CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals and Materials

2.1.1 Chemical

1. Acetaldehyde	AR grade	BDH
2. Acetic acid	AR grade	Merk.
3. Dichloromethane	AR grade	Merk.
4. 2,2- diphenyl-1-picrylhydrazyl (DPPH)	AR grade	Fluka
5. Ethanol	HPLC grade	Merk
6. Ethanol 95%	Commercial grade	Ayudhyh
7. Ethyl acetate	AR grade	Merk
8. Wine Sample	Shiraz - Black Beauty wine obtain from Maechan Winery, Chiang Rai, Thailand.	

2.1.2 Material

1. Black poum	Thon Tong Chai, Lampang, Thailand
2. Drumstick wood	Ban Lao, Lampang, Thailand
3. Longan wood	Ban Lao, Lampang, Thailand
4. Luna nut wood	Ban Lao, Lampang, Thailand
5. Neem wood	Chang Peuk, Chiang Mai, Thailand
6. Oak wood (chip)	D.I wine, Bangkok, Thailand

2.2 Instrument

2.2.1 GC – MS	Allgilent model 6890 GCsystem
2.2.2 UV-spectrophotometer	Shimadzu Model UV-160

2.3 Methods

2.3.1 Wine Aging

Well dried the wood, Longan, Drumstick, Luna nut, Neem, Black poum and Oak were cut, then sliced into small pieces of about of 0.5 x 1.0 cm size. The wood chips were roasted for 30 min using a hot plate. The roasted wood chips become dark and a burnt smell. The six kinds of the wood chips were kept in sealed containers for storage until being used for wine aging.

Fresh wine, produced from Shiraz - Black Beauty grapes and provided from Maechan Winery, Chiang Rai, Thailand was used in this study. For wine aging step, the roasted wood chips were used of ratio of wood to wine was 5g/1000mL of wine. The wines were finally stored in glass bottles closed with cork at room temperature in a dark room for 120 days.

2.3.2 Examiners training

Fifty volunteer students and staffs (between 20 – 55 years old) from different disciplines in Chiang Mai Rajabhat University, Chiang Mai province, were selected and trained for more experience in sensory evaluation of wine aroma. Nineteen types of flavor and aroma materials responsible in wine naming ethanol, acetaldehyde, acetic acid, ethyl acetate, rose, hydrogen sulfide, yogurt, spinach, grape, jasmine, wine, oxidized wine, pickle vegetable, brandy, coffee, pine apple, garlic, paper and passion fruit were prepared with proper concentration. Chemicals, ethanol, acetaldehyde, acetic acid, ethyl acetate was prepared in 0.1%v/v concentration. Moreover, in case of wine, oxidized wine, yogurt, brandy, coffee, samples were prepared in 1% concentration. Otherwise, rose, spinach, grape, jasmine, pickle vegetable, pine apple, garlic, paper and passion fruit were prepared by crushing of the materials (1.0 g) followed by filtering and diluting to 100mL with water. The imitation of sulfur dioxide odor was achieved by using mineral water form Sankampaeng hot spring. After training, well trained volunteers were able to describe precisely all flavor and aroma character of wine.

2.3.3 Wine sample preparation and sensory evaluation

Six types of woods, Longan, Drumstick, Luna nut, Neem, Black poum and Oak were used for preparation of aged wine. The woods were sliced and cut to small pieces and roast for 30 min. Red wine used in this research were obtain from Mae-Chan winery, Chiang Rai province.

Wines were aged with roasted woods with the ratio of 5 g wood chips in 1 l of wine. Wines were aged for 4 month before tasting examination.

Evaluation of wine flavor and aroma was done in well-ventilated room without blowing wind. Six wine samples were divided into three bottles (total of 18 bottles) and was numbered so that the examiners could not know the type of wood chips used. Appearance, taste and odor test were assigned to be done by the examiners. Those 30 well trained examiners were experienced how to detect, taste and sniff in the test for appearance, taste and odor. The evaluation was carried out using 5 - 10 ml wine samples in clean glass. All wine samples were scored and recorded in a table design by Pradit (2002), sample of table showed in appendix B. Appearance was detected for clarity and color of wine samples. Flavor and aroma of wine samples were identified as vinegar odor, sour, sweet, texture, flavor, and tannin and the overall quality. The average score of wine sample are analyzed which computer application, ANOVA and SPSS statistic program.

2.3.4 Volatile compound analysis

All wine samples were analyzed for the volatile chemical compositions by SPME-GC-MS, especially, headspace solid phase micro-extraction (HS-SPME) was used according to the following protocol: 10 ml of wine were placed in a 25 ml vial capped with a silicon septum, containing a small magnetic stirrer. A polydimethylsiloxane (PDMS) coated SPME fiber was manually inserted through the vial septum and exposed to the headspace at 30°C for 30 min under stirring (140 rpm). Afterwards, the fiber was removed from the vial and inserted into the GC injector, and held at 250°C for 7 min, in split-less mode for thermal desorption of the aromatic compounds, which were then analyzed by GC.

Chromatographic analyses were carried out through a Trace gas chromatograph, equipped with MS detector, a DB – WAX, 60 m x 0.25 mm x 0.25 μm capillary column, with helium as a carrier gas at a flow-rate of 0.9 ml/min. The oven temperature was kept at 40 °C for 7 min then was increased to 230°C at a rate of 3 °C/min.

MS condition:

Ionization	:	EI (Electron ionization)
Electron voltage	:	70 eV
Ion source temperature	:	200°C

GC interface temperature : 300°C

Injection module : splitless

Mass range : 40 - 800

2.3.5 Antioxidant activity analysis

In this research, the DPPH technique were determine the antioxidant activity of wine samples, 3mL of DPPH reagent was filled in test tubes of 3mL wine samples in different concentration (0.5, 1, 3, 5, 7 and 9% v/v respectively) and methanol was use as blank solution (0% v/v of wine sample) and standed for 30 minutes. The mixtures were then analyzed by UV-VIS Spectrophotometer (Shimadzu Model UV-160) at 517 nm. Data were corrected and analyzed with computer spread sheet application for IC_{50} value calculated from relation graph between sample concentration and Absorbance. The IC_{50} value was the point at the concentration be able to reduce the Absorbance value to 50%.