

Research Article

Identification of volatile compounds released from dry scented Thai flowers and their potential application in flower-mixed tea

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Abstract

Scented Thai flowers have been traditionally used for several applications. One traditional practice is to use flowers in food and drink, for example, adding scented flowers in sweets and beverages. Scented flowers provide good aroma, resulting in good emotions and commonly known as aromatherapy. The purpose of this research was to develop a new tea beverage with increased aroma of scented Thai flowers. The experiments were conducted in two steps (i) identification of volatile chemicals of dried scented flowers and (ii) consumer acceptance test of flower-mixed tea. Four scented flowers: Champak (*Michelia champaca* L.), Indian Cork flower (*Millingtonia hortensis* L.), Jasmine (*Jasminum sambac* L.), and Ylang Ylang (*Cananga odorata* Lam.) were selected. The headspace volatile compounds of the dried scented flowers were sampled by solid phase microextraction using Carboxen/Polydimethylsiloxen fibre and characterised using gas chromatography-mass spectrometry. The identified volatile compounds were grouped as alcohols, aldehydes, ketones, esters, ethers and terpenes. In every sample, the four sesquiterpenes, caryophyllene, β -bergamotene, α -cubebene and β -cubebene were found in high amounts. Characteristic compounds were also found in each sample. Champak consisted of β -terpinene, eucalyptol and epoxy linalool; Indian cork flower consisted of 1-octen-3-ol and linalyl anthranilate; Ylang Ylang consisted of germacrene D and α -longipinene; and Jasmine consisted of linalool and (Z)- β -farnesene. The consumer acceptance test (n = 406) of dried flower tea was performed by 9-point hedonic scale. The overall liking, the flower aroma preference and the acceptance of flower-mixed tea of those four flowers were significantly different. The most preferred was green tea mixed with Jasmine flowers (6.14 ± 1.68) obtaining the highest overall liking score, while green tea mixed with Champak (6.63 ± 1.85) resulted in the highest rating of

flower aroma. Consumer acceptance test showed a preference for green tea mixed with Jasmine (87.4%), followed by Champak, Indian Cork flower and Ylang Ylang, with 84.2%, 83.5% and 80.0%, respectively. This was probably due to the consumer acquaintance to jasmine tea in the market. This investigation is the first comparative study showing the potential use of different scented Thai flowers in green tea.

Keywords: aroma, beverage, SPME, GC-MS, consumer test, green tea

Introduction

The scented flowers of Champak (*Michelia champacca* L.), Indian Cork (*Millingtonia hortensis* L.), Ylang Ylang (*Cananga odorata* Lam.) and Jasmine (*Jasminum sambac* L.) have unique and good aroma. The aroma mainly originates from the petals [1], and enhances good emotions and sensation to consumers. Moreover, it has been proved that flowers added to green tea stimulate blood circulation and help the respiration system [2]. In the traditional lifestyle of Thai people, these flowers have been used for centuries and are part of Thai tradition in many aspects. During last few decades the main use of these flowers has been as natural flavourings for sweets and beverages [3, 4]. In the latter case dried scented flowers are mixed with green tea [5] and it is of interest to produce more varieties of scented green tea to meet consumer preferences. In addition, scented flowers are also used for garlands and wreaths [6], cosmetic products, air fresheners and aroma therapy [7].

This research aims to identify the volatile compounds released from four dry-scented flowers: Champak, Indian cork, Ylang Ylang and Jasmine. In addition, a comparative study of the potential use of these dry flowers in flower-mixed green tea was carried out to determine consumer acceptability.

Materials and Methods

Preparation of flower samples

The three scented flowers of Champak, Ylang Ylang and Jasmine were purchased from a local fresh market (Chiang Mai city, Thailand) and the Indian Cork flower was picked from Indian Cork trees around the main campus of Chiang Mai University. Following collection, the four scented flowers were washed with fresh and cool water and were then air-cooled on a tray. Subsequently, they were dried by using a hot air oven (R3-Controller series, Binder, Germany) at 45°C for 25 hours [8]. The obtained samples were collected, packed in zipped-lock bags and stored at 4°C for further experiments.

Volatile compound analysis using gas chromatography – mass spectrometry

The volatile compounds released to headspace of each sample were determined using solid phase microextraction technique (SPME) [9]. The 85 µm Carboxen™/Polydimethylsiloxane StableFlex™ type fibre (Car/PDMS, Supelco, USA) was used. The Car/PDMS fibre was exposed for 30 min in a headspace of a septum-capped vial containing 5 g of dry flower. Following this, the fibre was directly injected into an injection port of a gas chromatograph (GC, HP 6890N, Agilent Technologies, USA) coupled with a mass spectrometer (MS, 5973, Agilent Technologies, USA).

The GC was operated on an HP-5MS column (30 m x 0.25 mm, i.d., 0.25 μm film thickness) and helium was used as the carrier gas at a flow rate of 1.0 ml min⁻¹. The temperature program was started with an initial temperature of 40°C and kept for 1.5 min at this temperature, then heated up to 200°C with a heating rate of 6°C min⁻¹ and held for 5 min. The MS was operated in the electron impact mode with electron energy of 70 eV and scan over range 20-300 amu, 230°C source temperature. The obtained mass spectra were preliminarily interpreted by comparing with those of Enhance Chemstation Version D00.00.38 (Agilent Technologies), the Mass Spectral Search Library of the National Institute of Standards and Technology (NIST, Gaithersburg, USA).

Sensory evaluation

The 9-point hedonic scale was used for sensory evaluation and consumer acceptance of flower-mixed green tea, where 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely [10]. Overall liking, colour, tea aroma, flower aroma, tea flavour, flower flavour, astringency, bitterness and aftertaste of flower-mixed tea were evaluated. The samples were prepared by mixing dried flower sample with a commercial green tea (Raming Tea Co., Ltd., Thailand) at 1:1 w/w in a tea bag and soaking in boiled water at 100 \pm 2°C for 5 minutes [10]. The prepared tea was kept in a thermo container at 60 – 65°C. Thirty ml of the four flower-mixed green teas in plastic cups was separately served for consumer test and the samples were coded by three-digit numbers [11, 12]. There were 406 consumers involved in this study.

Data analysis

The consumer acceptance and sensory data were statistically analysed using SPSS version 10.1 (SPSS Inc., USA). The data of consumer acceptance were decoded and calculated to mean and standard deviation. The significance of the results was evaluated by an analysis of variance (ANOVA) and Tukey's test with 5% significance level. Logistic regression analysis was used to indicate the model of acceptance towards flower-mixed green tea to the attributes of the mixed-flower green tea as mentioned before.

Results and Discussion

Volatile compound analysis using gas chromatography – mass spectrometry

In this experiment, SPME sampling technique was utilised because it is uncomplicated and artefact can be excluded from sample preparation. The chromatograms of volatiles of each dried flower are shown in Figure 1. There were a total of 70 volatile compounds identified from the four scented flowers. The detected compounds are listed in Table 1. The identified volatiles belonged to aldehydes, alcohols, esters, N-containing compounds and terpenes. The majority compounds were terpenes of monoterpenes and sesquiterpenes. The volatile compounds released from these scented flowers showed that some flowers shared some volatile compounds. The two sesquiterpenes of caryophyllene and β -bergamotene were detected in relatively high abundance in all samples. A bibliographic comparison showed that these compounds were also present in an essential oil of fresh Champak flower [13] and other scented flowers, for example, bread flower [14], bacuripari [15] and chastetree flower [16]. However, it is considered that these sesquiterpenes are not likely contributors to typical odour of the flower samples. Linalool-related compounds could be more important, having a typical sweet odour. In addition, there were different volatile compounds which presented in each flower sample: Champak consisted of β -terpinene, eucalyptol and epoxy linalool in relatively high abundance; Indian cork flower consisted of linalyl anthranilate,

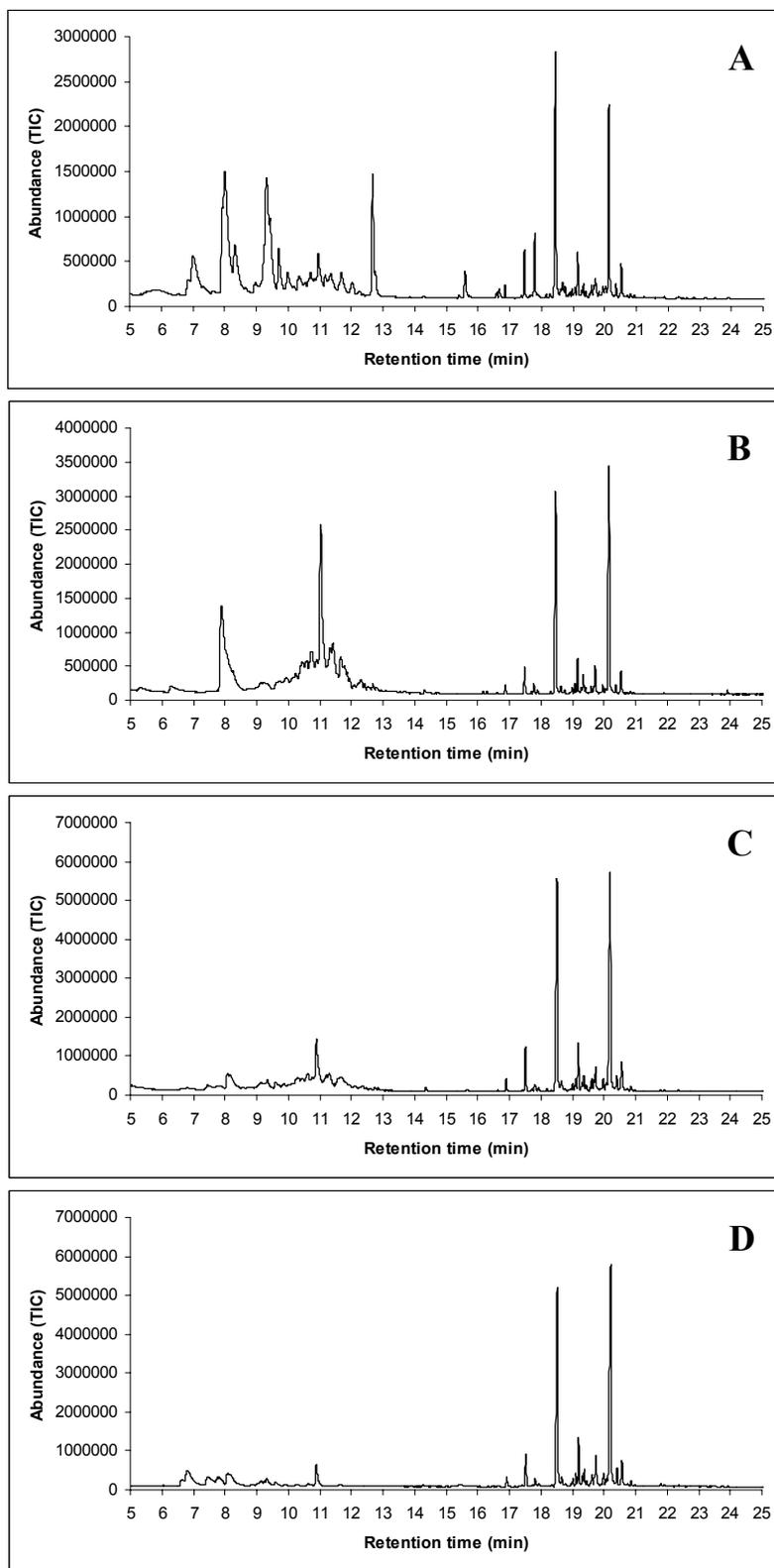


Figure 1. Chromatograms of dried flowers.

(A) Champak (*Michelia champaca* L.), (B) Indian cork flower (*Millingtonia hortensis* L.), (C) Jasmine (*Jasminum sambac* L.) and (D) Ylang Ylang (*Canaga odorata* Lam.).

caryophyllene and α -bergamotene in high abundance but 1-octen-3-ol was also detected in low abundance; Ylang Ylang consisted of caryophyllene, Z,Z,Z-1,5,9,9-tetramethyl-1,4,7-cycloundecatriene, germacrene D, α -bergamotene, and α -longipinene in relatively high abundance. Finally, characteristic compounds of Jasmine flowers were linalool, caryophyllene, α -bergamotene and cis- β -farnesene in relatively high abundance.

Table 1. Volatile compounds detected in headspace of the four dried flower samples.

Volatile compound	LRI	Characteristic odour [reference]	CP	IC	JM	YY
hexanal	903	grassy, leafy ^[a]	-	+(1.67)	+(2.73)	-
β -pinene	1039	pine-like ^[a]	-	-	-	+(7.89)
α -thujene	1039	woody green ^[a]	+(1.60)	-	-	-
α -pinene	1045	pine tree ^[a]	+(3.01)	-	-	-
benzaldehyde	1072	burnt sugar ^[a]	-	-	-	+(3.73)
1-octen-3-ol	1081	mushroom-like ^[a]	-	+(8.13)	-	-
β -terpinene	1087	NA	+(7.97)	-	-	-
sulcatone	1089	pepper, mushroom ^[a]	-	-	+(2.72)	+(4.37)
β -myrcene	1100	balsamic, spice ^[a]	+(3.59)	-	-	+(11.60)
eucalyptol	1139	minty, sweet ^[a]	+(7.63)	-	-	-
benzyl alcohol	1145	floral ^[a]	-	-	-	+(2.27)
β -ocimene	1154	citrus-like ^[a]	+(5.19)	-	-	+(2.01)
5-ethyldecane	1166	NA	-	+(1.63)	-	-
γ -terpinene	1167	gasoline, turpentine ^[a]	+(2.02)	-	-	-
2,2,4,6,6-pentamethyl-heptane	1176	NA	-	+(2.18)	-	-
hexadecane	1178	NA	-	-	+(2.24)	-
linalool oxide	1180	floral, leafy ^[a]	+(1.76)	-	-	-
triacontane	1185	NA	-	+(3.22)	-	-
hexyl pentyl ether	1190	pungent ^[b]	-	+(3.43)	-	-
2,4,4-trimethyl-2-hexene	1191	None	-	-	+(2.76)	-
α -terpinolene	1194	citrus, pine ^[a]	+(2.03)	-	-	-
linalool	1201	lavender-like ^[a]	+(3.12)	-	+(7.17)	-
linalyl anthranilate	1206	gardenia, woody ^[a]	-	+(15.13)	-	-
dihydro-2,2,6-trimethyl-6-vinyl-2H-pyran-3(4H)-one	1213	NA	+(1.83)	-	-	-
3-methyl undecane	1219	NA	-	+(4.46)	-	-
benzyl carbinol	1220	rose-like, honey ^[a]	+(1.95)	-	-	-
6-methyl undecane	1223	NA	-	-	+(1.40)	-
2,3-dimethyl octane	1223	NA	-	+(4.86)	-	-
2,7-dimethyl undecane	1232	NA	-	+(3.70)	-	-
4-picoline	1235	unpleasant ^[c]	-	-	-	+(1.85)

<i>N</i> -ethyl-aniline	1236	characteristic odour ^[d]	-	+ (2.86)	-	-
alloocimene	1247	fresh herbal ^[b]	+ (2.01)	-	-	+ (1.69)
benzyl acetate	1269	floral, Burnt ^[a]	-	-	-	+ (1.64)
epoxylinalool	1274	flower ^[a]	+ (7.84)	-	-	-
3,4-dimethoxytoluene	1342	NA	-	-	-	+ (1.76)
bornyl acetate	1390	pine, camphor ^[b]	+ (0.66)	-	-	-
indole	1398	burnt, mothball ^[a]	+ (2.02)	+ (0.53)	-	-
γ -elemene	1443	fruity, dry ^[a]	+ (0.78)	-	-	-
elixene	1444	NA	-	-	-	+ (1.40)

Table 1. cont.

Volatile compound	LRI	Characteristic odour ^[reference]	CP	IC	JM	YY
methyl anthranilate	1447	fruity ^[a]	+ (1.01)	+ (0.55)	-	-
α -cubebene	1484	herb, wax ^[a]	+ (3.21)	+ (2.86)	+ (6.23)	+ (15.98)
epi bicyclosesquiphellandrene	1497	ashy, sulphur ^[a]	+ (1.36)	-	-	+ (2.72)
β -cubebene	1498	citrus, fruit ^[a]	+ (1.01)	+ (2.91)	+ (1.27)	+ (3.08)
β -elemene	1498	herb, wax ^[a]	+ (4.29)	-	-	-
methyl eugenol	1511	clove, spice ^[a]	-	-	-	+ (1.51)
β -caryophyllene	1529	wood, Spice ^[a]	+ (14.32)	+ (17.47)	+ (25.83)	+ (3.16)
dihydro- β -ionone	1543	earthy, woody ^[a]	+ (1.23)	-	-	-
α -muurolene	1558	herb, wood ^[a]	+ (1.14)	-	-	-
(<i>Z</i>)- β -farnesene	1559	citrus, green ^[a]	-	+ (1.39)	+ (2.18)	-
β -caryophellene	1562	fruity, oily ^[a]	+ (3.00)	+ (3.67)	+ (6.66)	-
(<i>Z,Z,Z</i>)-1,5,9,9-tetramethyl 1,4,7-cycloundecatriene	1567	NA	-	-	-	+ (2.18)
α -curcumene	1575	herb ^[a]	-	+ (1.20)	+ (1.27)	-
α -amorphene	1584	NA	-	-	+ (1.94)	-
germacrene D	1595	wood, spice ^[a]	-	-	-	+ (1.99)
β -cedrene	1601	cedar wood ^[b]	-	+ (2.44)	-	-
α -copaene	1602	wood, spice ^[a]	-	-	+ (2.14)	-
α -bergamotene	1611	tea-leaf-like ^[a]	+ (11.91)	+ (13.12)	+ (26.64)	+ (2.89)
γ -cadinene	1624	thyme, wood ^[a]	-	-	+ (2.51)	-
α -longipinene	1628	NA	-	-	-	+ (2.22)
calamenene	1631	herb, spice ^[a]	+ (2.50)	+ (2.54)	+ (4.32)	+ (2.20)
2-isopropyl-5-methyl-9-methylene-bicyclo(4.4.0)dec-1-ene	1634	sweet, wood, floral ^[b]	-	-	-	+ (4.40)
1,4-cadinadiene	1646	fruity, spicy ^[a]	-	-	-	+ (3.74)
α -cadinene	1651	NA	-	-	-	+ (1.63)
α -calacorene	1656	woody ^[a]	-	-	-	+ (1.70)
elimicene	1660	flowery, spice ^[a]	-	-	-	+ (1.76)
aristolene epoxide	1666	NA	-	-	-	+ (1.55)
caryophyllene oxide	1697	sweet, spice ^[a]	-	-	-	+ (1.70)
<i>trans</i> -6-dimethyl-3,6-diethyl-3-tricyclo(3.1.0.0(2,4))-hexane	1725	NA	-	-	-	+ (2.23)

9-methyl-S-octahydro anthracene	1732	NA	-	-	-	+(1.55)
	1749	NA	-	-	-	+(1.60)
4,4-dimethyltetracyclo (6.3.2.0(2,5).0(1,8) tridecan-9-ol			-	-	-	+(1.60)

Flower samples: CP = Champak, IC = Indian cork flower, JM = Jasmine, YY = Ylang Ylang

- not detected; + (percentage of peak area), detected

LRI = Calculated linear retention index

NA: data not available

^a <http://www.flavornet.org>

^b <http://www.pherobase.com>

^c <http://www.cityu.edu.hk/fse/safety/msds/eng/msds317.pdf>

^d http://www.ilo.org/public/english/protection/safework/cis/products/icsc/dtasht/_icsc13/icsc1385.pdf

Sensory evaluation

To evaluate the consumer acceptance of flower-mixed green tea, a Jasmine-mixed green tea was used to obtain basic data for comparison since it is available in the market and accepted by consumers. As shown in Table 2, the sensory panel preferred green tea mixed with Jasmine followed by Champak, Ylang Ylang and Indian Cork flowers. This was probably due to their prior acquaintance with Jasmine-mixed green tea. Champak-mixed green tea possessed some sensory attributes which were better than jasmine-mixed green tea. These characters were colour, flower aroma and flower flavour. The tea aroma preference of the two flower-mixed green teas was similar. Flower flavour of green tea mixed with Indian cork and Ylang Ylang flowers were least favoured. More details of consumer test are given in Table 2.

Table 2. Mean hedonic ratings of flower-mixed green tea as rated by consumers.

Attribute	Flower sample			
	Champak	Indian cork	Jasmine	Ylang Ylang
Overall liking	5.53 ± 1.87 ^b	5.15 ± 1.96 ^c	6.14 ± 1.68 ^a	5.35 ± 1.90 ^{bc}
Colour	6.79 ± 1.36 ^a	6.33 ± 1.84 ^c	6.44 ± 1.47 ^{bc}	6.57 ± 1.37 ^b
Tea aroma	6.14 ± 1.59 ^a	5.66 ± 1.71 ^b	6.21 ± 1.55 ^a	5.43 ± 1.81 ^c
Flower aroma	6.63 ± 1.85 ^a	5.52 ± 1.85 ^b	5.74 ± 1.72 ^b	5.48 ± 2.04 ^b
Tea flavour	5.53 ± 1.82 ^b	5.18 ± 1.81 ^c	5.97 ± 1.65 ^a	5.19 ± 1.92 ^c
Flower flavour	5.82 ± 1.83 ^a	5.26 ± 1.82 ^c	5.75 ± 1.75 ^b	5.11 ± 2.09 ^c
Bitterness	4.46 ± 2.11 ^b	3.74 ± 2.20 ^c	5.05 ± 2.05 ^a	4.42 ± 2.29 ^b
Astringency	4.62 ± 2.05 ^b	3.87 ± 2.05 ^c	5.23 ± 1.89 ^a	4.67 ± 2.08 ^b
Aftertaste	4.92 ± 1.95 ^b	4.64 ± 2.12 ^b	5.77 ± 1.96 ^a	4.77 ± 2.14 ^b

The different letters in the same row mean significantly different ($p \leq 0.05$)

The acceptance model of flower-mixed green tea (1.1) was created according to logistic regression analysis (hit rate at 77.4%). The factors that affected the consumer acceptance were flower aroma, flower flavour, bitterness, astringency and aftertaste (Table 3).

Logistic regression of acceptance (Hit rate = 77.4%)

$$Y = -4.469 - 0.007 * \text{Colour} + 0.059 * \text{Tea aroma} + \mathbf{0.143 * \text{Flower aroma}} - 0.089 * \text{Tea flavour} + \mathbf{0.179 * \text{Flower flavour}} + \mathbf{0.282 * \text{Bitterness}} + 0.142 * \text{Astringency} + \mathbf{0.299 * \text{Aftertaste}} \quad (1.1)$$

Table 3. Hedonic attributes affecting flower-mixed green tea acceptance by logistic regression.

Hedonic attribute	Beta	Wald's chi-square	Significance (p ≤ 0.05)	Odd ratio [Exp(B)]
Colour	-0.007	0.026	0.872	0.993
Tea aroma	0.059	1.487	0.223	1.061
Flower aroma	0.143	8.611*	3.34E-3	1.154
Tea flavour	-0.089	2.861	0.091	0.915
Flower flavour	0.179	11.597*	0.001	1.195
Bitterness	0.282	30.522*	3.30E-8	1.326
Astringency	0.142	6.248*	0.012	1.152
Aftertaste	0.299	46.635*	8.55E-12	1.349
Constant	-4.469	158.600	2.29E-36	0.011

Conclusions

The identified compounds of the four dried flower samples belonged to many chemical groups, but they were mostly sesquiterpenes. However, it is still unclear which of the identified volatile compounds contribute to the typical aroma of each of the four flowers. Therefore, it will be of interest to continue this study, particularly in regard to human olfaction.

The hit rate of flower-mixed green tea acceptance was 77.4 %, indicating the potential application of scented flower in flower-mixed tea. The sensory evaluation revealed that the Champak flower showed higher potential to be used for flower-mixed green tea.

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