contents (TPC)

Total phenolic contents of the mulberry wines were determined by the Folin-Ciocalteu method (Kahkonen et al., 1999). The sample solution (200 μ l) was transferred into a test tube and then mixed thoroughly with 1 ml of Folin-Ciocalteu reagent. After mixing for 3 min, 0.8 ml of 7.5% (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer then allowed to stand for a further 30 min in the dark, and centrifuged at 3300g for 5 min. The absorbance of extracts and a prepared blank were measured at 765 nm using a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as grams of gallic acid equivalents (GAE) per liter, which was determined from known concentrations of gallic acid standard prepared similarly.

### Determination of total flavonoid contents (TFC)

Total flavonoids were measured using a colorimetric assay developed by Dewanto et al. (2002). An aliquot of diluted sample or standard solution of (+)-catechin was added to 75 μl of $NaNO_2$ solution (7%), and mixed for 6 min, before adding 0.15 ml $AlCl_3$ (10%). After 5 min, 0.5 ml of 1 M NaOH solution was added. The final volume was adjusted to 2.5 ml, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm. Total flavonoids were expressed as mg (+)-catechin equivalent $L^{-1}$ ($mgCEL^{-1}$), through the calibration curve of (+)-catechin (range from 0 - 400 $\mu gml^{-1}$). All samples were analyzed in three replications.

### High performance liquid chromatography (HPLC) conditions

HPLC method has been developed to determine the organic acids in mulberry wine (Samappito and Butkhup, 2008). HPLC apparatus consisting of a 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 was used to record online UV spectra of the organic acids in the samples. The data were collected and analyzed with a Shimadzu computing system. The column used was Apollo $C_{18}$ (Alltech) (ø 4.6 x 250 mm, 5 μm) protected with guard column Inertsil ODS-3 (4.0, 10 mm, 5 μm). Twenty-microlitre samples of each sample were analysed using an HPLC system. Elution was effected using an isocratic elution of the solvent, 25 mM phosphate buffer (pH 2.5) at a flow rate of 0.9 $mlmin^{-1}$ and column temperature was at 40°C. The UV–Vis spectra were recorded from 190 to 400 nm, with detection at 210 nm. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions, and quantification was carried out by the integration of the peak using external standard method.

### Headspace–solid phase microextraction (HS–SPME) procedure

HS–SPME procedure for determination of flavor volatiles was carried out as described in detail elsewhere (Camara et al., 2007). Mulberry wine sample was adjusted to pH 3.3 and the ionic strength was increased to improve the extraction efficiency using NaCl (30%), because demethylated pectate anion in mulberry wine will combine with sodium cations to form a gel, and reduce the concentration of analyze in the headspace. A 20 ml vial containing 5 ml of sample, spiked with 50 μl of 2-octanol (Sigma–Aldrich),

**Table 1.** General composition of the mulberry wine.

| Parameter | Skin contact time | | | | Significant |
|---|---|---|---|---|---|
| | Control | 6 h | 12 h | 24 h | |
| pH | 3.44 ± 0.02 | 3.50± 0.03 | 3.52± 0.10 | 3.48± 0.13 | NS |
| Reducing sugar ($gl^{-1}$) | 4.20 ± 0.21 | 3.41± 0.15 | 3.76± 0.24 | 3.10± 0.10 | NS |
| TFC ($mgL^{-1}$eq. (+)-catechin) | 26.78 ± 4.06c | 40.35 ± 3.11b | 54.06± 2.37a | 52.32± 3.02a | * |
| TPC ($mgL^{-1}$ eq. gallic acid) | 152.04 ± 5.18d | 217.08 ± 5.28b | 237.15 ± 3.96a | 241.90 ± 4.10a | * |
| Ascorbic acid ($gl^{-1}$) | 1.17 ± 0.12 | 1.24± 0.10 | 1.46± 0.05 | 2.19± 0.14 | NS |
| Citric acid ($gl^{-1}$) | 0.72 ± 0.24 | 0.70± 0.13 | 0.64± 0.12 | 0.68± 0.17 | NS |
| Lactic acid ($gl^{-1}$) | 0.35 ± 0.02 | 0.31± 0.03 | 0.32± 0.02 | 0.23± 0.05 | NS |
| Malic acid ($gl^{-1}$) | 0.44 ± 0.10 | 0.42± 0.11 | 0.40± 0.04 | 0.41± 0.06 | NS |
| Oxalic acid ($gl^{-1}$) | 0.11 ± 0.01 | 0.14± 0.02 | 0.15± 0.03 | 0.13± 0.03 | NS |
| Benzoic acid ($gl^{-1}$) | 5.14 ± 0.20 | 5.23± 0.15 | 5.68± 0.30 | 5.65± 0.24 | NS |
| Alcohol level (% v/v) | 12.60 ± 0.14 | 12.31 ± 0.08 | 11.76 ±0.12 | 11.50 ± 0.10 | NS |
| Volatile acidity ($gl^{-1}$ eq. acetic acid) | 0.49 ± 0.05 | 0.46± 0.03 | 0.45± 0.04 | 0.42± 0.02 | NS |
| Titratable acidity ($gl^{-1}$ eq. tartaric acid) | 6.40 ± 1.00 | 5.60± 0.84 | 4.82± 0.66 | 4.67± 0.72 | NS |
| Total $SO_2$ ($mgl^{-1}$) | 76.32 ± 5.10 | 70.14 ± 6.54 | 72.61 ± 4.12 | 75.03 ± 3.15 | NS |

*Scheffe's test, significance at $p < 0.05$ (within the rows, means followed by the same letters (a - d) are not significantly different). NS, not significant.

which was used as internal standard, was placed in a thermostatic block on a stirrer. The fiber was then exposed to the gaseous phase for an appropriate time period at temperature of 40°C. As stirring usually improves the extraction, because the static layer resistant to mass transfer is destroyed (facilitate mass transport between the bulk of the aqueous sample and the fiber), all the experiments were performed under constant stirring velocity (750 rpm). Headspace sampling involved automatically exposing the fibers through a Teflon-lined cap of the vial (with the stirrer con-stantly stirring). After extraction, the SPME fiber was inserted into the hot injector port (240°C) of the GC–MS system for 6 min where the extracted chemicals were desorbed thermally and transferred directly to the analytical column. All SPME samplings were carried out in triplicate unless otherwise noted.

#### Gas chromatography–mass spectrometry (GC–MS) conditions

Gas chromatographic-mass spectrometric analysis (EI) was performed using a Shimadzu GC 2010 gas chromatograph (Shimadzu Cooperation Analytical & Measuring Instruments Division Kyoto, Japan) series equipped with a split/splitless injector, coupled to a GCMS–QP2010 mass spectrometer. Data acquisition was performed by a GC- MSsolution software (Shimadzu, Kyoto, Japan). The separation was achieved using a Restek RTX–5MS fused silica capillary column, 30 m x 0.25 mm i.d., 0.25 m film thickness (Superchrom, Milan, Italy). GC oven temperature was programmed from 50°C (6 min) to 240°C at a rate of $2.0°Cmin^{-1}$, then to 280°C at a rate of $20°Cmin^{-1}$. Helium was used as carrier gas; inlet pressure was 25 kPa; linear velocity: 28.6 cm/sec at 50°C. Injector temperature: 250°C. Injection mode: splitless. MS scan conditions: source temperature, 200°C; interface temperature, 250°C; Energy, 70 eV; mass scan range, 39 - 350 amu. The GC oven temperature was held at 35°C for 10 min. It was raised at 5°C/min to 100°C, followed by an increase of temperature to 210°C at a rate of $3°Cmin^{-1}$ . Then the temperature was held at 210°C for 40 min. The total run time was 99.65 min. The injection port temperature was 250°C, and the detector transfer line was 250°C.

The identification was based on comparison of the GC retention times and mass spectra with authentic standards from Sigma–Aldrich when standards were available; for these compounds, calibration curves were calculated with the purpose of quantification. The data were collected and analyzed with a Shimadzu computing system. When the authentic standards were not available, the identification was based on comparison with the spectral data of the Wiley Spectral Library and NIST Library.

The odour activity value (OAV) for each compound was calculated by dividing its wine concentration by the concentration corresponding to its odour threshold.

#### Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA), if justified by the statistical probability ($p < 0.05$), by Scheffe's test using the SPSS software

## RESULTS AND DISCUSSION

### General mulberry wine composition and effect of skin contact time

General composition of wines obtained with different skin contact times from mulberry fruit is given in Table 1. Skin contact treatment had no significant effect on the most general composition of wines, in agreement with others studied (Darias-Martin et al., 2000; Selli et al., 2006). The total flavonoid and total phenolic contents were affected by the skin contact treatment. The wines made with skin contact with 6, 12 and 24 h treatments had higher values for total flavonoids and total phenolics than did to the control wine, due to the consequence of skin compounds being extracted into the juice. It has been reported that skin contact caused an increase in flavonoid content

(Selli et al., 2006) and total phenol content (Sanchez Palomo et al., 2007) of the wine. On the other hand, the wines with skin contact treatments had lower values for ethanol and titratable acidity values. Similar results were reported in the literature (Darias-Martin et al., 2000; Sanchez Palomo et al., 2007; Selli et al., 2006).

**Volatile compounds of mulberry wines and effect of skin contact time**

Quantitative data of the aroma compounds found in the mulberry wine as affected by skin contact treatment are shown in Table 2. The data are expressed as means ($mgL^{-1}$) of the three analytical replicates. A total of 73 volatile compounds were identified in the mulberry wine, including twenty two alcohols, twenty esters, twenty eight acids, two carbonyl compounds and two volatile phenols. Among the compounds found, well known by-products of yeast metabolism were the most abundant substances. Thus, volatile compounds which reached the highest levels were alcohols, acids (mainly fatty acids) and esters. Higher alcohols and esters, produced during alcoholic fermentation, play an important role in the flavor of wines, depending on the types of compounds and their concentrations (Valero et al., 2002).

**Alcohols**

As indicated in Table 2, higher alcohols were the largest group of volatile compounds in mulberry wines. The concentration of higher alcohols was generally dependent of with skin contact times. The skin contact increased the concentrations of sensorially valuable compounds in wines, e.g., 1-heptanol, 1-hexanol, isobutyl alcohol, 2-butanol, 1-propanol, isoamyl alcohol, hexanol, 3-ethoxy-1-propanol, and 6,10-dodecadien-1-ol, and these differences were statistically significant, especially in wines macerated for 12 h. Several studies of contents in higher alcohols showed a higher content in macerated wines than in non-macerated wines (Rodrıguez-Bencomo et al., 2008; Selli et al., 2006). Most of these compounds are formed by yeast during the alcoholic fermentation. The increase of these compounds in wines elaborated with skin contact could be related to yeast metabolism. Higher alcohols positively affect the quality of wines in quantities not above 400 $mgL^{-1}$ (Selli et al., 2006). The total concentrations of these components in control and skin contact wines were below 400 $mgL^{-1}$ (Table 2). These alcohols are characterized by solvent, fruity and floral attributes.

**Acids**

Within the family of fatty acids, acetic, hexanoic, octanoic, hexadecanoic, 9-octadecenoic acid and octadecanoic acids were the major fatty acids in mulberry wines. In general, fatty acid concentrations increased slightly in skin-contact wines (Rodrıguez-Bencomo et al., 2008; Sanchez- Palomo et al., 2006; Selli et al., 2006). The total fatty acid levels were significantly higher with a 24 h skin contact treatment compared to control, 6, and 12 h treatment (Table 2) . However, the increase in fatty acid levels may not have a direct impact on wine aroma since the concentration of fatty acids is far below their threshold values. The production of fatty acids depends on the composition of the must and fermentation conditions (Selli et al., 2006) . Although the presence of fatty acids is related usually to the appearance of negative odors, they are very important for the aromatic equilibrium in wines because they are opposed to the hydrolysis of the corresponding esters (Gil et al., 2006). Long chain fatty acids, decanoic, dodecanoic, tetradecanoic, and hexadecanoic acid have less strong effect on the flavor of the wine.

**Esters**

Skin contact treatment resulted in significant increases in the concentrations of esters, including ethyl acetate, isobutyl acetate, isoamyl acetate, ethyl butanoate, ethyl hexanoate, methyl salicylate, isobutyl decanoate, isoamyl laurate, ethyl laurate, phenylethyl acetate and ethyl 9-hexadecanoate, and these differences were statistically significant, especially in wines macerated for 12 h. Skin contact treatment increased the total concentration of esters in wines compared to the control wine. These results were similar to observe in the previously studied (Sanchez Palomo et al., 2007; Selli et al., 2006). The control, skin contact for 6, 12 and 24 h wines contained 22.36, 23.73, 52.26 and 41.58 $mgL^{-1}$ of total esters, respectively. Esters are one of the major components of wine aroma. These compounds make a positive contribution to the general quality of wine being responsible for their “fruity” and “floral” sensory properties of wines. By contrast, ethyl decanoate and methyl laurate levels declined significantly with skin-contact treatment.

**Other compounds**

Among carbonyl compounds, acetaldehyde was found in wines. This compound increased with skin contact treatments, especially in wines macerated for 12 and 24 h.

Phenols present in mulberry wines arise from the fruit and from the yeast metabolism. These compounds may contribute to the overall aroma and form the body of the wine. The remarkable compounds, such as volatile phenols, have been detected. Among volatile phenols, significant increases occurred in 4-ethylphenol level with skin contact times. Volatile phenols are considered among the usual components of the aroma of a wine.

The volatile phenols play an important role in wine aroma. 4-Ethylphenol is responsible for phenolic, smoky, horsy, medicinal and barnyard odors (Selli et al., 2006). Depending on their concentration, they contribute

**Table 2.** Effect of skin contact on the aroma compound levels of mulberry wines ($mgL^{-1}$).

| Compound | Skin contact time | | | | Significant |
|---|---|---|---|---|---|
| | Control | 6 h | 12 h | 24 h | |
| **Alcohols** | | | | | |
| -Butanol | 4.84 ± 1.05 | 4.12 ± 1.30 | 3.59 ± 4.10 | 2.31 ± 2.02 | NS |
| Isobutanol | 15.01 ± 1.10 a | 14.15 ± 0.12 a | 10.22 ± 0.10 b | 7.34 ± 0.01 c | * |
| 1-Heptanol | 0.22 ± 0.01 b | 0.22 ± 0.01 b | 1.67 ± 0.02 a | 1.24 ± 0.01 a | * |
| 1-Hexanol | 3.59 ± 1.20 c | 4.12 ± 1.02 c | 23.48 ± 1.10 a | 19.38 ± 1.35 b | * |
| Isobutyl alcohol | 1.64 ± 0.40 c | 1.05 ± 0.31 c | 14.20 ± 0.80 a | 10.42 ± 0.52 b | * |
| 2-Butanol | 2.30 ± 0.12 b | 2.18 ± 0.20 b | 9.40 ± 0.31 a | 10.16 ± 0.20 a | * |
| 2,3-Butanediol | 27.70 ± 1.03 a | 26.14 ± 1.25 a | 18.30 ± 1.25 b | 19.51 ± 1.50 b | * |
| 1-Octanol | 1.22 ± 0.14 | 1.30 ± 0.13 | 1.12 ± 0.01 | 1.10 ± 0.02 | NS |
| 1-Propanol | 10.54 ± 1.35 c | 14.32 ± 1.61 b | 28.02 ± 1.20 a | 29.26 ± 1.23 a | * |
| Isoamyl alcohol | 110.33 ± 2.57 c | 129.57 ± 2.52 b | 143.81 ± 4.30 a | 131.40 ± 2.13 b | * |
| 1-Nonanol | 9.10 ± 1.25 a | 8.33 ± 1.06 a | 6.97 ± 1.44 b | 6.15 ± 1.53 b | * |
| 1-Pentanol | 1.80 ± 0.14 | 1.84 ± 0.12 | 1.90 ± 0.02 | 1.96 ± 0.03 | NS |
| Cyclohexanol | 6.04 ± 1.16 | 6.57 ± 0.25 | 6.82 ± 0.50 | 7.30 ± 0.04 | NS |
| Hexanol | tr | 0.02 ± 0.00 b | 1.04 ± 0.02 a | 1.20 ± 0.05 a | * |
| Phenylethyl alcohol | 7.25 ± 1.62 d | 18.53 ± 1.12 c | 35.24 ± 3.10 a | 30.01 ± 1.41 b | * |
| 1-Decanol | 1.01 ± 0.02 | 1.04 ± 0.10 | 1.25 ± 0.03 | 1.64 ± 0.06 | NS |
| 3-Ethoxy-1-propanol | ND | tr | 0.85 ± 0.02 a | 1.40 ± 0.12 a | * |
| 2-Phenylethyl alcohol | 2.53 ± 0.22 | 2.41 ± 0.14 | 2.36 ± 0.15 | 2.10 ± 0.04 | NS |
| 6,10-Dodecadien-1-ol | trace | tr | 0.40 ± 0.01 b | 1.28 ± 0.02 a | * |
| 4-Morpholineethanol | ND | tr | 1.00 ± 0.00 | 1.02 ± 0.01 | NS |
| 1-Hexadecanol | 1.61 ± 0.32 | 1.50 ± 0.06 | 1.47 ± 0.10 | 1.68 ± 0.20 | NS |
| 1-Octadecanol | 1.80 ± 0.11 | 1.95 ± 0.13 | 2.83 ± 0.23 | 3.00 ± 0.10 | NS |
| Subtotal ($mgL^{-1}$) | 208.53 d | 239.36 c | 315.94 a | 290.86 b | * |
| Subtotal (%) | 72.93 | 73.76 | 69.47 | 63.50 | |
| **Esters** | | | | | |
| Ethyl acetate | 11.24 ± 1.08 c | 11.29 ± 1.40 c | 21.40 ± 1.22 a | 14.06 ± 1.55 b | * |
| Isobutyl acetate | 1.33 ± 0.14 b | 1.30 ± 0.21 b | 6.23 ± 1.02 a | 6.12 ± 0.30 a | * |
| Ethyl octanoate | 0.40 ± 0.05 | 0.47 ± 0.03 | 0.58 ± 0.03 | 0.73 ± 0.04 | NS |
| Isoamyl acetate | 1.37 ± 0.58 b | 1.06 ± 0.44 b | 3.21 ± 0.14 a | 3.91 ± 0.30 a | * |
| Ethyl butanoate | 2.18 ± 0.24 c | 2.90 ± 0.20 c | 9.70 ± 0.56 a | 5.42 ± 0.15 b | * |
| Ethyl hexanoate | 0.42 ± 0.02 b | 0.99 ± 0.04 b | 2.23 ± 0.18 a | 2.19 ± 0.14 a | * |
| Ethyl 9-decenoate | 1.10 ± 0.02 | 1.18 ± 0.03 | 1.15 ± 0.04 | 1.22 ± 0.30 | NS |
| Ethyl butyrate | 0.22 ± 0.03 | 0.19 ± 0.01 | 0.26 ± 0.02 | 0.28 ± 0.04 | NS |
| Cyclopentyl ester | 0.56 ± 0.01 | 0.58 ± 0.02 | 0.66 ± 0.04 | 0.89 ± 0.03 | NS |
| Methyl salicylate | ND | tr | 0.83 ± 0.03 a | 0.98 ± 0.02 a | * |
| Isobutyl decanoate | 0.09 ± 0.01 c | 0.12 ± 0.02 bc | 0.40 ± 0.02 ab | 0.64 ± 0.03 a | * |
| Ethyl decanoate | 0.78 ± 0.03 a | 0.66 ± 0.26 a | 0.22 ± 0.01 b | 0.18 ± 0.01 b | * |
| Ethyl heptanoate | 0.53 ± 0.02 | 0.55 ± 0.05 | 0.50 ± 0.02 | 0.48 ± 0.03 | NS |
| Isopropyl myristate | 0.40 ± 0.02 | 0.42 ± 0.02 | 0.44 ± 0.04 | 0.34 ± 0.02 | NS |
| Methyl laurate | 1.16 ± 0.05 a | 1.23 ± 0.02 a | 0.34 ± 0.03 b | 0.16 ± 0.02 b | * |
| Isoamyl laurate | 0.14 ± 0.05 b | 0.32 ± 0.05 b | 1.04 ± 0.10 a | 1.28 ± 0.10 a | * |
| Ethyl laurate | tr | 0.04 ± 0.00 c | 0.31 ± 0.01 b | 0.90 ± 0.06 a | * |
| Phenylethyl acetate | 0.24 ± 0.01 b | 0.26 ± 0.02 b | 1.13 ± 0.13 a | 1.20 ± 0.04 a | * |
| Ethyl 9-hexadecanoate | 0.13 ± 0.02 c | 0.11 ± 0.03 c | 1.52 ± 0.03 a | 0.52 ± 0.03 b | * |
| Ethyl linoleate | 0.07 ± 0.00 | 0.06 ± 0.00 | 0.11 ± 0.02 | 0.08 ± 0.01 | NS |

**Table 2.** Contd.

| | | | | | |
|---|---|---|---|---|---|
| Subtotal ($mgL^{-1}$) | 22.36 c | 23.73 c | 52.26 a | 41.58 b | NS |
| Subtotal (%) | 7.82 | 7.31 | 11.53 | 9.08 | |
| **Acids** | | | | | |
| 4-Ethylbenzoic acid | 0.18 ± 0.02 b | 0.14 ± 0.03 b | 2.02 ± 0.10 a | 2.68 ± 0.12 a | * |
| Acetic acid | 26.84 ± 1.62 c | 27.26 ± 1.22 c | 35.81 ± 1.59 b | 68.25 ± 4.10 a | * |
| Linoleic acid | 0.61 ± 0.13 | 0.64 ± 0.10 | 0.67 ± 0.04 | 0.74 ± 0.03 | NS |
| Benzenebutanoic acid | ND | tr | 0.51 ± 0.02 | 0.56 ± 0.03 | * |
| Silicic acid | 1.86 ± 0.17 | 1.95 ± 0.04 | 1.98 ± 0.15 | 2.10 ± 0.10 | NS |
| Hexanoic acid | 6.12 ± 0.34 b | 8.54 ± 0.25 a | 9.73 ± 0.12 a | 6.04 ± 0.20 b | * |
| Dodecanoic acid | 0.10 ± 0.04 b | 0.13 ± 0.02 b | 1.40 ± 0.11 a | 1.48 ± 0.02 a | * |
| Propanoic acid | 0.71 ± 0.01 | 0.73 ± 0.03 | 0.75 ± 0.04 | 0.80 ± 0.06 | NS |
| Pentadecanoic acid | 0.30 ± 0.03 b | 0.35 ± 0.02 b | 1.12 ± 0.02 a | 1.24 ± 0.04 a | * |
| Heptanoic acid | 0.61 ± 0.06 | 0.56 ± 0.01 | 0.58 ± 0.04 | 0.64 ± 0.03 | NS |
| Octanoic acid | 5.47 ± 1.20 b | 6.89 ± 1.34 b | 10.91 ± 1.42 a | 12.14 ± 1.42 a | * |
| Tetradecanoic acid | 0.79 ± 0.01 | 0.82 ± 0.05 | 0.86 ± 0.02 | 0.97 ± 0.03 | NS |
| Nonanoic acid | 0.57 ± 0.01 c | 0.60 ± 0.02 c | 0.93 ± 0.02 b | 1.69 ± 0.02 a | * |
| Butanoic acid | 0.66 ± 0.04 | 0.69 ± 0.05 | 0.76 ± 0.03 | 0.81 ± 0.07 | NS |
| Hexadecanoic acid | 0.85 ± 0.12 c | 0.90 ± 0.10 c | 1.42 ± 0.21 b | 3.58 ± 0.40 a | * |
| Phenylacetic acid | 0.80 ± 0.04 b | 0.84 ± 0.07 b | 1.25 ± 0.13 a | 1.18 ± 0.02 a | * |
| 9-Octadecenoic acid | 1.36 ± 0.06 c | 1.57 ± 0.08 c | 3.20 ± 0.10 b | 5.60 ± 0.21 a | * |
| Propiolic acid | 0.46 ± 0.03 | 0.40 ± 0.04 | 0.38 ± 0.06 | 0.43 ± 0.01 | NS |
| 9-Decenoic acid | 0.35 ± 0.02 | 0.34 ± 0.04 | 0.23 ± 0.01 | 0.28 ± 0.02 | NS |
| Hydrocinnamic acid | 0.68 ± 0.03 | 0.67 ± 0.06 | 0.55 ± 0.03 | 0.64 ± 0.04 | NS |
| Benzoic acid | 0.28 ± 0.07 b | 0.26 ± 0.02 b | 0.90 ± 0.03 a | 0.96 ± 0.04 a | * |
| Octadecanoic acid | 1.04 ± 0.05 c | 2.48 ± 0.12 b | 3.50 ± 0.32 a | 3.88 ± 0.41 a | * |
| n-Decanoic acid | tr | tr | 0.31 ± 0.01 b | 1.02 ± 0.04 a | * |
| Nonadecanoic acid | 1.46 ± 0.02 | 1.51 ± 0.04 | 1.57 ± 0.05 | 1.61 ± 0.02 | NS |
| Decanoic acid | 0.21 ± 0.01 | 0.20 ± 0.03 | 0.22 ± 0.01 | 0.25 ± 0.02 | NS |
| Decanedioic acid | 0.58 ± 0.03 | 0.63 ± 0.06 | 0.60 ± 0.04 | 0.61 ± 0.05 | NS |
| 6-Decenoic acid | 0.26 ± 0.02 | 0.27 ± 0.05 | 0.22 ± 0.03 | 0.24 ± 0.02 | NS |
| 9-Octadecanoic acid | 0.47 ± 0.06 c | 0.50 ± 0.04 c | 1.01 ± 0.09 b | 1.53 ± 0.01 a | * |
| Subtotal ($mgL^{-1}$) | 53.62 d | 59.87 c | 83.39 b | 121.95 a | * |
| Subtotal (%) | 18.75 | 18.45 | 18.39 | 26.63 | |
| **Carbonyl compounds** | | | | | |
| Acetaldehyde | 0.44 ± 0.03 b | 0.50 ± 0.02 b | 1.38 ± 0.03 a | 1.52 ± 0.04 a | * |
| Benzaldehyde | 0.13 ± 0.02 | 0.12 ± 0.01 | 0.09 ± 0.01 | 0.10 ± 0.02 | NS |
| Subtotal ($mgL^{-1}$) | 0.57 c | 0.62 c | 1.47 a | 1.62 a | * |
| Subtotal (%) | 0.20 | 0.20 | 0.32 | 0.35 | |
| **Volatile phenols** | | | | | |
| 4-Ethylphenol | 0.14 ± 0.02 c | 0.16 ± 0.01 c | 0.58 ± 0.03 b | 1.12 ± 0.04 a | * |
| 4-Vinylphenol | 0.70 ± 0.03 | 0.74 ± 0.04 | 0.71 ± 0.02 | 0.89 ± 0.03 | NS |
| Subtotal ($mgL^{-1}$) | 0.84 c | 0.90 c | 1.29 b | 2.01 a | * |
| Subtotal (%) | 0.29 | 0.28 | 0.28 | 0.44 | |

Results are the means ± standard deviation of three replications. *Scheffe's test, significance at $p < 0.05$ (within the rows, means followed by the same letters (a–d) are not significantly different). tr, ≤ 0.001 $mgl^{-1}$; ND, not detected; NS, not significant.

positively or negatively to wine aroma, but the levels of the volatile phenols detected in mulberry wines had the lower concentration in this wine and above its odor perception threshold (Table 3).

**Odor activity values for volatile compounds**

Odour activity value (OAV) is a measure of importance of a specific compound to the odor of a sample (e.g. food). It is calculated as the ratio between the concentration of individual substance in a sample and the threshold concentration of this substance (= odor threshold value, = minimal concentration that can be detected by human nose). One way of quantification of the odour activity of a compound is to determine its OAV. Such a value is calculated by dividing the concentration of the compound in the wine into its perception threshold (Cabaroglu et al., 2002; Guth, 1997). Thus, the odour impact of a substance increases in proportion to its OAV when this value is >1. Based on these criteria, the above mentioned compounds (particularly those exhibiting the highest OAVs) can be assumed to be those with the strongest odour impact, thereby contributing to a great extent to the aroma of mulberry wines and reasonably being largely responsible of the sensory profile of these wines.

In order to relate the quantitative results of each compound with its sensorial importance in wine aroma, the OAVs of each compound were calculated. Table 3 shows the OAV for each compound. OAV was obtained as the ratio of compound concentration to its odor perception threshold (OPT) value. The OPTs used have been previously reported by other authors (Munoz et al., 2007; Peinado et al., 2004; Santos et al., 2004; Selli et al., 2003; Zea et al., 2001). OAV of the major components increased with skin contact treatments, especially in wines macerated for 12 and 24 h. As can be seen, seven alcohols (1-hexanol, 1-octanol, isoamyl alcohol, cyclo-hexanol, phenylethyl alcohol, 1-decanol, and 3-ethoxy-1- propanol), nine esters (ethyl acetate, isobutyl acetate, ethyl octanoate, isoamyl acetate, ethyl butanoate, ethyl hexanoate, ethyl butyrate, ethyl laurate, and phenylethyl acetate), five acids (benzenebutanoic acid, hexanoic acid, octanoic acid, phenylacetic acid, and 9-decenoic acid), and two other compounds (acetalde-hyde, and 4-vinylphenol) had higher than 1 OAV. These compounds were the most markedly contributing to mulberry wine odorant series. Compounds with higher OAV are frequently considered as essential for the aroma, although there are exceptions where odorants with high OAVs are suppressed and compounds with lower OAVs are revealed as important contributors (Grosch, 2001). Esters are responsible for fruity aromas in wines and acids contribute to freshness and to equili-brate the fruity aroma. The origin of these compounds is principally fermentative. As for the differences found between maceration times, these could be related to the different composition of must that may affect fermentation.

**Odorant series**

By combining the OAV for each individual compound in an aroma series, the global OAV for each series (Table 3) was obtained. An odor profile for the wines was obtained by grouping the volatile aroma compounds with similar descriptors in odorant series. The value for each odorant series was calculated as the sum of the OAV of the compounds in it. Each compound was assigned to one or several aroma series, depending on its principal odor descriptors; the fruity, balsamic, solvent, floral, herbaceous, phenolic, fatty, roasty and spicy odorant series were chosen for this purpose on account of their extensive use for describing and distinguishing mulberry wine in terms of aroma by specialized journals (Munoz et al., 2007; Peinado et al., 2004; Santos et al., 2004; Selli et al., 2003; Zea et al., 2001). As can be seen in Figure 1, shows ‘‘spider webs’’, diagrams for the odorant series of aroma attributes of the control and skin contact for 6, 12 and 24 h wines. The mulberry wine showed the highest contribution of the fruity series (followed by the floral, fatty, solvent, phenolic, roasty, herbaceous, spicy and balsamic series) to the global aroma. Skin contact slightly increased some aroma attributes, such as fruity and floral series, correlated with the increase of alcohols and esters concentrations in skin contact wines, such as phenylethyl alcohol, 1-decanol, 3-ethoxy-1-propanol, ethyl acetate, isobutyl acetate, ethyl octanoate, isoamyl acetate, ethyl butanoate, ethyl hexanoate, and ethyl butyrate. Attributes such as fruity, solvent, floral and fatty had a significant increase ($p < 0.05$) in wines elaborated with skin contact, especially after 12 h. No significant changes were observed in the balsamic, herbaceous, phenolic, roasty and spicy series when the control and after 12 h of skin contact wines were compared.

**Conclusion**

The mulberry wines made with skin contact treatments had higher values for total flavonoids and total phenolics contents than did to the control wine, due to the consequence of skin compounds being extracted into the juice. In addition, in the present work, the aromatic profile of mulberry wine was first characterized. The mulberry wine showed the highest contribution of the fruity series. Skin contact slightly increased some aroma attributes, such as fruity and floral series, correlated with the increase of alcohols and esters concentrations in skin contact wines, such as phenylethyl alcohol, 1-decanol, 3-ethoxy-1-propanol, ethyl acetate, isobutyl acetate, ethyl octanoate, isoamyl acetate, ethyl butanoate, ethyl hexanoate, and ethyl butyrate. Attributes such as fruity,

**Table 3.** Odor activity values OAVs , odor descriptions, odor perception thresholds (OPT), and odorant series (OS) for the aroma compounds in mulberry wines.

| Compound | OAVs | | | | Odor descriptor[a] | OPT[a] ($mgl^{-1}$) | OS[b] |
|---|---|---|---|---|---|---|---|
| | Control | 6 h | 12 h | 24 h | | | |
| **Alcohols** | | | | | | | |
| -Butanol | 0.032 | 0.027 | 0.024 | 0.015 | Medicinal, wine-like | 150.00 | 6 |
| Isobutanol | 0.200 | 0.189 | 0.136 | 0.098 | Alcohol, nail polish | 75.00 | 3 |
| 1-Hexanol | 0.449 | 0.515 | 2.935 | 2.423 | Herbaceous, grass, woody | 8.00 | 5 |
| Isobutyl alcohol | 0.041 | 0.026 | 0.355 | 0.261 | Sweet, whiskey-like | 40.00 | 3 |
| 2-Butanol | 0.046 | 0.044 | 0.188 | 0.203 | Wine-like, solvent | 50.00 | 3 |
| 2,3-Butanediol | 0.185 | 0.174 | 0.122 | 0.130 | Floral, waxy, fruity, herbal | 150.00 | 1,4 |
| 1-Octanol | 1.525 | 1.625 | 1.400 | 1.375 | Orange-rose, jasmine, lemon, herbaceous | 0.80 | 1,4,5 |
| 1-Propanol | 0.034 | 0.047 | 0.092 | 0.096 | Alcohol-like, ripe fruit | 306.00 | 1,3 |
| Isoamyl alcohol | 1.839 | 2.160 | 2.397 | 2.190 | Solvent, sweet, nail polish | 60.00 | 3 |
| 1-Pentanol | 0.028 | 0.029 | 0.030 | 0.031 | Fruity | 64.00 | 1 |
| Cyclohexanol | 20.133 | 1.900 | 22.733 | 24.333 | Camphor like odor, pungent | 0.30 | 3 |
| Phenylethyl alcohol | 0.725 | 1.853 | 3.524 | 3.001 | Rose, honey | 10.00 | 4 |
| 1-Decanol | 2.525 | 2.600 | 3.125 | 4.100 | Floral, fruity, alcohol | 0.40 | 1,3,4 |
| 3-Ethoxy-1-propanol | – | – | 8.500 | 14.00 | Fruity | 0.10 | 1 |
| 2-Phenylethyl alcohol | 0.530 | 0.241 | 0.236 | 0.210 | Flowery, rose, honey | 10.00 | 4 |
| **Esters** | | | | | | | |
| Ethyl acetate | 0.937 | 0.941 | 1.783 | 1.172 | Pineapple, fruity, balsamic | 12.00 | 1,2 |
| Isobutyl acetate | 0.831 | 0.813 | 3.894 | 3.825 | Fruity, apple, banana | 1.60 | 1 |
| Ethyl octanoate | 1.667 | 1.958 | 2.417 | 3.042 | Floral, fruity, banana, pear | 0.24 | 1,4 |
| Isoamyl acetate | 8.563 | 6.625 | 20.063 | 24.438 | Banana, fruity, sweet | 0.16 | 1 |
| Ethyl butanoate | 5.450 | 7.250 | 24.250 | 13.550 | Strawberry, pineapple | 0.40 | 1 |
| Ethyl hexanoate | 5.250 | 2.375 | 27.875 | 27.375 | Green apple, banana | 0.08 | 1 |
| Ethyl butyrate | 11.000 | 9.500 | 13.000 | 14.000 | Fruity, apple | 0.02 | 1 |
| Ethyl decanoate | 3.900 | 3.300 | 1.100 | 0.900 | Pleasant, soap | 0.20 | 7 |
| Ethyl laurate | – | 1.000 | 7.750 | 22.500 | Oily, fatty, floral | 0.04 | 4,7 |
| Phenylethyl acetate | 0.960 | 1.040 | 4.520 | 4.800 | Roses, flowery | 0.25 | 4 |
| **Acids** | | | | | | | |
| Benzenebutanoic acid | – | – | 2.040 | 2.240 | Fatty-rancid, cheesy | 0.25 | 7 |
| Hexanoic acid | 2.040 | 2.847 | 3.243 | 2.013 | Cheese, fatty, grass, fruity | 3.00 | 1,7 |
| Octanoic acid | 0.547 | 0.689 | 1.091 | 1.214 | Fatty acid, rancid, dairy | 10.00 | 7 |
| Butanoic acid | 0.300 | 0.314 | 0.345 | 0.368 | Cheese, fatty, rancid | 2.20 | 7 |
| Phenylacetic acid | 0.800 | 0.840 | 1.250 | 1.180 | Honey, floral, flowery | 1.00 | 4 |
| 9-Decenoic acid | 8.750 | 8.500 | 5.750 | 7.000 | Waxy, fatty, soapy | 0.04 | 7 |
| Decanoic acid | 0.150 | 0.143 | 0.157 | 0.178 | Fatty acid, rancid, woody | 1.40 | 5,7 |
| **Carbonyl compounds** | | | | | | | |
| Acetaldehyde | 3.667 | 4.167 | 11.500 | 12.667 | Ripeness apple | 0.12 | 1 |
| Benzaldehyde | 0.065 | 0.060 | 0.045 | 0.050 | Almond, fragrant, cherry | 2.00 | 1 |
| *Volatile Phenols* | | | | | | | |
| 4-Ethylphenol | 0.230 | 0.262 | 0.951 | 1.836 | Shoe polish, phenolic, leather, smoky, horsy, medicinal | 0.61 | 6,8,9 |
| 4-Vinylphenol | 3.889 | 4.111 | 3.944 | 4.944 | Pharmaceutical, meaty, smoky | 0.18 | 6,8 |

[a]Odor description and odor threshold reported in the literature (Munoz et al., 2007; Peinado et al., 2004; Santos et al., 2004; Selli et al., 2003; Zea et al., 2001).
[b]Odorant series: (1), fruity; (2), balsamic; (3), solvent; (4), floral; (5), herbaceous; (6), phenolic; (7), fatty; (8), roasty; (9), spicy.

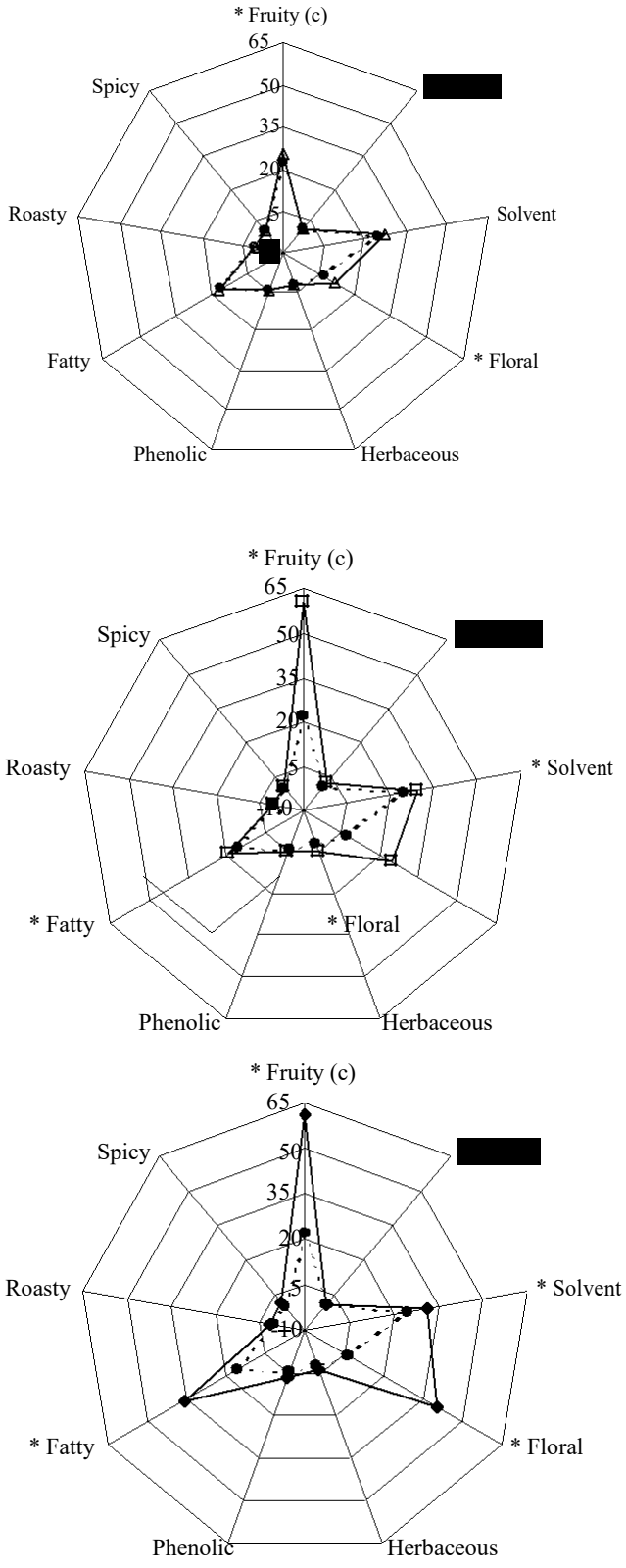


**Figure 1.** Odorant series [b] ($p$ < values obtained by ANOVA: *$p$ < 0.05, [c] for better visualization, values shown for the fruity series are the second part of the real values) calculated by adding the odor activity values (OAVs) of the compounds grouped in the control (•), skin contact for 6 h (Δ), 12 h ( ) and 24 h (♦) wines.

solvent, floral and fatty had a significant increase in wines elaborated with skin contact, especially after 12 h when compared to the immediate pressing and 6 h skin contact. This may be due to the fact that the volatiles of wines were released to the musts during the maceration. With regard to the overall aromatic characteristics and quality, the skin contact wines gave better than the control wine.

## ACKNOWLEDGMENTS

The authors would like to acknowledge Silk Innovation Center, Mahasarakham University, which allowed us to benefit from their knowledge of the mulberry fruit studied. We would like to gratefully acknowledge the staff of the Microbiology Laboratory, Department of Biotechnology, Technology Faculty, Mahasarakham University, Thailand, for their collaboration, providing laboratory facilities, excellent guidance and constructive criticisms throughout this investigation.

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