

Article

Evaluation of the Optimum Harvesting Maturity of Makhwaen Fruit for the Perfumery Industry

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Abstract: Harvesting makhwaen (*Zanthoxylum myriacanthum* Wall. ex Hook. f) fruits at the appropriate maturity is the key to ensure that the essential oil quality meets the need of consumers. In common practice, the fruits are usually harvested when their pericarps start to open and fruits are greenish-red in colour depending on the judgment of the farmers. This leads to inconsistencies in the essential oil quality. This research aims at characterising the aromatic profiles of makhwaen essential oil thereby for consumers to choose the quality that best fits their need and eventually identify the optimum harvesting index of the fruits. The effects of maturity states viz. 15, 36, 45 and 60 (MK15–60) days after fruiting on chemical and sensorial quality of the essential oil was evaluated. Fruit sizes ranged from ~3.3–3.7 mm and fruits appeared to dry initially when they reached 45 days. Essential oils were extracted from these fruits after they had been oven dried (60 °C) to the same moisture content, about 10%. The chemical profiles of the essential oil were different. L-limonene and sabinene were evaluated as key components for good quality essential oil and they were found to be higher in MK45 and MK60 (max = 139.04 µg·mL^{−1} and max = 146.27 respectively). NIR spectral patterns of pure extracted oil for every different harvesting time (of every different harvesting time of MK60 and MK36) were similar. Sensorial descriptive analysis by semi-trained panellists defined six terms for characteristics (woody, citrus, herb, sweet, pine and spice). The panels provided the highest rating score (15 numeric scale) of citrus and pine scents at MK45, while sweet and woody aromas were the highest at MK15. The spice scent was maximum when the fruits were harvested at 36 days after fruiting. From this study we suggest that the optimum harvesting index for the distinctive aroma of essential oil ought to be at late harvesting (45–60 days after fruiting). The findings contribute to our understanding of the harvesting maturity, which can also provide significant benefit for the perfumery industry, i.e., the optimum harvesting stage that imparts the essential oil with highest quality.

Keywords: aromatic profile; maturity; spice; utilisation; *Zanthoxylum*

1. Introduction

The *Zanthoxylum* genus of the Rutaceae family comprises ~250 plant species [1]. These aromatic crops can be found in tropical and temperate regions [2]. In Thailand, most *Zanthoxylum* species are known for not only uses as spices, but also to cure many ailments. For examples, *Zanthoxylum acanthopodium* DC. and

Zanthoxylum armatum DC. are known for their medicinal properties such as aromatic tonic, and they are used to treat illness including fever, dyspepsia, toothache and cholera [3,4]. Nine species of *Zanthoxylum* are found in Thailand and four of them, viz. *Z. acanthopodium*, *Z. armatum*, *Zanthoxylum rhetsa* (Roxb.) DC. and *Z. myriacanthum*., are commonly used as spices and condiments in the Northern part of the country [5].

Z. myriacanthum is locally known in Northern Thai as makhwaen and its fruits yield essential oils as high as 4% dry weight [5,6]. The essential oils are used as ingredients in food flavouring, traditional medicine and pharmaceuticals, and has recently gained commercial attention, particularly in the fragrance industry [4–7]. The major volatile component of makhwaen essential oil is L-limonene, comprising as high as ~60% [8,9]. The other constituents are α -pinene (6.49%), β -myrcene (3.87%) and linalool (2.96) representing citrus, herb, spicy and floral characteristics [9,10]. The harvesting index for most horticulture species is based principally on visual parameters such as fruit size and colour. Proper harvesting maturity is, however, the key for fresh plant products in order to adequately ensure good quality of the raw material, which ultimately ensures high quality of the finish processed product [11]. In essential oil-bearing plants, particularly of the Lamiaceae family, the maximum levels, both in yield and essential oils quality, are when they are close to the blooming stage [12]. Harvesting of Patchouli leaves (*Pogostemon cablin*) at the appropriate stage when the leaves are semi-mature affects the amount of essential oil and chemical composition [13]. In addition, harvesting Kekik (*Thymbra spicata*) after the flowering stage affects the volatile components of essential oil, as observed by the carvacrol element representing the spice aroma from after flowering (63%), which had greater volume than before flowering (59%) and full flowering states (53%) [14].

Near infrared spectroscopy (NIR) is a physical and non-destructive technique used to monitor the quality and quantity of agricultural and food products, as well as medicinal plants [15,16]. The application of NIR to quantify important substances in the essential oils of plant origins has been well documented in many cases such as that of *Ravensara aromatic* [15], pennycress [17], various basil [18], citrus oils [19] and some aromatic herbs (thyme, oregano and chamomile) [20]. It can also be used to predict the content of valuable components, such as aldehydes, nootkatone and limonene, as well as other terpene hydrocarbons rapidly and accurately in the essential oils [19]. However, there is a limited literature on the appropriate harvesting index of other essential oil-bearing parts such as fruits, particularly those from the *Zanthoxylum* species. In northern Thailand, the fruit of makhwaen is harvested from November to December [21]. However, the effect of harvesting maturity of makhwaen fruit on the quality of the essential oils has not been described. Therefore, in this research, we outline the aromatic profiles, both chemically and sensorially, of essential oils of the fruit at different maturity stages. The information we provide can be used as a guideline harvesting index for high quality raw material for the perfumery industry.

2. Materials and Methods

2.1. Plant Materials

Makhwaen fruits were randomly harvested from an orchard in the Papea subdistrict, Mae Tang district, Chiang Mai province, Thailand. Fruit branches from 12 trees within the same orchard were harvested 15, 36, 45 and 60 days after the fruiting period (MK15, MK36, MK45 and MK60). They were placed in a styro-foam box (covered with wet cloths) to maintain the temperature ~20 °C inside the box. Size and weight of individual fruit from harvested branches were averaged from 1200 fruits randomly selected on each harvesting day [22]. Fruits without stalks were dried at 60 °C (hot air oven, ED56, BINDER, Tuttlingen, Germany) and dried samples were removed from the oven after the constant weight was reached (10% dry weight), then they were ready for essential oil extraction [23].

2.2. Essential Oils Extraction

The essential oil was extracted via hydro-distillation for 4 h, from 100 g dried fruits in 600 mL of water in a 2 L flask Clevenger-type apparatus. The extraction was repeated twice and yield (mean

value) was reported as the percentage of essential oil from dry plant material [16]. The oil was dried over anhydrous sodium sulphate and kept at 4 °C until analysis (usually within 3 days).

2.3. Chemical Composition Analysis

Essential oil samples (2 µL at the dilution of 1% *v/v* in dichloromethane with 0.003% *w/v* toluene as an internal standard) were injected in a split mode (1:20) [10]. The gas chromatography mass spectrometry (GC-MS) analysis was performed on using a Bruker - scion 436 GC a Rxi 5Sil MS (30 m × 0.25 mm; 0.25 µm film thicknesses). The temperature program oven temperature was held for 2 min at 60 °C and was enhanced to 150 °C at 3 °C·min⁻¹. Then, temperature enhancement was programmed up to 270 °C at 5 °C·min⁻¹ and held at this temperature for 15 min. Other operating conditions were helium as the carrier gas with a flow rate of 1.1 mL·min⁻¹; injector and detector temperatures were 300 °C, and split ratio, 1:50. Mass spectra were taken at 70 eV. The mass spectra and retention indices of essential oil components were identified by comparison to published literature as presented in the MS computer library, supplied by Adams [24]. The standard solution of C₈-C₂₀ n-alkane (Fluka® Analytical, Munich, Germany) in dichloromethane was also used for the calculation of retention indices (RI) [23]. The identification of the volatile compositions was by comparison with mass spectra in NIST 05.L and NIST 98.L libraries with >70% similarity. The compounds were confirmed by their RI as well as those from the literature [9]. The amount in µg·mL⁻¹ of essential oil was calculated as relative to that of toluene internal standard.

2.4. NIR Analysis

The pure essential oils extracted were scanned to analyse the basic physical properties of essential oils from the makhwaen essential oil using a FT-NIR spectrometer (MPA FT-NIR Series Bruker Optics, Stuttgart, Germany), averaging 32 scans per spectrum at 8 cm⁻¹ resolution to assess the makhwaen essential oil samples. Scanning was conducted in reflectance mode comparing the MPA integrated sphere and liquid probe against the Matrix-F with a fibre-coupled measurement head operating two tungsten halogen 12 V, 20 W light sources [25].

2.5. Sensory Analysis

A descriptive sensory analysis was carried out to profile the major odour characteristics of the essential oil. The sensory analysis was performed by five semi-trained panellists (2 male, 3 female, ages 20–37). During training sessions which lasted for 30 hours, the panellists smelled the different essential oil samples and discussed odour attributes [26]. From the discussion session and by using GC-MS analysed result, the descriptive terms were identified [10,27]. The odour attributes (Table 1), including citrus, herb, pine, spice, sweet and woody attributes, as well as general intensity and desirability were evaluated. The descriptive analysis was performed along a scoring line ranging from 0 (none) to 15 (very strong) in triplicates, and two samples were presented for each analysis [26,28].

Table 1. Attributes and references used in evaluating in 6 essential oil odours.

Odour Attributes	Reference Standards	n/15 *
Citrus	Lemon extract (McCormick), 200 µL	8/15
Herb	Thyme (McCormick), 0.5 g	10/15
Pine	Pine/cypress essential oil	10/15
Spice	Ground allspice (McCormick), 0.5 g	8/15
Sweet	Vanilla flavour (McCormick), 200 µL	10/15
Woody	Peanut peel 2 g with 100 µL DI water	7/15

* As described by Tangpao et al. [10] and Sriwichai et al. [29].

2.6. Statistical Analyses

The data was statistically analysed using a comparison of the means of yield for essential oils evaluated by Duncan's multiple range test at 95% confidential level [30]. A principle component analysis (PCA) was used to identify the main sources of systematic variation in the sensory descriptive data [31].

3. Results and Discussion

3.1. Fruit Size, Weight and Colour

The size and weight of harvested makhwaen fruits of different maturity stages were measured (Table 2). Fruit sizes and weight ranged from ≈ 3.4 – 3.7 mm and 0.016 – 0.034 g, and is consistent with the report from Suksathan et al. [21] who reported that the size of fruit was about 3–4 mm and 0.010 – 0.030 g. Harvest time was important for the accumulation of chemical substances in essential oils, as immature harvesting may cause the yield and accumulation of essential substances to be lower [32]. Variation of fruit colour from 15–60 days after initial fruiting are varied (Figure 1). It can be observed that the colour of the fruits altered from light greenish (MK15) to reddish (MK45) and finally to brownish (MK60 or over mature). However, in some species of *Zanthoxylum*, such as *Zanthoxylum bungeanum* Maxim. [1] and *Z. armatum* [33], the colour of the fruits is clear when fruits are over mature.

Table 2. Size and weight of makhwaen fruits.

Treatment	Size (mm)	Weight (g)
MK15	3.73 ± 0.08^b	0.026 ± 0.006^{ab}
MK36	3.72 ± 0.08^b	0.034 ± 0.024^b
MK45	3.55 ± 0.07^{ab}	0.023 ± 0.005^{ab}
MK60	3.45 ± 0.06^a	0.016 ± 0.010^a

Mean scores ($n = 100$) for each size and weight within a column with different superscript letters (a, b) are significantly different at $\alpha = 0.05$ using Duncan's multiple comparison test.

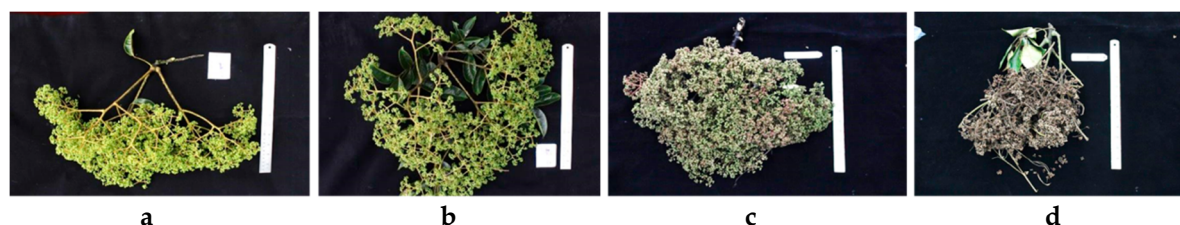


Figure 1. The branches of makhwaen fruits at different harvesting time, (a) 15 days after fruiting, (b) 36 days after fruiting, (c) 45 days after fruiting and (d) 60 days after fruiting.

3.2. Chemical Compounds

Forty-four volatile compounds were detected using GC-MS (Table 3). The chemical profiles of the essential oils from makhwaen fruits at different maturities were variable. Based on the results of GC-MS analysis, the main components of different harvesting time could be identified in the following sequence: MK15; sabinene ($85.81 \mu\text{g}\cdot\text{mL}^{-1}$), methyl (1-methylethyl) benzene ($49.34 \mu\text{g}\cdot\text{mL}^{-1}$) and β -phellandrene ($45.22 \mu\text{g}\cdot\text{mL}^{-1}$) essential oil, MK36; limonene ($139.04 \mu\text{g}\cdot\text{mL}^{-1}$), sabinene ($118.89 \mu\text{g}\cdot\text{mL}^{-1}$) and β -phellandrene ($28.48 \mu\text{g}\cdot\text{mL}^{-1}$) essential oil, MK45; limonene ($140.56 \mu\text{g}\cdot\text{mL}^{-1}$), sabinene ($115.48 \mu\text{g}\cdot\text{mL}^{-1}$) and L-phellandrene ($52.41 \mu\text{g}\cdot\text{mL}^{-1}$) essential oil and MK60; sabinene ($146.27 \mu\text{g}\cdot\text{mL}^{-1}$), limonene ($135.64 \mu\text{g}\cdot\text{mL}^{-1}$) and β -phellandrene ($125.30 \mu\text{g}\cdot\text{mL}^{-1}$) essential oil, respectively. The dominant component in MK60 was sabinene ($146.27 \mu\text{g}\cdot\text{mL}^{-1}$) and MK45 was L-limonene ($140.65 \mu\text{g}\cdot\text{mL}^{-1}$). Sabinene was also the major component in the essential oil of other *Zanthoxylum* species, including that found in *Zanthoxylum rhoifolium* L. and *Z. myriacanthum* [29,34]. Other literature suggests that L-limonene is the important volatile compound from the essential oil

of fresh *Z. myriacanthum* var. *pubescens* fruits [35], as well as *Zanthoxylum schinifolium* S.et Z., *Z. bungeanum* and *Zanthoxylum piperitum* (L.) DC. [9,36,37]. For the assessment of the essential oil quality for these particular species, sabinene and limonene as the representatives of woody and citrus aromas are then used as evaluating indicators [29]. Zhang et al. [1] noted that fruit maturity may influence the chemical compositions of the essential oil.

Table 3. Chemical profiles of makhwaen fruit essential oil.

No.	Chemical Compounds	Amount of Chemical ($\mu\text{g}\cdot\text{mL}^{-1}$ Essential Oil ^a)					
		RI ^{cal}	RI ^{ref}	MK15	MK36	MK45	MK60
1	α -thujene	800.5	926	2.21 $\pm$ 0.01	ND	4.30 $\pm$ 0.02	ND
2	α -phellandrene	800.5	1004	4.26 $\pm$ 0.01	ND	ND	2.23 $\pm$ 0.01
3	α -pinene	800.6	937	2.01 $\pm$ 0.01	5.16 $\pm$ 0.01	11.66 $\pm$ 0.01	6.10 $\pm$ 0.01
4	cis-ocimene	886.2		3.91 $\pm$ 0.37	1.92 $\pm$ 0.01	3.45 $\pm$ 0.01	1.96 $\pm$ 0.01
5	sabinene	900.0	1132	85.81 $\pm$ 0.02	118.89 $\pm$ 0.05	115.48 $\pm$ 0.11	146.27 $\pm$ 0.07
6	β -mycrene	900.1	992	22.45 $\pm$ 0.18	14.69 $\pm$ 0.01	23.47 $\pm$ 0.02	20.30 $\pm$ 0.01
7	octanal	900.2		ND	ND	1.20 $\pm$ 0.01	1.10 $\pm$ 0.01
8	L-phellandrene	900.2		14.18 $\pm$ 0.07	14.81 $\pm$ 0.01	52.41 $\pm$ 0.02	22.72 $\pm$ 0.02
9	α -terpinene	900.3	1196	6.03 $\pm$ 0.03	6.37 $\pm$ 0.03	10.78 $\pm$ 0.01	9.21 $\pm$ 0.01
10	benzene, methyl (1-methylethyl)	900.4		49.34 $\pm$ 0.30	4.82 $\pm$ 0.02	2.77 $\pm$ 0.01	2.78 $\pm$ 0.01
11	L-limonene	900.4	1035	25.31 $\pm$ 0.18	139.04 $\pm$ 0.09	140.65 $\pm$ 0.08	135.64 $\pm$ 0.01
12	β -phellandrene	900.5	1227	45.22 $\pm$ 0.29	28.48 $\pm$ 0.14	39.13 $\pm$ 0.02	125.30 $\pm$ 0.06
13	1,3,6 octatriene, 3,7-dimethyl	900.6		38.45 $\pm$ 0.35	23.71 $\pm$ 0.02	30.49 $\pm$ 0.02	12.83 $\pm$ 0.01
14	γ -terpinene	900.7	1017	22.04 $\pm$ 0.18	11.76 $\pm$ 0.06	17.81 $\pm$ 0.01	14.80 $\pm$ 0.01
15	p-menth-2-en-1-ol	967.1		34.49 $\pm$ 0.22	ND	ND	ND
16	α -terpinolene	920.7		2.14 $\pm$ 0.01	2.93 $\pm$ 0.01	4.89 $\pm$ 0.01	3.94 $\pm$ 0.01
17	cis-sabinenehydrate	900.6	1578	37.31 $\pm$ 0.18	ND	ND	ND
18	trans-sabinene hydrate	900.7		ND	2.33 $\pm$ 0.01	ND	4.27 $\pm$ 0.01
19	α -terpineol	900.9	1594	ND	2.16 $\pm$ 0.01	ND	ND
20	cyclohexane, 1-methyl-4-(1-methylethylidene)	920.7		30.84 $\pm$ 0.15	5.39 $\pm$ 0.03	4.03 $\pm$ 0.01	ND
21	L-linalool	925.7	1566	3.93 $\pm$ 0.01	5.10 $\pm$ 0.01	14.12 $\pm$ 0.01	8.77 $\pm$ 0.01
22	1-terpineol	1000.1		ND	ND	3.44 $\pm$ 0.03	ND
23	Δ -3-carene	900.6	1011	ND	9.86 $\pm$ 0.10	ND	ND
24	3-cyclohexane-1-ol, 4-methyl-1-(1-methylethylidene)	1000.5		9.01 $\pm$ 0.04	27.06 $\pm$ 0.13	39.49 $\pm$ 0.03	35.65 $\pm$ 0.02
25	2-cyclohexane-1-ol, 1-methyl-4-(1-methylethylidene)	901.1		ND	2.18 $\pm$ 0.11	ND	ND
26	2- β -pinene	901.3		ND	1.35 $\pm$ 0.03	ND	ND
27	cryptone	1000.5		2.20 $\pm$ 0.01	6.39 $\pm$ 0.01	1.34 $\pm$ 0.01	2.69 $\pm$ 0.01
28	β -fenchyl alcohol	1013.0		42.98 $\pm$ 0.38	11.03 $\pm$ 0.01	11.19 $\pm$ 0.04	12.69 $\pm$ 0.01
29	decanal	1000.7		7.59 $\pm$ 0.02	5.85 $\pm$ 0.03	10.72 $\pm$ 0.03	5.97 $\pm$ 0.01
30	acetic acid, octyl ester	1025.6		16.76 $\pm$ 0.14	10.65 $\pm$ 0.01	11.86 $\pm$ 0.01	9.36 $\pm$ 0.01
31	2-undecanone	1128.8		2.78 $\pm$ 0.01	4.30 $\pm$ 0.02	12.82 $\pm$ 0.01	5.28 $\pm$ 0.01
32	benzaldehyde, 4-(1-methylethylidene)	1066.9		1.45 $\pm$ 0.01	1.30 $\pm$ 0.01	ND	1.02 $\pm$ 0.01
33	2-decanone	1050.8		ND	9.49 $\pm$ 0.01	ND	ND
34	1-decanol	1100.2		ND	ND	ND	1.10 $\pm$ 0.01
35	2,6-octadiene-1-ol, 3,7-dimethyl-, acetate	1001.8		ND	3.23 $\pm$ 0.02	ND	ND
36	tetradecanal	1150.7		ND	1.87 $\pm$ 0.01	1.95 $\pm$ 0.01	ND
37	D-germacrene	1226.3	1490	ND	1.45 $\pm$ 0.01	2.50 $\pm$ 0.01	ND
38	neryl acetate	1200.0		ND	2.34 $\pm$ 0.01	6.56 $\pm$ 0.01	3.65 $\pm$ 0.01
39	dodecanal	1200.2		ND	ND	2.32 $\pm$ 0.01	2.02 $\pm$ 0.01
40	1-tetradecanol	1299.8	1257	ND	1.80 $\pm$ 0.01	ND	ND
41	linalyl acetate	1051.2		5.51 $\pm$ 0.04	ND	ND	ND
42	geranyl acetate	1233.3	1385	14.55 $\pm$ 0.12	ND	ND	ND
43	2-tridecanone	1288.7	1820	5.97 $\pm$ 0.02	2.18 $\pm$ 0.01	1.89 $\pm$ 0.01	1.35 $\pm$ 0.01
44	hexadecanoic acid	1501.1		ND	2.69 $\pm$ 0.01	3.57 $\pm$ 0.01	10.18 $\pm$ 0.04

RI^{Ref}: Retention index from the referent [9,34]. RI^{cal}: Calculated retention index. ^a Values are calculated as reference to internal standard toluene (0.003% w/v), makhwaen fruit were harvested at 15, 36, 45 and 60 days after fruiting (MK15, MK36, MK45 and MK60). ND: not detectable. The column with different superscript letters (a,b) were significantly different at $\alpha = 0.05$ using Duncan's multiple comparison test.

The PCA between the volatile composition and the harvesting stage revealed two clustering groups. The first group (MK15 and MK60) had the dominant β -phellandrene representing a minty

aroma (Figure 2). As with the experimental work of Jiang and Kubota [26], they reported that Japanese pepper (*Z. piperitum*) contained β -phellandrene as the main substance that provided a minty aroma. The second cluster was of the MK36 and MK45 with L-limonene (citrus aroma) as the most distinctive compound. This group represented the citrus aroma, for use as a flavouring agent and is found in the food flavour and fragrance aroma ingredient industry [36]. The optimum harvesting index of makwhaen fruits is therefore suggested to be 45 to 60 days after fruiting, as indicated by the greater contents of sabinene and limonene.

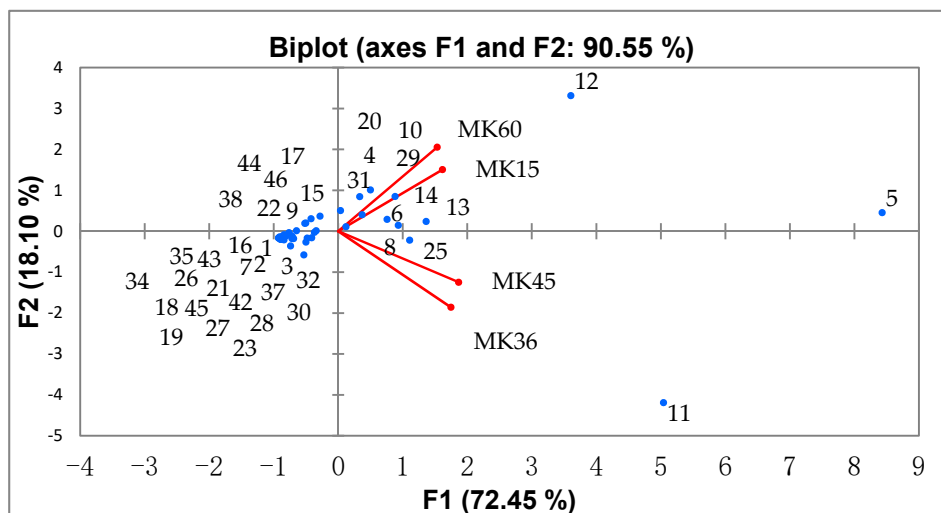


Figure 2. Principal component analysis (PCA) biplot (axes F1 and F2: 90.55%) illustrating the relationships among the chemical components and different harvesting day of makhwaen fruit essential oil.

3.3. NIR Analysis

According to the NIR spectra analysis, oil samples were dominated by overtones and different combinations of C–H stretching and bending vibrations occurring between $9090\text{--}5555\text{ cm}^{-1}$ ($1600\text{--}1800\text{ nm}$) and $4545\text{--}4000\text{ cm}^{-1}$ ($2200\text{--}2500\text{ nm}$), respectively (Figure 3). An NIR spectrum scan of makhwaen essential oil of different fruit maturities (MK15, MK36, MK45 and MK60) absorbed light at wavelengths of $8750\text{--}8000$, $6500\text{--}7400$, 6250 and $6000\text{--}5500\text{ cm}^{-1}$, respectively, therefore illustrating similar light transmission patterns. Essential oil containing multiple chemical compositions usually presents a much more complex peak pattern than that of the pure chemical [18]. Moreover, NIR spectrum patterns are generally used to distinguish the different types of essential oils from different plant species [15]. For instance, there was a similarity in the NIR pattern of fresh and dried lavender essential oil, but when compared with the tea tree essential oil, the spectrum peaks were different [38]. Our results illustrated that even though there were variations in chemical compositions from the essential oils of different matured fruits, there was still the similarity in NIR pattern suggesting that these essential oils were of the same plant species with the corresponding chemical finger prints.

3.4. Sensory Analysis

The terms selection from GC-MS results, following descriptors viz. citrus, herb, pine, spice, sweet and woody, were selected (Table 4). The citrus aroma is also a primary aromatic scent in many *Zanthoxylum* spp. essential oils including that of *Z. piperathum* [36], *Z. myriacanthum* [9], *Z. bungeanum* [37] and *Zanthoxylum xanthoxyloides* [39]. From the results, the panellists gave citrus and pine scents a high rating score for MK45, while sweet and woody aromas were the highest scents at MK15. The harvesting at 36 days after fruiting of makhwaen fruit showed essential oil at the maximum value of spice scent. The panellists could significantly tell the different among the samples for woody, sweet and pine scents (Table 4). However, the descriptive scoring in citrus, herb and spice aroma was

not significantly different. Descriptive data by the semi-trained panellists on PCA analysis showed the relationship of the odour attributes and different harvesting day of makhwaen fruit essential oil, to be divided into two groups (Figure 4). The first group was MK36 and MK45 with the dominant pine scent. The second group was MK15 and MK60 with the leading citrus scent. From the results of panellists regarding Japanese pepper (*Z. piperitum*) essential oil, it was found that the odour characteristics were similar to makhwaen essential oil viz. citrus-like, sweet and pine, and different harvesting stages affecting the different odour intensities of essential oil [26]. Based on the sensory analysis results from panellists, the optimal time for harvesting makhwaen fruit is at 60 days because the citrus and woody aromas were noticeably higher.

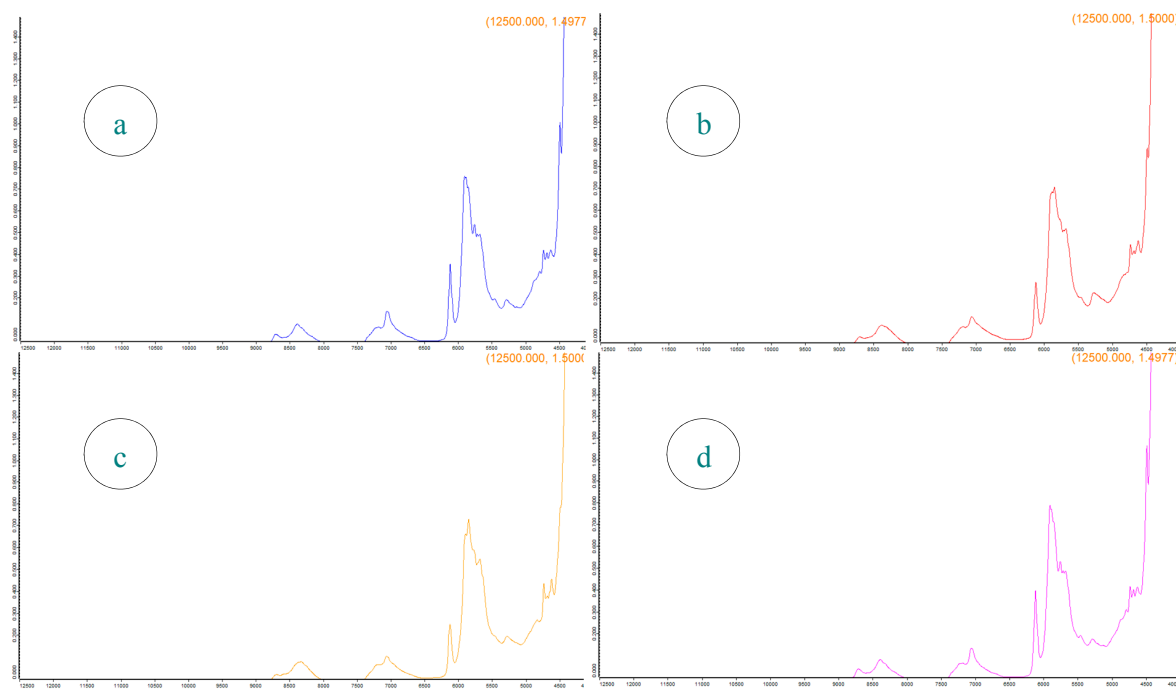


Figure 3. Near infrared spectra of the essential oils from four different harvesting time. The insertion is the inset evidence of the peaks between 4000–12,500 cm^{-1} : (a) MK15, (b) MK36, (c) MK45 and (d) MK60.

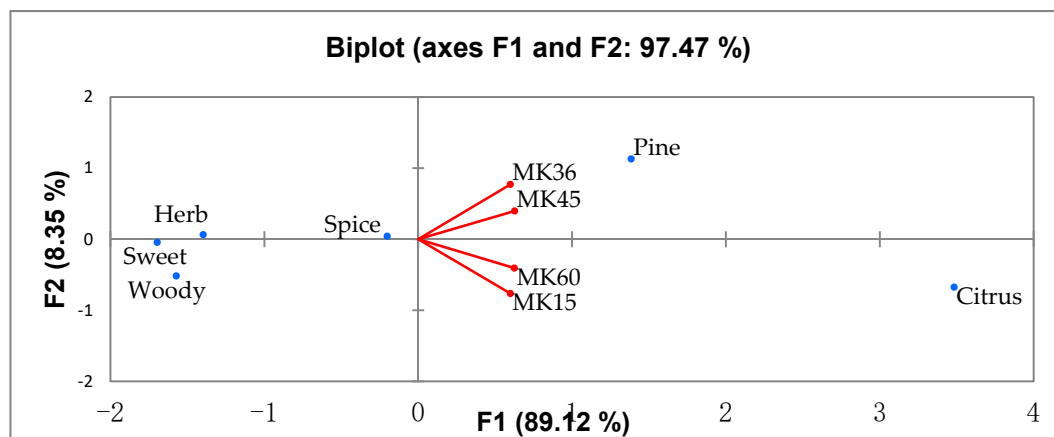


Figure 4. Principal component analysis (PCA) biplot (axes F1 and F2: 97.47%) illustrating the relationships among the odour attributes and different harvesting day of makhwaen fruit essential oil.

Table 4. Odour attributes from sensory analysis of makhwaen fruit essential oil.

Day/Odour Attributes	Citrus	Herb	Pine	Spice	Sweet	Woody
MK15	10.0 ± 1.10 ^a	3.1 ± 1.03 ^a	5.2 ± 1.38 ^a	5.2 ± 1.24 ^a	3.9 ± 0.90 ^b	4.2 ± 0.49 ^b
MK36	8.1 ± 1.17 ^a	3.5 ± 0.77 ^a	8.4 ± 0.51 ^b	5.6 ± 1.03 ^a	2.8 ± 0.73 ^{ab}	2.6 ± 0.53 ^a
MK45	10.1 ± 0.95 ^a	2.7 ± 0.73 ^a	8.5 ± 0.45 ^b	3.7 ± 0.89 ^a	3.1 ± 0.40 ^{ab}	1.8 ± 0.58 ^a
MK60	9.5 ± 0.58 ^a	3.6 ± 0.93 ^a	5.4 ± 1.34 ^a	4.3 ± 1.34 ^a	1.7 ± 0.44 ^a	3.2 ± 0.37 ^{ab}

Mean scores ($n = 5$) for each attribute within a column with different superscript letters (a,b) are significantly different at $\alpha = 0.05$ using Duncan's multiple comparison test.

4. Conclusions

The main volatile components of makhwaen essential oil were sabinene, L-limonene and β -phellandrene. The physical characteristics of makhwaen essential oil from different harvesting times using an NIR method illustrated similar light transmission patterns. The semi-trained panellists could distinguish the scent of essential oil from makhwaen fruit using the six attributes of citrus, herb, pine, spice, sweet and woody. Sensory information of essential oil from makhwaen fruits at 36 and 45 days after its initial fruiting had the highest pine aroma, and the citrus scent was dominant in all treatments. This report provides the patterns of aromatic profiles that can be used for future product development of the natural product industries.

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