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Chemical composition and comparison of genetic variation of commonly available Thai garlic used as food supplement

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Summary

In order to classify true garlic cultivars, comparisons of oil composition and genetic of three garlic cultivars (*Allium sativum* L.) commonly used for essential oil production in the northern Thai market [viz., Thai (TH), Chinese (CH) and Pingpong (PP) cultivars] were carried out. Garlic essential oils were obtained by hydrodistillation and microwave hydrodistillation which were then analysed for chemical components by gas chromatography-mass spectrometry. The RAPD data suggests similarity (>95 %) of the three cultivars in chemical compositions, and the major compounds are trisulphide, di-2-propenyl, the disulphide, di-2-propenyl, and the trisulphide, methyl 2-propenyl. Sulphur-containing compounds (R_f = 0.18-0.2) were detected by thin-layer chromatography (TLC) with ninhydrin staining reagent. The essential oil of CH from hydrodistillation and microwave hydrodistillation showed the highest alliin content. The RAPD analysis of the three garlic cultivars presents 45 fragments. A dendrogram shows genetic similarity between the garlic cultivars. The TH and the CH showed similarity value as 0.93, while the PP was classified as a different cluster. Though there was considerable similarity between the chemical and the genetic profiles of the TH and the CH, the CH demonstrated high potential as an ingredient in food supplement products due to its high alliin content.

Abbreviations: CAS, chemical abstracts service; CH, garlic cv. Chinese; GC-MS, gas chromatography-mass spectrometry; MOG, garlic essential oil from microwave hydrodistillation; OG, garlic essential oil from hydrodistillation; PP, garlic cv. Pingpong; TH, garlic cv. Thai; TLC, thin layer chromatography.

Introduction

Besides being an important spice in many cultures, garlic (*Allium sativum* L.) is also well known for its excellent medicinal properties (Velíšek et al., 1997). The major components found in garlic cloves are sulphur-containing compounds such as allicin, alliin, ajoene, diallyl disulphide, dithiin and S-allylcysteine. These compounds are responsible for the pungent smell and taste of garlic (Gafar et al., 2012). Among those, allicin is the most abundant (70 % of the overall thiosulphates) present in fresh garlic which can be activated upon mechanical crushing (Miron et al., 2004). This compound possesses powerful antibiotic, antimicrobial, anticancer and other properties. Essential oil of garlic also contains a number of sulphides such as diallyl disulphide and dilly trisulphide; however, allicin can be completely eliminated during thermal or chemical extraction processes (Block, 1985).

Food supplement products made from garlic are available in plenty, including, for example, garlic essential oil capsules, dehydrated garlic powder, garlic essential oil macerate and aged garlic extract (Amagase et al., 2001; Gafar et al., 2012). The challenge for food processors is to control the products in terms of quality, and, thus, at the starting point, searching for a suitable raw material is vitally important (Velíšek et al., 1997). By dealing with major fresh garlic suppliers in Thailand, we identified that there was a minimum of three garlic cultivars in the market. These types include locally grown cultivars, such as 'Thai' and 'Pingpong'. The latter is named after its colour and shape. The 'Chinese' cultivar is said to be imported through the Thai-Burmese border; however, its real origin is unknown. Classification of 'true' variety of garlic can be done by using different techniques including morphological comparison and chemical analyses such as volatile components, DNA fingerprints and contents of bioactive compounds (Trirongjitmoah et al., 2015).

In this study, we compared three garlic specimens generally available in Thai markets based on their morphology, bioactive components, volatile profiles of essential oils and variation in their DNA fingerprints. The aim was to classify the sources of raw materials and to control for quality the final products produced by Thai food producers.

Materials and methods

Plant material

Garlic bulbs were supplied by Chiang Mai Harvest Co., Ltd., the major garlic supplier from Chiang Mai, Thailand. Three garlic cultivars, commonly known as (1) garlic cv. Thai -(TH), (2) garlic cv. Chinese -(CH) and (3) garlic cv. Pingpong -(PP) were examined for morphology and stored in commercial warehouse conditions until use.

Extraction of garlic essential oil

Essential oil of garlic was extracted by hydrodistillation from fresh garlic cloves (200 g) with 1 L of water for 3 h. The essential oil was collected from a Clevenger trap and dried over anhydrous sodium sulphate. The yield of the oil obtained (OG) was calculated as a percentage (v/w) (Sowbhagya et al., 2009). The same amount of garlic was also used for garlic essential oil extraction using microwave hydrodistillation and gravity unit at 700 W without the addition of water (Sommano et al., 2015). The yield of the extract (MGO) was also accessed.

Analysis of chemical composition of garlic essential oil by gas chromatography-mass spectrometry (GC-MS)

The oil was diluted in hexane (0.01%) prior to injection onto a GC-MS system (BRUKER, SCION SQ 436-GC). The gas chromatographic column was RESTEK (Rxi®-5sil MS, 30 m, 0.25 mmID, 0.25 um). The column was maintained at 45 °C for 1 min, and then heated to 245 °C at 4 °C min^{-1} using helium as the carrier gas (2 cm^3/min) (Weinberg et al., 1993). The mass spectrometer was run in electron ionization mode (EI 70 eV); source temperature, 100-325 °C; quadrupole temperature, 90 °C; mass scan range, m/z 1-1200 Da; and scan rate, up to 14,000 Da s^{-1}, in a complete scan

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acquisition mode. The comparison of MS fragmentation patterns with those from the National Institute of Standards and Technology (NIST) MS 98 library was performed. The relative peak areas (RAs) of a single compound were expressed in relation to the total peak area of the identified compounds. Cluster analysis was performed based on 75 chemical compositions of garlic essential oil using the statistical package XLSTAT version 2015.5.01.23234 software. The coefficients of genetic similarity for all pair-wise comparisons were computed using Jaccard's coefficient of similarity and then the distance matrix was subjected to cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to produce a dendrogram.

Identification of sulphur-containing compounds by Thin Layer Chromatography

Separation of chemical compositions from essential oil (5-10 µl) was achieved by Thin Layer Chromatography (TLC silica gel 60 F254, Merck) with glacial acetic acid:propanol:water:ethanol (20:20:20:20) as the mobile phase. Sulphur-containing compounds were visible after spraying the TLC plates with ninhydrin reagent (Belemkar et al., 2013; Keusgen, 1997). The retention factor (R_f) is the result of the distance travelled by the compound divided by the distance travelled by the mobile phase.

Analysis of alliin content by spectrophotometry

The alliin content was analysed using the spectrophotometry method with some adaptations (Miron et al., 2002). Briefly, garlic essential oil (4 µl) was incubated with 1.1×10^{-4} M of mercaptopyridine (4-MP) in 50 mM sodium phosphate buffer, pH 7.2, containing alliinase. The decrease in the optical density (OD) at 324 nm was determined after 30 min of incubation at room temperature. The alliin concentration was calculated using the following equation:

$$[\text{alliin}] = \Delta A_{324} \times \text{dilution} \times [\varepsilon_M]^{-1},$$

where ΔA_{324} = [OD without extract] – [OD with extract]
ε_M = Molar extinction coefficient at 324 (4MP) = 19,800

DNA analysis

Total genomic DNA was extracted using the CTAB (hexadecyltrimethylammonium bromide) method with some modification (Doyle and Doyle, 1987). Into the ground sample, 1000 µl of the extraction buffer [100 mM Tris–HCl pH 8.0, 20 mM EDTA (ethylenediaminetetracetate) pH 8.0, 1.4 mM NaCl and 4% (w/v) CTAB] was added and the sample was incubated at 65 °C for 1 h. Thereafter, the sample was further extracted with 600 µl chloroform:isoamyl alcohol [24:1 (v/v)] and centrifuged at 13,000 rpm for 10 min. The resultant supernatants were transferred to a new microcentrifuge tube. The air-dried pellet was re-suspended in 50 µl TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA and pH 8.0). The DNA samples were stored at -20 °C prior to RAPD analysis.

DNA quantification

DNA was quantified by using nano-drop spectrophotometer (ND-1000, spectrophotometer). For re-quantification, the extracted DNA was run on 1% agarose gel electrophoresis using 1× TBE buffer at 5-8 V/ml for 30 min and visualised under BLook LED transilluminator (Genedirex, Taiwan) by staining with MaestroSafe™ (Maestrogen, USA). The DNA solution was diluted with sterile distilled water to a concentration of 50 ng/µl for PCR analysis and kept in -20 °C until use.

RAPD-PCR protocols

For RAPD analysis of the genomic DNA, 10-base primers from Operon Technologies (Alameda, USA) and UBC (University of British Columbia, Canada) were chosen (Tab. 1). A total of 10 primers were screened. The polymerase chain reaction (PCR) was adjusted to 10 µl containing 8 µl of OnePCR™ Plus (Genedirex, Taiwan), 1 µl of 1 µM RAPD primer and 1 µl of 10 ng genomic DNA. All the reactions were carried out on a Flexcycler^2 thermal cycler (Analytik Jena, Germany) using the following profile: 1 cycle, 94 °C, 4 min; 40 cycles, 94 °C, 30 s; 37 °C, 30 s; 72 °C, 60 s; 1 cycle, 72 °C, 10 min. The sample was separated in a 1.5% agarose gel in 1× TBE buffer. The samples were run at 70 V for 90 min. The gels were then visualised using the BLooK LED transilluminator (Genedirex, Taiwan).

Tab. 1: Sequences of oligonucleotide primers used for RAPD analysis

Primer name	Sequence	Number of score bands	Number of polymorphic bands	Percent polymorphism
OPA08	GTGACGTAGG	8	7	87.5
UBC106	AGGAGTCGGA	6	4	66.6
UBC120	AGACCCTTGG	7	7	100
UBC155	CTGGCGGCTG	8	3	37.5
UBC184	CAAACGGCAC	6	5	83.3
UBC215	TCACACGTGC	5	2	40
UBC237	CGACCAGAGC	5	0	0
UBC275	CCGGGCAAGC	5	4	80

Statistical analysis

The extractions were performed in triplicate. The data were analysed by using the IBM SPSS statistical software version 22. The means were subjected to comparison by using the one-way analysis of variance (ANOVA) and the Ducan's post hoc multiple comparisons at the 99% confidence level. The banding pattern for each primer was scored as diallelic (1 = band present, 0 = band absent), and stored in an Excel (Microsoft) spreadsheet file in the form of a binary matrix. In order to assess the genetic differentiation between the three garlic accessions, eight RAPD markers were analysed using the statistical package XLSTAT version 2015.5.01.23234 software. The coefficients of genetic similarity for all the pair-wise comparisons were computed using the Jaccard's coefficient of similarity, and then the distance matrix was subjected to cluster analysis by using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to produce a dendrogram.

Results and discussion

Garlic bulb is solitary with globose to applanate-globose shapes depending upon the variety. In general, garlic is 2-6 cm in diameter, consisting of several bulblets. The bulblets are covered with white to purple tunic and underneath the tunic are layers of scales which are held together by the basal plate. The basal plate is located at the bottom of the bulb (Wu et al., 1998). Morphological differences of the Thai garlic bulbs used in this experiment are shown in Tab. 2 and Fig. 1.

The major chemical components of garlic are sulphur-containing compounds which are measured in the forms of cysteine sulfoxides [i.e., alliin (1)] and non-volatile γ-glutamylcysteine peptides (>82%) (Sendl, 1995). The representatives of this group are as shown in Fig. 2: thiosulphinates [i.e., allicin (2)], ajoenes [i.e., *E*-ajoene (3)

Tab. 2: The morphological descriptions of three garlic cultivars

Cultivars	Bulb (cm)	Weight (g)	Shape	Colour of tunic	No. of bulblets	Clove size (cm)	Weight (g)	Garlic flavour
TH	3.7–4.3	14.78–20.85	Round	Pinkish white	21–23	1×2–2.3	0.79–1.33	++
CH	1.8–2.5	7.91–12.87	Oval	Opaque white–purple	4–11	0.9-2.1×1.9-3.0	0.68–2.35	+++
PP	5–5.5	32.23–51.33	Round	Pinkish white–purple	12–14	1.3-1.7×3.2-3.5	2.28–5.97	++

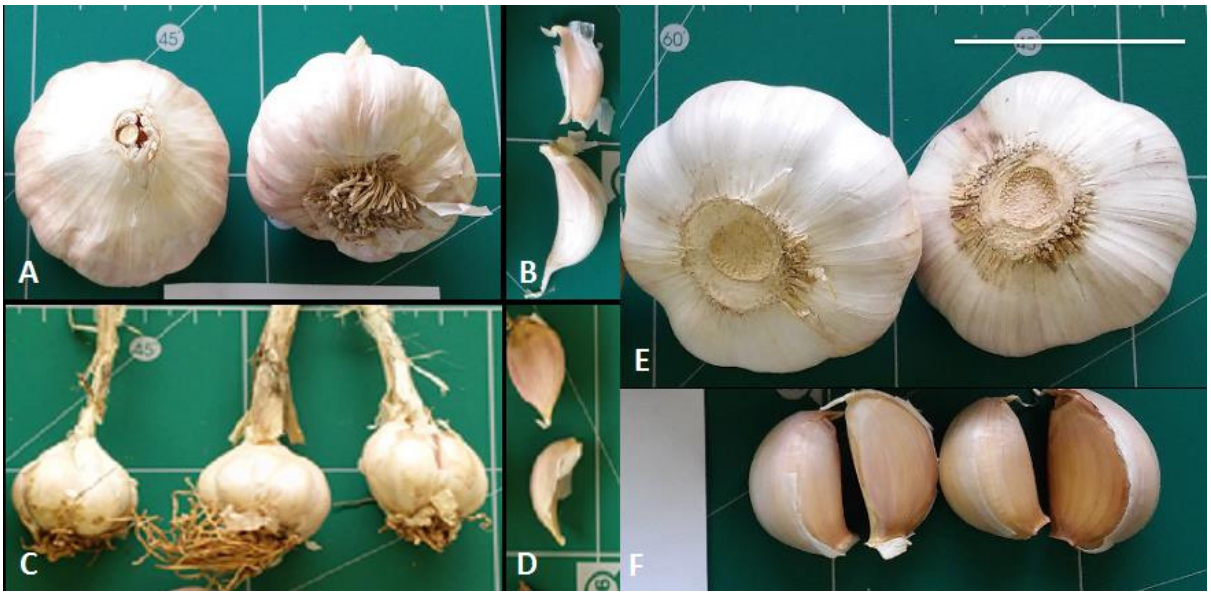

Fig. 1: Garlic bulb and bulblets of *A. sativum* L. cv. Thai (A-B), cv. Chinese (C-D) and cv. Pingpong (E-F). Scale bar = 5 cm.

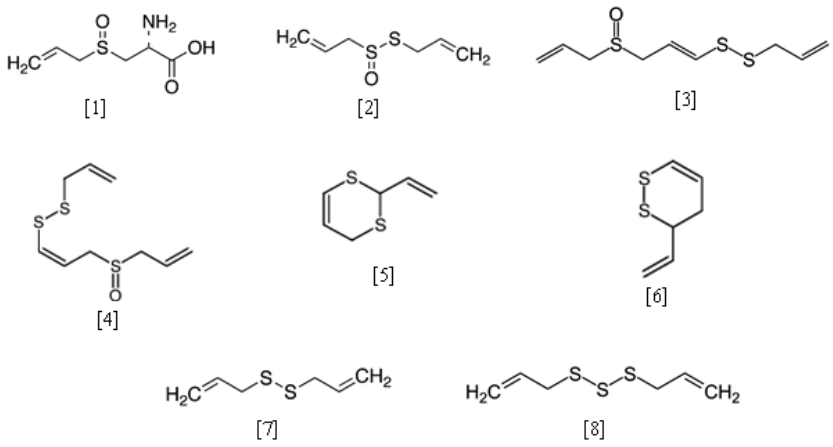

Fig. 2: The chemical structures of sulphur-containing compounds found in garlic.

and *Z*-ajoene (4)], vinyldithiins [i.e., 2-vinyl-(4*H*)-1,3-dithiin (5) and 3-vinyl-(4*H*)-1,2-dithiin (6)] and sulphides [i.e., diallyl disulphide (7) and diallyl trisulphide (8)]. Among those, diallyl sulphides (57%), allyl methyl (37%) and dimethyl mono- to hexasulphides (6%) are most commonly found in garlic essential oil (Cerella et al., 2011).

Essential oil from garlic specimens of the TH, CH and PP was analysed for the chemical profiles by GC-MS. All the tested samples consisted of similar major chemical components, which were trisulphide, di-2-propenyl (40.97-45.17%), disulphide, di-2-propenyl (CAS) (16.95-25.59%) and trisulphide, methyl 2-propenyl (CAS) (13.41-14.43%) (Tab. 3).

These results are in line with the findings of the work reported by Lalla (2013). The garlic essential oil from *A. sativum* for. *pekinense* Makino had disulphide, di-2-propenyl, 32.82%, and trisulphide, di-2-propenyl, 29.12%, and essential oil of *A. sativum* var. Jam Nagar gave trisulphide, di-2-propenyl, as high as 60%, and disulphide, di-2-propenyl, 13.07% (Pyun and Shin, 2006; Sowbhagya

Tab. 3: Chemical profiles of garlic essential oil analysed by GC-MS

No.	Compounds	% of total compounds of garlic essential oil										
		TH[1]	CH[1]	PP[1]	Morocco origin[2]	Chinese origin[3]	Egyptian origin[3]	Mexican origin[3]	for. *pekinense* Makino[4]	Tunisian origin[5]	var. Jam Nagar[6]	var. sativum[7]
1	1,2-Ditriacyclo-pentane	0.83±0.05a	0.85±0.11a	0.81±0.14a	-	-	-	-	-	-	-	-
2	1-Propene,3,3-thiobis (CAS)	0.74±0.06a	0.45±0.06a	1.39±0.12b	3.93	-	-	-	-	-	-	-
3	Disulphide, methyl 2 – propenyl (CAS)	2.07±0.20a	1.63±0.10a	4.12±0.14b	1.71	-	-	-	-	-	-	-
4	3-Chlorocrotonal-dehyde	1.28±0.46a	0.97±0.22a	0.99±0.38a	-	-	-	-	-	-	-	-
5	Ethoxycyclohexyldi-methylsilane	-	-	-	-	-	-	-	-	-	-	-
6	Allyl thiol	-	-	-	-	0.15	0.21	0.14	-	-	-	-
7	Methyl allyl sulphide	-	-	-	-	0.64	1.21	3.15	-	-	-	-
8	Dimethyl disulphide	-	-	-	-	0.23	0.41	0.88	-	-	-	1
9	Diallyl sulphide	-	-	-	-	4.25	3.69	6.96	-	2.30	-	1.20
10	Methyl allyl disulphide	-	-	-	-	2.23	5.37	15.25	-	1.70	-	-
11	Allyl methyl sulphide	-	-	-	-	-	-	-	-	-	-	-
12	Dimethyl disulphide	-	-	-	-	-	-	-	-	-	-	-
13	Diallyl sulphide	-	-	-	-	-	-	-	-	-	-	-
14	Allyl methyl disulphide	-	-	-	-	-	-	-	-	-	-	-
15	3,3-Thio bis-1-propene	-	-	-	-	-	-	-	0.87	-	-	-
16	2,4-Dimethyl thiophene	-	-	-	-	-	-	-	0.03	-	-	-
17	2,5-Dimethyl thiophene	-	-	-	-	-	-	-	0.06	-	-	-
18	Methyl cis-propenyl disulphide	-	-	-	-	-	-	-	0.13	-	-	-
19	Methyl trans-propenyl disulphide	-	-	-	-	-	-	-	0.24	-	-	-
20	N,N'-Dimethyl thiourea	-	-	-	-	-	-	-	1.46	-	-	-
21	Trisulphide, dimethyl (CAS)	0.80±0.17a	0.58±0.11a	0.85±0.14a	-	0.18	1.3	1.44	0.51	-	-	-
22	Disulphide, di-2-propenyl (CAS)	14.74±3.72a	16.00±0.66a	18.06±4.74a	14.30	-	-	-	32.82	-	13.07	-
23	Diallyl disulphide	2.19±0.90a	0.92±0.10a	1.60±0.44a	0.46	-	-	-	-	-	-	-
24	Diallyl disulphide	1.64±0.11a	1.25±0.11a	3.19±0.40b	-	-	-	-	-	-	-	-
25	Methyl isopentyl disulphide	-	-	-	-	-	-	-	-	-	-	19.00
26	Methyl allyl disulphide	-	-	-	-	-	-	-	-	-	-	-
27	Dipropyl disulphide	-	-	-	-	-	-	-	0.11	-	-	-
28	Diallyl disulphide	-	-	-	-	-	-	-	-	-	-	-
29	Trans-propenyl propyl disulphide	-	-	-	-	-	-	-	0.30	-	-	-
30	Trisulphide, methyl 2-propenyl (CAS)	11.34±1.37a	13.14±0.17a	11.12±1.54a	10.88	-	-	-	7.40	-	-	-
31	Ally1 methyl trisulphide	-	-	-	-	-	-	-	-	-	-	-
32	Methyl propyl trisulphide	-	-	-	-	-	-	-	0.15	-	-	-
33	1,3,5-Trithiane	-	-	-	-	-	-	-	0.26	-	-	-
34	3-Methyl thio 1-propene	-	-	-	-	-	-	-	0.03	-	-	-
35	4,5-Dimethyl thiazole	-	-	-	-	-	-	-	0.02	-	-	-
36	1-Methyl thio-1-propene	-	-	-	-	-	-	-	0.12	-	-	-
37	3-Vinyl-1,2-dithiane	-	-	-	-	-	-	-	-	-	1.46	-

[1]Data are expressed as mean ± SD of triplicate hydrodistillation (OG) extracts. Means in the same row with different letters (a–b) are significantly different (P<0.01), ANOVA, Ducan, IBM SPSS Statistics. [2]Garlic essential oil of Morocco origin (DOUIRI et al., 2014) [3]Garlic essential oil of Chinese, Egyptian and Mexican origin (SHAATH and FLORES, 1995) [4]Garlic essential oil of for. *pekinense* Makino of Seoul (PYUN and SHIN, 2006) [5]Garlic essential oil of Tunisian origin (DZIRI et al., 2014) [6]Garlic essential oil of Jam Nagar variety of India (SOWBHAGYA et al., 2009) [7]Garlic essential oil of sativum variety of Iran (KHARAZI, 2005). Data was compared without consideration of different GCMS conditions from different reports.

Tab. 3: Chemical profiles of garlic essential oil analysed by GC-MS (continued)

No.	Compounds	% of total compounds of garlic essential oil										
		TH[1]	CH[1]	PP[1]	Morocco origin[2]	Chinese origin[3]	Egyptian origin[3]	Mexican origin[3]	for. *pekinense* Makino[4]	Tunisian origin[5]	var. Jam Nagar[6]	var. sativum[7]
38	2-Vinyl-1,3-dithiane	-	-	-	-	-	-	-	-	-	3.42	-
39	3-Vinyl-[4H]-1,2-dithiin	2.00±0.43a	1.83±0.09a	1.43±0.22a	2.76	-	-	-	1.99	-	-	-
40	Diallyl trisulphide	-	-	-	-	-	-	-	-	-	-	-
41	2-Vinyl-[4H]-1,3-dithiin	-	-	-	1.64	-	-	-	-	-	1.85	-
42	2-Vinyl+H)- 1,3-dithiin	-	-	-	-	-	-	-	5.87	-	-	-
43	1,2,3-Thiadiazole,5-methyl-(CAS)	2.12±0.10a	2.42±0.30a	1.71±0.23a	-	-	-	-	-	-	-	-
44	3-Vinyl-1,2-dithiacyclohex-5-ene	4.60±0.54a	4.61±0.24a	3.44±0.13a	-	-	-	-	-	-	-	-
45	2,5-Dimethylthiazole	-	-	-	-	-	-	-	0.01	-	-	-
46	Trisulphide, di-2-propenyl	21.59±11.05a	42.57±2.60a	14.03±8.44a	46.52	-	-	-	29.12	-	60.00	-
47	Isobutyl isothiocyanate	1.57±0.30a	1.28±0.20a	1.73±0.16a	-	-	-	-	-	-	-	-
48	3,5-Diethyl-1,2,4-Trithiolane	-	-	-	-	-	-	-	-	-	1.00	-
49	5-Methyl-1,2,3,4-tetrathia-cyclohexane	-	0.72±0.09b	0.45±0.11b	-	-	-	-	-	-	-	-
50	Disulphide, methyl 2-propenyl (CAS)	-	0.90±0.21b	0.64±0.15b	-	-	-	-	-	-	-	-
51	1,2-Dihydrocyclobutabenzene	0.64±0.21a	1.00±0.27a	0.50±0.11a	-	-	-	-	-	-	-	-
52	Diallyl disulphide	4.34±0.81a	4.74±1.10a	4.01±1.02a	-	28.6	27.45	42.46	-	-	-	-
53	trans-Propenyl methyl disulphide	-	-	-	-	-	-	-	-	0.40	-	-
54	SULPHUR,MOL.(S8)	1.15±0.18a	1.28±0.13a	0.87±0.16a	-	-	-	-	-	-	-	-
55	Allyl propyl disulphide	-	-	-	-	0.57	0.74	0.28	-	-	-	-
56	$C_6H_{10}S_2$ (Tent ID)	-	-	-	-	0.91	1.92	0.03	-	-	-	-
57	Methyl allyl trisulphide	-	-	-	-	6.77	16.82	10.36	-	-	-	-
58	Dimethyl trisulphide	-	-	-	-	-	-	-	-	0.20	-	-
59	Diallyl disulphide	-	-	-	-	-	-	-	-	29.10	-	-
60	Methyl allyl trisulphide	-	-	-	-	-	-	-	-	10.40	-	-
61	2-Vinyl-1.3-dithiane	-	-	-	-	-	-	-	-	3.90	-	-
62	1.4-Dimethyl tetrasulphide	-	-	-	-	-	-	-	-	0.40	-	-
63	Diallyl trisulphide	-	-	-	-	50.92	35.30	12.52	-	37.30	-	3.20
64	Eugenol	-	-	-	-	-	-	-	-	0.40	-	-
65	α-Caryophyllene	-	-	-	-	-	-	-	-	0.30	-	-
66	α-Guaiene	-	-	-	-	-	-	-	-	1.00	-	-
67	Aromadendrene	-	-	-	-	-	-	-	-	1.70	-	-
68	α-Bisabolene	-	-	-	-	-	-	-	-	2.10	-	-
69	γ-Cadinene	-	-	-	-	-	-	-	-	4.30	-	-
70	Di-2-propenyl tetrasulphide	-	-	-	-	-	-	-	-	-	5.01	-
71	Diallyl tetrasulphide (Tent ID)	-	-	-	-	0.74	0.70	1.09	6.35	3.00	-	-
72	1,4-Dimethyl tetrasulphide	-	-	-	-	-	-	-	0.28	-	-	-
73	1,2-Dithiane-4-one	-	-	-	-	-	-	-	0.05	-	-	-

[1]Data are expressed as mean ± SD of triplicate hydrodistillation (OG) extracts. Means in the same row with different letters (a–b) are significantly different ($P<0.01$), ANOVA, Ducan, IBM SPSS Statistics. [2]Garlic essential oil of Morocco origin (DOUIRI et al., 2014) [3]Garlic essential oil of Chinese, Egyptian and Mexican origin (SHAATH and FLORES, 1995) [4]Garlic essential oil of for. *pekinense* Makino of Seoul (PYUN and SHIN, 2006) [5]Garlic essential oil of Tunisian origin (DZIRI et al., 2014) [6]Garlic essential oil of Jam Nagar variety of India (SOWBHAGYA et al., 2009) [7]Garlic essential oil of sativum variety of Iran (KHARAZI, 2005). Data was compared without consideration of different GCMS conditions from different reports.

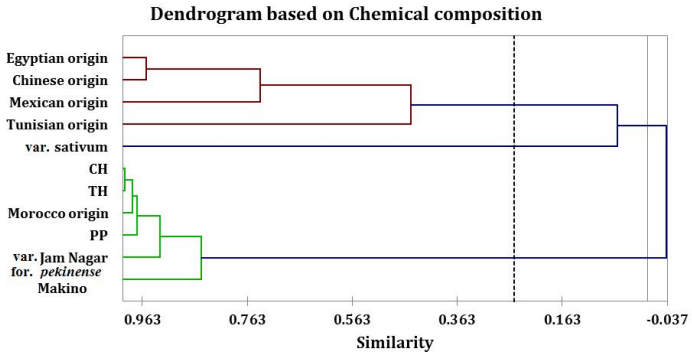

Fig. 3: The dendrogram of the chemical composition of garlic oil obtained from *A. sativum* L. cv.Thai (TH), cv. Chinese (CH) and cv. Pingpong (PP), of Morocco origin (DOUIRI et al., 2014), var. Jam Nagar (SOWBHAGYA et al., 2009), for. *pekinense* Makino (PYUN and SHIN, 2006), var. sativum (KHARAZI, 2005), Egyptian origin, Chinese origin, Mexican origin (SHAATH and FLORES, 1995) and Tunisian origin (DZIRI et al., 2014) derived by UPGMA from the similarity matrix of 75 chemical compositions.

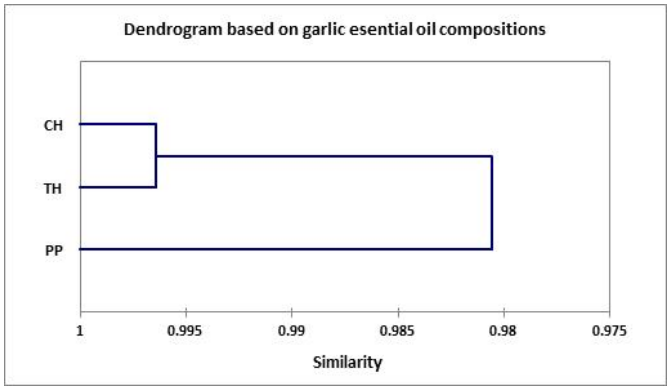

Fig. 4: The dendrogram of three garlic cultivars, cv. Thai (TH), cv. Chinese (CH) and cv. Pingpong (PP), derived by UPGMA from the similarity matrix of 75 chemical compositions.

et al., 2009). Garlic essential oils of different origins (viz., Chinese, Egyptian, Mexican and Tunisian) also contained two major compounds, diallyl trisulphide (12.52-50.92%) and diallyl disulphide (28.6-42.46%) (SHAATH and FLORES, 1995; DZIRI et al., 2014).

Cluster analysis of 75 chemical compositions of garlic essential oil from the three cultivars and eight references divided garlic into three groups on a dendrogram (Fig. 3). Group 1 includes garlic cv. CH, cv. TH, of Morocco origin (DOUIRI et al., 2013), cv. PP, var. Jam Nagar (SOWBHAGYA et al., 2009), and for. *pekinense* Makino (PYUN and SHIN, 2006). Group 2 includes garlic var. sativum (KHARAZI, 2005) and group 3 includes garlic of Egyptian, Chinese, Mexican (SHAATH and FLORES, 1995) and Tunisian origin (DZIRI et al., 2014) (Fig. 3). The TH, CH and PP were classified into the same group where the CH and the TH showed 0.997, while the PP gave 0.981 similarity values (Fig. 4).

Identification of sulphur-containing compounds from the three cultivars was achieved on TLC. Staining these compounds with ninhydrin revealed the compounds with R_f to be in the range of 0.18-0.2 in all the tested cultivars and extraction methods (Tab. 4). This result is in agreement with the report by BELEMKAR (2013), which suggested the R_f of sulphur-containing compounds as being about 0.2, and those compounds were identified as diallyl disulphide, diallyl sulphide, alliin and thiosulphinate. However, the oil extracted by the hydrodistillation of the PP showed no evidence of the presence of such compounds. The chemical component of garlic essential oil varied, depending largely upon the method of extraction: for example, allylsulphides were apparent in hydrodistillated oil, while solvent extraction using ethanol at the ambient temperature gave thiosulphinates, and temperatures below 0 °C were suitable for alliin and amino acid (LI et al., 2010; SENDL, 1995). As one of the heat stable compounds found in garlic, the alliin content was analysed using spectrophotometry (MIRON et al., 2002). The alliin content of the oil extract by hydrodistillation of the three cultivars was in the range of 40.0-90.0 mg/100 ml, while the content was less in the oil extracted using microwave-assisted apparatus (15.0-60.0 mg/100 ml) (Tab. 4). In any case, the CH gave the highest alliin content among the tested cultivars. Variations in the alliin content among the cultivars and because of the types of processing as well as products have also been observed in previous studies (BHANDARI et al., 2014; VELÍŠEK et al., 1997).

Alliin is a bioactive compound that shows high anti-human colon cancer anti-stomach cancer activities and stimulates peripheral blood cell immune functions (KHANUM et al., 2004; SALMAN et al., 1999). In addition, several reports have illustrated its health benefits including biological activities such as anti-platelet aggregatory effect, in addition to decreasing of systolic blood pressure, lipid peroxides, HL-60 human leukaemia uric acid, blood glucose, total lipid, triglyceride and cholesterol, glycemic controlling, antioxidant system modulation of red blood cell and anti-atherogenic effect (YUN et al., 2014; ANWAR and MEKI, 2003; LIU et al., 2005; WU et al., 2001; JAIN and KONAR, 1978). In this study, RAPD-PCR fingerprints were generated from three garlic cultivars (cv. TH, cv. CH and cv. PP). Ten randomly designed 10-mer oligonucleotide primers were initially used for screening the DNA samples to obtain reproducible RAPD fingerprints. Out of the ten primers tested, only eight primers provided consistent well-resolved and reproducible band patterns, and were therefore selected for further analysis.The total number of fragments observed among the three garlic cultivars based on the RAPD analysis with eight polymorphic primers was 45 fragments (Fig. 5). A dendrogram based on the similarity matrix generated with the RAPD primers is presented in Fig. 6. The dendrograms at a similarity value of 0.93 grouped the TH and the CH together, while the PP was separated into different clusters with 0.31 genetic similarity.

Tab. 4: Quantitative and qualitative comparisons of sulphur-containing compounds in garlic bulbs Thai, Chinese and Pingpong cultivars

Garlic essential oil	Time of extraction (min)	% Yield			Sulphur-containing compounds retention factor (Rf)			Alliin content (mg 100/ml)		
		TH	CH	PP	TH	CH	PP	TH	CH	PP
OG	120	0.36 ± 0.01b	0.39 ± 0.02b	0.45 ± 0.02a	0.20	0.19	-	88.70 ± 0.20b	91.53 ± 0.11a	40.76 ± 0.20c
MOG	45	0.22 ± 0.02a	0.21 ± 0.01a	0.16 ± 0.01b	0.19	0.20	0.18	15.04 ± 0.29c	60.43 ± 0.18a	22.91 ± 0.09b

All data are expressed as mean±SD of triplicate measurements. Means in the same row with different letters (a–c) are significantly different (P<0.01), ANOVA, Ducan, IBM SPSS Statistics.

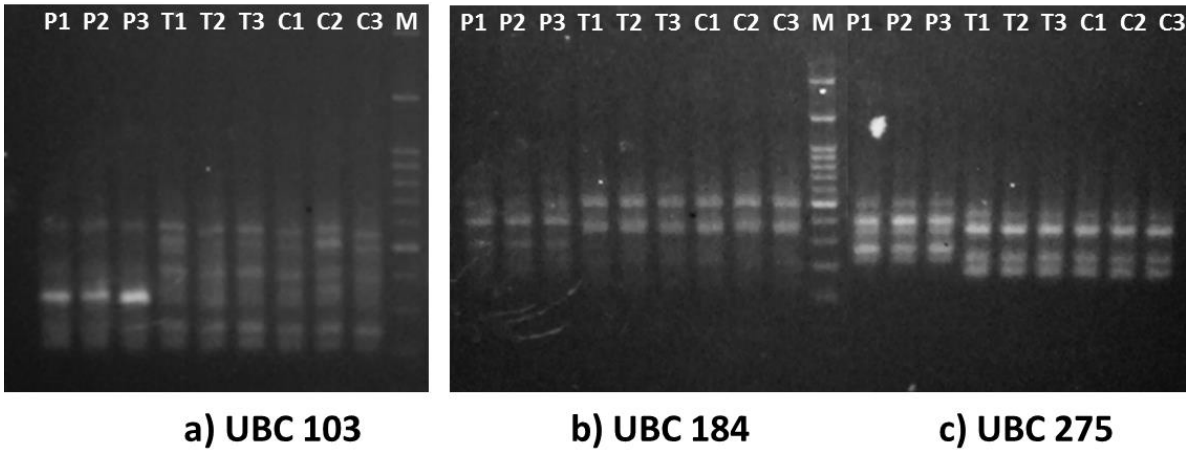

Fig. 5: Sample DNA fingerprints obtained from three RAPD makers: (a) UBC 106 (b) UBC 184 and (c) UBC 275 of *A. sativum* L. of the Thai (TH), Chinese (CH) and Pingpong (PP) cultivars.

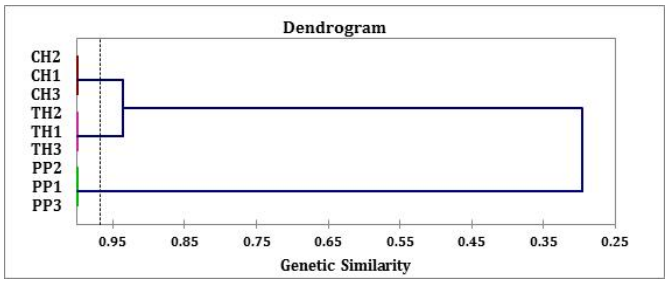

Fig. 6: The dendrogram of *A. sativum* L. of the Thai (TH), Chinese (CH) and Pingpong (PP) cultivars derived by UPGMA from the similarity matrix of the RAPD data.

It is noteworthy that the alliin content of the garlic essential oil from the CH was higher than that of the TH from both hydrodistillation and microwave hydrodistillation. The PP of garlic was genetically classified in clusters different from the TH and the CH. The extraction yield and the alliin content of the PP were also significantly different from the other two cultivars.In summary, garlic cv. TH and cv. CH were similar in chemical and genetic profiles. However, considering the higher content of bioactive alliin, the CH of garlic can be considered as the potential cultivar that can be used as raw material in food supplements and for further study.

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