

Aroma profiles and preferences of *Jasminum sambac* L. flowers grown in Thailand

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Synopsis

Comparison of volatile constituents and odor preference of *Jasminum sambac* cultivated in Thailand was performed by enfleurage and solvent extractions. Enfleurage bases consisting of spermaceti wax, olive, sunflower, and rice bran oils were prepared. The defleurage flower was daily replaced with fresh jasmine for a period of 12 days. The absolute de pomades and extraits of each base were subjected to gas chromatography mass spectrometry (GC/MS) analysis, comparing with the concrete and absolute values obtained from maceration of jasmine in *n*-hexane for 24 h. Linalool, benzyl acetate, and α -farnesene were found as the main volatile compounds in the jasmine extracts. Spermaceti wax and olive oil gave the best quality base, exhibiting the most preferred resemblance of jasmine odor with the least difference from fresh jasmine, as evaluated by 103 Thai volunteers.

INTRODUCTION

Fragrance applications, including perfumes and aromatherapy are increasing and are presented in a wide range of products for personal care, cleansing, washing, as well as consumer goods such as toilet paper, paper tissues, candles, etc. In addition, fragrance applications for indoor air modification, such as in shopping malls, offices, and restaurants are gaining importance in the present society. Therefore, production value to supply higher demand is increasing (1) and the number of fragrances in each application product is increasing accordingly, especially perfumes (2). In particular, floral notes are mainly in demand (3), although those of marine fragrance is becoming of interest in the perfumery business (4,5).

Enfleurage or cold fat extraction is one of the classical methods for aroma preparation from flowers. It is based on the absorption of volatile oil in fragrant flower onto fat for a period of time depending on the flowers. The fragrant saturated fat (pomade) is removed afterward. Thus, the fat base is of importance in enfleurage, which must be odorless with high consistency offering a semihard surface to allow sufficient absorption of fragrances

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and simply removal of the flower, retaining the fat surface. Although there are complicated and laborious steps in enfleurage, several advanced techniques have been archived in aroma preparation in addition to those of synthetic aroma materials. True natural fragrances, particularly flower oils of a certain high quality are necessary to be produced by enfleurage. Countries and regions such as Bulgaria, Egypt, Algeria, and Sicily (Italy), and in particular Grasse (France), produce high-quality natural flower oils by this method (6).

Enfleurage has been revorded since the ancient Egypt, and was largely used to prepare perfumed oils. Documented evidence shows that the pomade was applied directly to the ancient Egyptian's hair (7). However, the pomade is inconvenient to use with respect to the current living style. Therefore, further extraction with absolute ethanol producing absolute de pomade and, consequently, removal of solvent yields an extrait that has more applications (8).

Jasmine, similar to the other oriental floral note flowers, has a unique and pleasant odor, and is gaining high interest in aromatherapy and the spa business (9). These fragrance flowers are scarce and consist of heat-sensitive aromatic oil that could not be distilled. In this study, we attempted to prepare fragrance materials from jasmine cultivated in Thailand. Aroma profiles of *Jasminum sambac* were subjectively verified because it is the major cultivar cultivated in Thailand. In addition to the cultivar effect, the environmental condition and agricultural practices, including isolation method, play a major role in the variation of jasmine aroma (10). The quality of the flower oil extract was further comparatively evaluated with those of solvent extraction, and preference test of the prepared fragrance extracts and fresh jasmine was also carried out.

MATERIALS AND METHODS

PLANT MATERIALS

Jasmine (*J. sambac*) was harvested from the organic farm located in Nakhonpathum province of central Thailand, in the morning at the beginning of its blossom stage.

ENFLEURAGE BASE PREPARATION

Spermaceti wax (Namsiang Trading, Thailand) was mixed with rice bran oil (Thai Edible Oil Co., Ltd., Thailand), sunflower oil (Thanakorn Vegetable Oil Products Co., Ltd., Thailand), and olive oil (Namsiang Trading, Thailand) at a 3:2 ratio, separately. The warm melt base containing wax and oil was poured into an aluminum tray (8 × 10 in), individually.

FLOWER OILS PREPARATION

All of the solvents used were of analytical grade unless otherwise stated.

ENFLEURAGE

Jasmine petals (100 g) were strewn by hand on the top of the enfleurage base layer and wrapped by aluminum foil and paraffin film to prevent the chassis from light exposure. The pile of the chassis was kept at 20°C for 24 h. The defleuraed jasmine petals were replaced daily with fresh jasmine petals in the morning for a period of 12 days. The obtained pomade was removed with a spatula and extracted by denatured alcohol (Merck, Germany) thrice in a separatory funnel. The alcoholic fragrance solution was kept under 20°C for 1 h before filtration. These processes were repeated until the obtained filtrate was a clear solution, giving an absolute de pomade. The absolute de pomade was equally divided. The first part was kept in a solution form in a light- and air-protected vessel, whereas the other was concentrated under *vacuo* at 35°C yielding an extrait.

MACERATION

Fresh blooming jasmine petals (200 g) were gently filled in an Erlenmeyer flask (1000 ml) in which 800 ml of *n*-hexane (Merck, Germany) was added. The maceration tank was sealed from light and air exposure, and kept at room temperature for 24 h. Subsequently, vacuum filtration was carried out, followed by removal of solvent under reduced pressure at 35°C to obtain a concrete. The concrete was further partitioned with denatured alcohol (60 ml \times 3), combined and concentrated as usual, yielding an absolute.

GAS CHROMATOGRAPHY MASS SPECTROMETRY

An aliquot (1 μ l) of each sample was diluted in CH₂Cl₂ (1:1, v/v; Fisher Scientific, UK) before analysis on a gas chromatography (GC; Agilent 6890N) equipped with DB5 (Agilent 122-5532, 30 m \times 250 μ m, 0.25 μ m film thickness) column and mass spectrophotometry (MS; Agilent 5973N). The oven program started from 60°C, rising to 300°C at a rate of 7°C/min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min with a pressure of 9.32 psi. Injector was kept at 220°C and was made in split mode (split ratio = 100:1). The reference mass spectrum was obtained from MS-Wiley7n.1 database. The bases were additionally analyzed for background cutoff, eliminating fat and oil components similar to the solvents.

PREFERENCE TEST

A total of 103 nonsmoking, healthy Thai males and females aged 20–45 years, without olfactory disorders as well as fragrance and pollen allergies, were recruited for preference test. All the recruited subjects were informed about the study both in writing and verbally, and signed a written consent form, which was approved by the ethical committee of the Mae Fah Luang University before enrollment.

Four aroma samples, comprising three extraits and one absolute (20 μ l), diluted in mineral oil (1:10, v/v), supplied by Namsiang Trading (Thailand), adsorbed on filter paper (Whatman no. 1, USA), and cut in a square shape (1 \times 1 cm), were placed in an amber

vial wrapped with an aluminum foil, separately, with a sniff port diameter of 1 mm. Sniff test of each sample (1 min) was done with 1 min of resting period before the next sniff. This preference test was performed in a controlled environment room without interference factors and was conducted by a recruited volunteer who refrained from fragrance and scent products application.

Likert scale was used for odor quality (1–3) and difference (1–5) evaluations. The odor quality, compared with fresh jasmine, was divided into three levels, i.e., less, equal, and better. The score interval was [(maximum means – minimum means)/3]. On the basis of the means time, the odor difference was separated into five levels, i.e., non, slightly, moderate, high, and extreme, and the score interval was calculated similar to that evaluated for the quality, but divided by 5 (11). Fresh jasmine was used as a reference, and was prepared in a similar container with those four samples. The data were analyzed and presented as means \pm SD.

RESULTS AND DISCUSSION

Jasmine is extensively used in perfumery because of its fine, sweet, and elegant fragrance impact. Its fresh flowers have been widely used in traditional Thai aroma materials, such as garland, mobile, as well as wreath. In addition, jasmine aroma essences are prepared using several methods and forms. Furthermore, the pleasant odor of jasmine exhibiting the oriental floral note is in high demand (9). Therefore, in this study the aroma profiles of this fragrant flower were studied. Harvesting of jasmine flowers for enfleurage and solvent extractions was conducted in the morning because of the presence of higher aroma compounds (12). The jasmine aroma preparations were carried out during February–June, which is the climax flowering season in Thailand.

Spermaceti wax was used in the present enfleurage because it is white, translucent, slightly unctuous, and free of rancidity due to the presence of cetyl palmitate, cetylic alcohol, and other fatty alcohols and fatty acids, which gave a clear colorless aroma extract because of less deterioration of the wax (13). Rice bran oil was chosen due to its versatile production in Thailand, similar to sunflower oil. These two oils were compared with the imported olive oil. Although odor of the base before aroma absorption blocked the oil character, strong fresh jasmine odor was obtained afterward. Although olive oil system gave fewer yields than rice bran oil (Table I), it deteriorated less. Therefore, enfleurage base composed of spermaceti wax and olive oil was found to be the most suitable base. This was further supported by the analysis of volatiles that was noted to be in accordance with the preference result and would be discussed later. However, the solvent extraction of jasmine gave wax that produced a pungent dry jasmine odor, particularly, in the concrete.

The quantitative results of volatile constituents of jasmine aroma extracts are shown in Table II. Samples A–C were the absolute de pomades, and Samples D–F were the extractions obtained from enfleurage bases, consisting of rice bran, sunflower, and olive oils, respectively. Solvent extraction gave the absolute (G) and concrete (H), which were compared. The enfleurage extraction gave the volatiles that were in higher quantities in the absolute de pomades than in the extractions. Furthermore, 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene, germacrene D derivative in alcohol form, which is a key component for differentiating *J. sambac* from *J. grandiflorum* (14), was found. This characteristic aroma of

Table I
Appearance and Yield of Jasmine Aroma Extractions

	Appearance	Weight (g)	Yield (%)
Rice bran oil	Clear brownish red liquid with jasmine and slightly rice bran odors	2.3637	0.20
Sunflower oil	Clear orange liquid with jasmine odor	1.2389	0.10
Olive oil	Clear yellow liquid with jasmine odor	1.9371	0.16
Solvent extraction	Yellowish green wax with strong dry jasmine odor	2.6530	0.22

J. sambac was especially isolated by the olive oil system. It was found in high quantities in Samples F and C. Linalool, benzyl acetate, and α -farnesene were found to be the main aroma compounds in each jasmine extract. Linalool was observed in highest quantity in the absolute de pomades (Sample C) from spermaceti wax and olive oil system, followed by the sunflower and rice bran oil bases, respectively, similar to benzyl acetate that was found in high quantity in the jasmine extract obtained from the olive oil system (Samples F and C). Although all the absolute de pomades contained β -ocimene, methyl salicylate was found in high quantity (9.46%) in the extract of the olive oil system (Sample F). However, this ester was undetectable in the absolute de pomades. Similarly, the highest amount of indole was found in Sample F (2.47%), in addition to β -phenyl ethyl acetate and ethyl salicylate. Furthermore, the presence of *E*- α -bisabolene, naphthalene, α -muurolene, and galaxolide in the extract of base containing rice bran oil (Sample D) was also noted. However, methyl anthranilate was found only in Samples G and F. Germacrene D, δ -cadinene, and α -farnesene were present in all of the absolute de pomades. Surprisingly, the extract from sunflower oil system (Sample E) did not contain germacrene D and δ -cadinene. Furthermore, *Z*-3-hexenyl benzoate was found in every sample, particularly in the concrete (Sample H), and was not detected in Sample B. The presence of γ -cadiene and its isomer, α -cadiene, in Sample F may be explained by the suitability of olive oil for the isolation of these compounds. Furthermore, olive oil was found to isolate more ethyl oleate, particularly in the extract (Sample F). In addition, β -caryophyllene was found only in Sample F. However, benzyl salicylate and methyl stearate could be isolated by the maceration of jasmine. Several essential fatty acids were found in the absolute de pomades and extracts, such as palmitic acid, palmitinic acid, and linoleic acid. Phytosterols, namely β -sitosterol and stigmasterol, were found in high amount in Samples C and B, respectively. However, spongesterol was detected only in Sample B. The presence of free fatty acids, fatty acid esters, and phytosterols in jasmine aroma extracts was found to be contributed by the jasmine flower wax, which is in agreement with the previous study on *J. grandiflorum* (15). In particular, palmitic acid was found to be in highest quantity in both the cultivars. Volatile nitrogen compounds, including indole, nitriles, anthranilate, and imide, may be derived from the bioconversion of amino acids containing nitrogen, such as phenylalanine during the picking process of jasmine flower (16).

Benzyl acetate and *Z*-3-hexenyl benzoate, which produce the characteristic jasmine odor, were also detected as the main volatile constituents in jasmine cultivated in Thailand. The aroma profiles of jasmine (*J. sambac*) grown in different geographical conditions were further compared, as shown in Table III. When compared with the Philippines, Indonesian, and Chinese jasmine, Thai jasmine was poor in indole (16,17). In particular, indole

Table II
Volatile Constituents (%) of Jasmine Aroma Extracts

Compound	RT	Sample							
		A	B	C	D	E	F	G	H
β-Aminocrotonitrile	5.52	0.52	—	—	—	—	—	—	—
β-Ocimene	6.31	0.63	0.87	1.03	—	—	0.46	—	—
Dihydromyrcenol	7.24	—	—	—	—	—	—	—	—
Linalool	7.33	3.25	5.34	6.55	2.35	2.05	3.81	0.39	0.72
α-Terpinolene	7.80	—	—	—	—	—	—	0.39	—
Phenyl ethyl alcohol	8.19	—	—	—	—	—	—	0.16	—
Benzyl acetate	8.44	0.46	0.78	1.11	0.46	0.35	1.85	0.65	—
Methyl salicylate	9.00	—	—	—	0.14	—	9.46	—	—
Phenylacetoneitrile	9.86	—	—	0.59	—	—	—	—	—
Phenyl methyl ester	9.96	—	—	—	—	—	—	—	0.75
Linalyl acetate	10.72	—	—	—	—	—	—	—	—
Furyl pentyl ketone	10.79	—	—	—	—	—	—	0.13	—
β-Phenyl ethyl acetate	10.80	—	—	—	—	—	0.20	—	—
Ethyl salicylate	11.13	—	—	—	—	—	0.16	—	—
Indole	11.75	0.67	—	—	—	—	2.47	—	—
E-α-Bisabolene	11.95	—	—	—	0.07	—	—	—	—
Naphthalene	12.13	—	—	—	0.16	—	—	—	—
Methyl anthranilate	12.58	—	—	—	—	—	—	—	—
α-Muurolene	12.90	—	—	—	0.18	—	0.33	1.38	—
Germacrene D	13.02	0.46	0.81	0.81	0.31	—	1.32	—	—
δ-Cadinene	13.91	2.08	1.54	1.04	1.81	—	0.89	0.51	—
α-Farnesene	14.02	9.27	9.72	9.42	8.13	1.48	20.21	6.68	13.05
β-Caryophyllene	14.89	—	—	—	—	—	0.17	—	—
Z-3-Hexenyl benzoate	15.28	1.63	—	1.86	1.49	0.70	3.76	7.39	15.11
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	15.71	—	1.34	2.23	—	—	2.63	—	—
γ-Cadinene	15.93	—	—	—	—	—	0.56	—	—
Isopropyl myristate	15.98	0.62	—	—	—	—	—	—	—

Table II
Continued

Compound	RT	Sample							
		A	B	C	D	E	F	G	H
α -Cadinene	16.10	—	—	—	—	—	0.11	0.03	1.15
α -Cadinol	16.22	0.60	—	0.39	0.71	—	0.33	0.65	1.37
Nerolidol	16.56	—	—	—	—	—	0.07	0.47	0.80
Amyl salicylate	16.98	—	—	—	—	—	—	—	—
Hexyl benzoate	17.06	—	—	—	—	—	—	0.10	—
Ethyl palmitate	17.75	—	—	0.61	—	—	1.16	—	—
Benzyl benzoate	18.87	—	—	—	0.07	—	0.17	1.63	3.21
Palmitic acid	19.41	5.37	—	0.73	8.28	13.61	—	0.99	—
Ethyl oleate	19.86	—	—	3.98	—	—	7.42	—	—
Palmitic acid	20.25	—	—	—	—	1.17	—	—	—
Benzyl salicylate	20.93	—	—	—	—	—	—	0.68	0.91
Methyl palmitate	21.37	—	—	—	—	—	0.23	0.68	6.60
Geranyl linalool	22.46	—	—	—	—	—	—	2.75	5.24
β -Sitosterol	23.10	—	—	9.87	—	—	—	—	—
Stigmasterol	23.13	—	8.33	—	—	—	—	—	—
Z-Linoleic acid methyl ester	23.24	—	—	—	—	—	0.17	—	—
Methyl oleate	23.33	—	—	—	—	—	1.06	—	—
Methyl stearate	23.71	—	—	—	—	—	—	—	5.27
Ethyl linoleate	24.15	—	—	—	—	—	0.74	—	—
Linoleic acid	24.27	—	—	0.46	—	0.07	—	—	—
Chorophenylmaleimide	24.54	0.67	—	—	—	—	—	—	—
Ethyl stearate	24.65	—	—	—	—	—	0.31	—	—
1,3-Dimethyl-4-azaphenanthrene	26.40	0.56	—	—	—	—	—	—	—
Spongesterol	25.87	—	1.10	—	—	—	—	—	—

Table III
Major Aroma Compounds of *J. sambac* Absolute Grown at Different Geography

Country	%						Reference
	Benzyl acetate	Indole	α -Farnesene	Z-3-Hexenyl benzoate	Linalool	Methyl anthranilate	
Thailand	0.7	–	6.7	7.4	0.4	1.4	–
Philippines	2.0	1.0–2.0	15.0–20.0	2.0	15.0–20.0	5.0	14
Indonesia	3.0	0.1	10.0–15.0	5.0	20.0–30.0	3.0	14
China	8.0	1.5	10.0	10.0	20.0	6.0	14
Egypt	14.2	13.4	13.1	9.4	6.3	4.7	15

in the absolute of Thai jasmine was far lesser than that in the Egyptian one, similar to those of benzyl acetate, α -farnesene, linalool, and methyl anthranilate. In contrary, Z-3-hexenyl benzoate was found to be higher, when compared with that noted in the Philippines and Indonesian jasmine absolute. Although Thai jasmine absolute contained less characteristic jasmine aroma as mentioned earlier, the quantities of benzyl acetate, indole, linalool, and methyl anthranilate were high in the absolute de pomades and extraits (Table II). The preference test comparing the samples with fresh jasmine flower was further conducted.

Odor quality and difference in the extraits, including the absolute, were compared with fresh jasmine, as shown in Table IV. Sample H was excluded from this test because of its pungent and unpleasant dry jasmine odor. Similarly, all of the absolute de pomades were excluded due to the presence of solvent and poor aroma profiles. Sample F, which was the extrait obtained from spermaceti wax and olive oil base system, showed the best quality with the least difference as compared to fresh jasmine. On the other hand, Sample G showed the lowest quality with the greatest difference from fresh jasmine. Furthermore, odor quality of the absolute was significantly different from those of the extraits ($p < 0.05$); however, odor quality of the extraits was not significantly different ($p > 0.05$).

Comparative preference toward the extraits in different genders was evaluated, and it was found that both the genders' preference was not different (Table V). However, dissimilar preferences was observed among different ages. Sample F presented a significant preference ($p = 0.01$), which was higher than Samples E ($p = 0.02$) and D ($p = 0.56$). Although Sample F exhibited better quality over other extraits, its differences from fresh jasmine was not significantly different from other extraits (Table V).

Table IV
Odor Quality and Difference Compared with Fresh Jasmine

Sample	Preference	
	Quality	Difference
D	1.69 \pm 0.57	2.65 \pm 0.74
E	1.93 \pm 0.42	2.13 \pm 0.75
F	2.13 \pm 0.56	1.94 \pm 0.75
G	1.00 \pm 0.00	4.47 \pm 0.52

Table V
Odor Quality and Difference of Jasmine Aroma Extracts

Demography		Quality										Difference					
		D		E		F		G		D		E		F		G	
Gender	n	Means	p	Means	p	Means	p	Means	p	Means	p	Means	p	Means	p	Means	p
Male	52	1.69	0.97	1.94	0.76	2.15	0.71	1.00	0.00	2.08	0.47	1.90	0.63	2.48	0.42	4.38	0.11
Female	61	1.69		1.92		2.11		1.00		2.18		1.97		2.38		4.54	
Age (years)			0.56		0.02		0.01		0.00		0.17		0.29		0.29		0.03
20–25	22	1.77		1.77		2.00		1.00		2.18		2.09		2.32		4.59	
26–30	23	1.83		1.96		2.13		1.00		1.87		1.91		2.26		4.61	
31–35	24	1.63		1.79		1.88		1.00		2.33		2.04		2.67		4.25	
36–40	24	1.58		2.04		2.38		1.00		2.00		1.71		2.42		4.58	
41–45	20	1.65		2.10		2.30		1.00		2.30		1.95		2.45		4.30	

The high preference of Sample F might be due to its high volatiles content, particularly, methyl salicylate (9.46%), indole (2.47%), γ -cadinene (0.56%), ethyl palmitate (1.16%), and ethyl oleate (7.42%), which was found to be higher than those observed in other extracts. In addition to the presence of β -phenylethyl acetate, ethyl salicylate, methyl anthranilate, β -caryophyllene, α -cadinene, nerolidol, ethyl palmitate, palmitic acid, methyl palmitate, *Z*-linoleic acid methyl ester, methyl oleate, ethyl linoleate, and ethyl stearate were detected only in Sample F. These combinations contributed to its nuance jasmine aroma producing the most preferred odor.

CONCLUSION

In summary, it can be concluded that spermaceti wax and olive oil gave the best enfleurage base for jasmine aroma extraction. In addition, the aroma profile of absolute de pomades, extracts, concrete, and absolute of Thai jasmine could aid in their applicable preparations that meet the consumers' expectation of jasmine odor.

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