Journal of Food and Drug Analysis

Volume 23 | Issue 1 | Article 13

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Recommended Citation
Chung, T.-Y.; Kuo, P.-C.; Liao, Z.-H.; Shih, Y.-E.; Yang, M.-L.; Cheng, M.-L.; Wu, C.-C.; and Tzen, J.T.C. (2015) "Analysis of lipophilic compounds of tea coated on the surface of clay teapots," *Journal of Food and Drug Analysis*: Vol. 23 : Iss. 1 , Article 13.
Available at: https://doi.org/10.1016/j.jfda.2014.05.005

JOURNAL OF FOOD AND DRUG ANALYSIS 23 (2015) 71–81

Available online at www.sciencedirect.com

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journal homepage: www.jfda-online.com

Original Article

Analysis of lipophilic compounds of tea coated on the surface of clay teapots

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ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form
11 April 2014
Accepted 12 May 2014
Available online 13 November 2014

Keywords:
clay teapot
oolong tea
palmitic acid
stearic acid
surface coating

ABSTRACT

The surface of a clay teapot tends to be coated with a waterproof film after constant use for tea preparation. The waterproof films of two kinds of teapots (*zisha* and *zhuni*) used for preparing oolong tea and old oolong tea were extracted and subjected to gas chromatography–mass spectrometry analysis. The results showed that comparable constituents were detected in these films; they were primarily fatty acids and linear hydrocarbons that were particularly rich in palmitic acid and stearic acid. To explore the source of these two abundant fatty acids, the fatty acid compositions of fresh tea leaves, granules, infusion, and vapor of infusion were analyzed by gas chromatography. Fresh tea leaves were rich in palmitic acid (C-16:0), unsaturated linolenic acid (C-18:3), linoleic acid (C-18:2), and oleic acid (C-18:1), which were presumably from the phospholipid membrane. During the process of manufacturing oolong tea, the three unsaturated fatty acids may be substantially degraded or oxidized to stearic acid (C-18:0), which was enriched with palmitic acid in the tea granules and in the infusion. The vapor of the tea infusion is primarily composed of palmitic acid and stearic acid. Thus, the coated films of teapots mostly originated from the lipophilic compounds of the tea infusions.

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http://dx.doi.org/10.1016/j.jfda.2014.05.005

1. Introduction

Tea is a very widely consumed beverage around the world, and many compounds in tea infusions have been identified with beneficial health functions [1–3]. Various teas are produced by versatile processes, and primarily classified as green tea (i.e., unfermented), oolong tea (i.e., partially fermented), and black tea (i.e., fully fermented), in which "fermentation" refers to natural browning reactions caused by oxidative enzymes in the cells of tea leaves [4]. Oolong tea, which possesses a taste and color between green tea and black tea, is manufactured primarily in Fujian and Guangdong in China, as well as in Taiwan. In the past few decades, different types of oolong teas with a fermentation degree in the range of 20–80% have been highly appreciated by Taiwanese people because of their special taste and flavor [5].

Old oolong tea generally refers to oolong tea that has been stored for >5 years and refined annually by a professional drying process at various desired temperatures [6]. Long-term storage (i.e., aging) and professional baking in a specialized oven at various desired temperatures are generally regarded as two major factors for the quality control of old oolong teas [7]. The longer oolong tea is stored and further oxidized gradually, the better it is empirically in taste and beneficial effects to human health. According to analyses by liquid chromatography tandem mass spectrometry (LC/MS/MS) and gas chromatography–mass spectrometry (GC/MS), the phenolic and volatile compounds of teas are significantly altered under the baking and aging processes [8–10].

To enhance the aroma, color, and taste of oolong tea, an infusion is traditionally prepared by immersing oolong tea granules with hot water in clay teapots. Clays such as *zisha* and *zhuni* are used to make teapots in Yixing, Jiangsu Province, China, and are fired at 1100–1200°C in an oxidizing atmosphere. In general, the major mineral composition of clay is silicon dioxide (SiO_2), followed by aluminum oxide (Al_2O_3) and iron (III) oxide (Fe_2O_3); however, iron influences the color of clay after firing. Of course, the mineral ratio varies substantially in clays dug from different areas [11]. Further observation of the clay teapot microstructure using a scanning electron microscope reveals continuous and discontinuous cavities of approximately 0.01–0.02 mm in the sections of teapots; they occupy approximately 20% of the clay volume. The presence of cavities in clay teapots seems to prevent the latent risk of breakage induced by the drastic thermal expansion during tea preparation with boiling water. Furthermore, water vapor and volatile compounds of the tea infusion in clay teapots probably pass through these cavities.

After frequent tea preparations, the inside of a clay teapot tends to be precipitated with dark brown scum while its surface is coated with a waterproof film. For tea consumers, it is part of enjoyment to observe the gradual change of the cumulative scum precipitation and surface coating in clay teapots, and the practice of this teapot maintenance is regarded as an art in tea culture called "Yang Hu." It has been proposed that the tea scum develops via the formation of complexes with tannin polymers and calcium (Ca^{2+}) ions in the tea infusion [12–15]. By contrast, the chemical constituents of the waterproof film coated on the surface of teapot are largely unknown.

In this study, we aimed to analyze the chemical constituents of the waterproof films coated on the surface of teapots. Because volatile compounds of tea infusions may pass through the cavities of the clay teapots, the waterproof films of *zisha* and *zhuni* teapots used for preparing oolong tea and old oolong tea were extracted and subjected to GC/MS analysis. To explore the source of the coated constituents, extracts of fresh tea leaves, granules, infusions, and vapor of infusion were also analyzed and compared.

2. Methods

2.1. Chemicals and materials

All chemicals were purchased from E. Merck Co. (Darmstadt, Germany), unless stated otherwise. Boron trifluoride, toluidine blue O, and *tert*-butanol were purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). Benzene was purchased from ALPS Chemical Co. (Taipei City, Taiwan). Water was purified using the Millipore Direct-Q clear water purification system (Millipore Corporation, Billerica, MA, USA). Fresh tea leaves (i.e., young green shoots) and tea granules were obtained from the same tea plant cultivar: *Camellia sinensis* L. Chin-Shin oolong was grown in Zhushan and Lugu, Nantou County, Taiwan. *Zisha* and *zhuni* teapots, which were manufactured in Yixing, Jiangsu Province, China, were used for the study of their surface coating.

2.2. Teapot maintenance

To analyze the chemical constituents of the waterproof film coated on the surface of the teapots by a scientific approach, three nearly identical used *zisha* or *zhuni* teapots were cleaned thoroughly with detergents, and were used for the following comparisons: teapot maintenance with water, oolong tea infusion, or old oolong tea infusion. In every tea preparation, 2 g of oolong tea granules or old oolong tea granules were set in a *zisha* or *zhuni* teapot, and 100 mL of boiling water was added to prepare the infusion. Boiling water without tea granules was concomitantly placed in a *zisha* or *zhuni* teapot as a background control. After 3 hours, the temperature of the tea infusion had cooled to room temperature. Thus, the infusions and expanded tea leaves in the teapots were decanted, and the teapots were cleaned by rinsing with running water. The aforementioned process of teapot maintenance was repeated 10 times per week. After 6 months (approximately 250 times), the waterproof films coated on the surface of teapots were subjected to GC/MS analysis.

2.3. GC/MS analysis and mass spectral identification

The constituents coated on the surface of teapots were dissolved in acetone, and the solvent was removed by a

Fig. 1 – Tea granules, infusion, and teapots. Granules of oolong tea and old oolong tea were separately used to prepare infusion in the two *zisha* teapots. The waterproof films coated on the surface of teapots were washed with acetone and used for the gas chromatography–mass spectroscopy analysis.

rotary evaporator at reduced pressure until the volume was reduced to 5 mL. The coated compounds were concentrated under a nitrogen stream to 30 μL, and subjected to GC/MS analysis using the Shimadzu GCMS-2010 Ultra gas chromatography/mass spectrometer (Shimadzu, Chiyoda-ku, Tokyo, Japan) that was equipped with a quadrupole mass analyzer. One-microliter samples were injected into the column. Analytical conditions were as follows: 30 m × 0.25 mm i.d.; 0.25 μm BP-20 column (SGE Forte; Ringwood, Victoria, Australia); and helium as the carrier gas at 3 mL/min. The column temperature was programmed for 50–260°C at a rate of 5°C/min in all runs. The injector temperature was 170°C. The column flow rate was 1 mL/min and the split ratio was 1:20. The mass spectrometer was used under the following conditions: ionization voltage, 70 eV; ion source temperature, 170°C; and interface temperature, 170°C. Mass spectral identification was achieved by comparing the spectra with the commercial mass spectral databases of the National Institute of Standards and Technology and LIBTX. Compounds were tentatively identified by the agreement of their retention times and mass spectra with published data and, if available, with those of authentic compounds [16].

2.4. *Slices of tea leaf samples at different manufacturing stages*

Samples of tea leaves at different stages of oolong tea manufacture were obtained from Mingchien, Nantou County, Taiwan. They were moisturized in precooled 100 mM sodium phosphate buffer at pH 7.0, cut into small pieces with a blade, and then fixed in glass vial with formalin–acetic acid–alcohol solution (prepared by mixing 100 mL of 95% ethanol, 70 mL of water, 20 mL of 37% formaldehyde solution, and 10 mL of glacial acetic acid) at 4°C overnight. After fixation, the tissues were dehydrated by a graded *tert*-butanol series and transferred into a *tert*-butanol/paraffin solution for embedding. The leaf samples in wax block were then sliced into 10-μm sections and stained with 1% toluidine blue O solution prior to observing them under a Nikon type E600 light microscope (Tokyo, Japan) [17].

2.5. *Fatty acid analysis*

Total lipids of the fresh tea leaves, granules, infusion, and vapor of infusion were extracted with chloroform and methanol (2:1, v/v) using the Bligh and Dyer method [18].

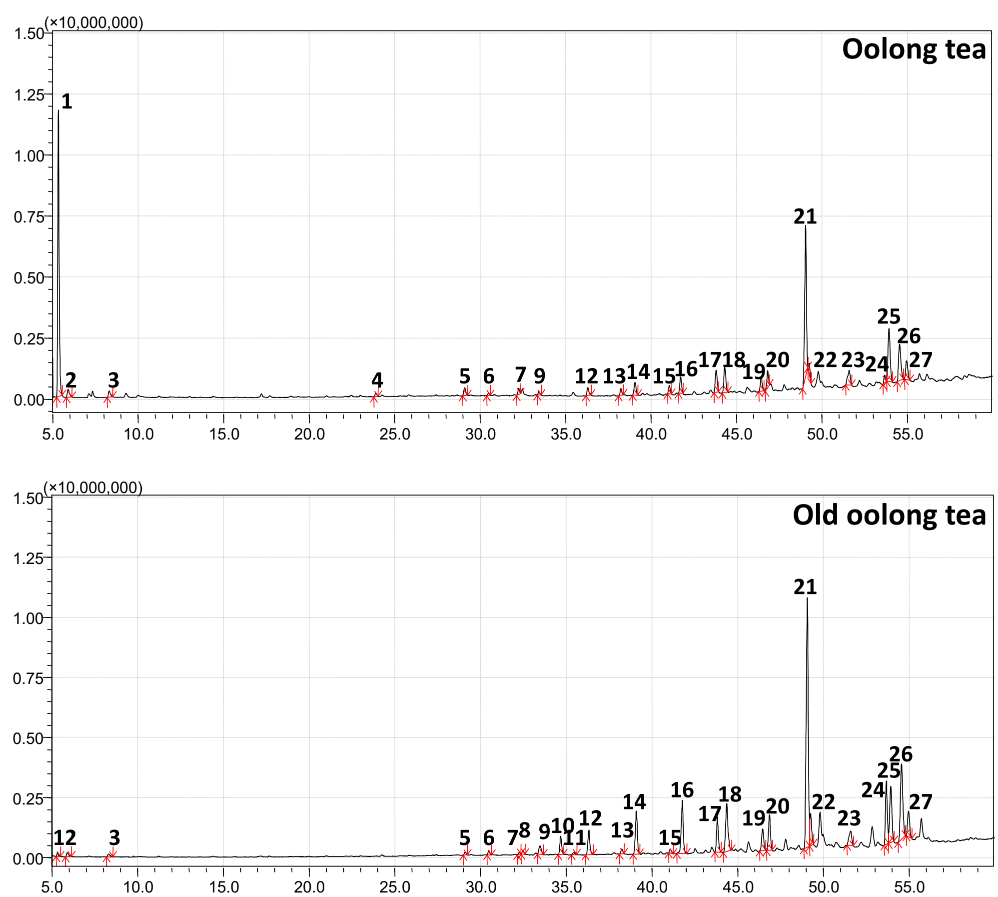

Fig. 2 – Gas chromatography–mass spectroscopy chromatograms of lipophilic compounds coated on the surface of two *zisha* teapots that were separately used to prepare infusions of oolong tea and old oolong tea. Table 1 lists the names of these compounds.

Twenty micrograms of heptadecanoic acid (C-17:0) were added to each sample as an internal standard. Fatty acids in the samples were transmethylated with methanol containing 14% boron trifluoride, based on the protocol described by Morrison and Smith [19]. They were then analyzed by a gas chromatograph (7890A Gas Chromatograph; Agilent Technologies, Santa Clara, CA, USA), which was equipped with a polar capillary column (HP-INNOWAX, 30 m × 0.25 mm i.d., 0.25 μm film; Agilent Technologies) and a flame-ionization detector. The injector and detector temperatures were set at 250°C. The oven temperature was initially set at 140°C, increased to 215°C at the rate of 15°C/min, and then increased to 235°C at the rate of 0.5°C/min. Fatty acids were identified by comparing their retention times with those of their commercial standards purchased from Nu-Chek-Prep (Elysian, MN, USA).

3. Results

3.1. *Chemical identification of the constituents coated on the surface of two* zisha *teapots*

As the initial approach of this research, the waterproof films coated on the surface of two *zisha* teapots were extracted for the analysis of their coated compounds; the teapots had been separately used for >3 years to prepare oolong tea and old oolong tea (Fig. 1). It was surprising to detect comparable levels of the constituents in these two waterproof films (Fig. 2), although the volatile compounds of the oolong tea infusions were substantially different from those of the old oolong tea infusions [10]. In total, 27 compounds were identified in the two waterproof films by GC/MS analysis (Table 1). Twenty-two of these compounds were structurally fatty acids and linear hydrocarbons, and the other compounds were diacetone alcohol, phenol, diethyl phthalate, caffeine, and di-*n*-octyl phthalate (Fig. 3). Three fatty acids—palmitic acid (C-16:0), stearic acid (C-18:0), and oleic acid (C-18:1)—were apparently abundant in both waterproof films (Fig. 2).

3.2. *Constituents coated on* zisha *and* zhuni *teapots after well-controlled maintenance*

For a scientific approach of equivalent tea maintenance, three nearly identical *zisha* teapots and three nearly identical *zhuni* teapots were gradually coated on their surface under a well-controlled process for the preparation of tea infusions. Similar patterns of constituents were again detected in the four waterproof films coated on the surface of two *zisha* and two *zhuni* teapots that were individually used for the preparation of oolong tea infusions and old oolong tea infusions

Table 1 – The lipophilic constituents on the surface of the teapots. Fig. 2 shows the chromatographic profile of these lipophilic compounds.

Peak no.	Retention time	Retention index (BP-20)	Compound	Oolong tea	Oolong tea aging
				Peak intensity (× 1,000,000)	
1	5.3	—	4-hydroxy-4-methyl-2-pentanone (diacetone alcohol)	11.8	0.2
2	5.9	—	Nonanal	0.4	0.2
3	8.3	—	Decanal	0.3	0.2
4	23.9	1990	Phenol	0.3	—
5	29.1	2155	*n*-Nonanoic acid	0.5	0.2
6	30.5	2200	*n*-Docosane	0.2	0.3
7	32.3	2260	*n*-Decanoic acid	0.4	0.2
8	32.5	2267	1,1′-Oxybis-octane	—	0.3
9	33.5	2300	*n*-Tricosane	0.3	0.5
10	34.7	2343	Diethyl phthalate	—	0.9
11	35.4	2368	1-Hexadecanol	—	0.2
12	36.3	2400	*n*-Tetracosane	0.5	1.1
13	38.2	2468	*n*-Dodecanoic acid	0.4	0.4
14	39.1	2500	*n*-Pentacosane	0.7	1.9
15	41.1	2577	1-Octadecanol	0.6	0.4
16	41.7	2600	*n*-Hexacosane	0.9	2.4
17	43.8	2683	*n*-Tetradecanoic acid	1.2	1.7
18	44.3	2700	*n*-Heptacosane	1.3	2.3
19	46.5	2788	*n*-Pentadecanoic acid	0.9	1.2
20	46.8	2800	*n*-Octacosane	1.2	1.8
21	49.1	2895	*n*-Hexadecanoic acid (palmitic acid)	7.1	10.9
22	49.2	2900	*n*-Nonacosane	1.5	1.8
23	51.6	3000	*n*-Triacontane	1.2	1.1
24	53.7	—	Caffeine	1.0	3.2
25	53.9	—	*n*-Octadecanoic acid (stearic acid)	2.9	3.0
26	54.5	—	Oleic acid	2.3	3.9
27	55.0	—	Di-*n*-octyl phthalate	1.6	1.9

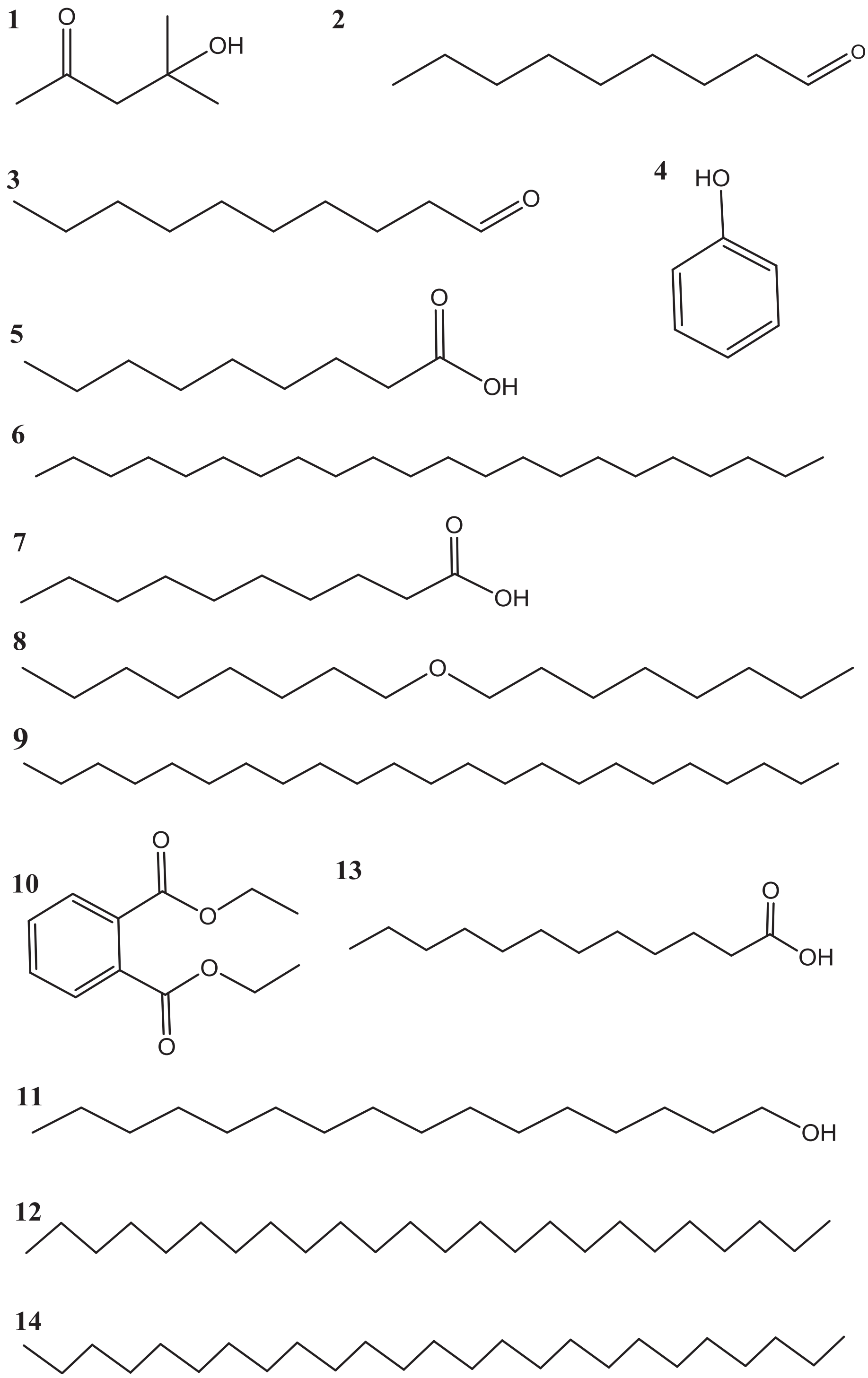

Fig. 3 – The chemical structures of the identified compounds coated on the surface of teapots, as indicated in Fig. 2.

(Fig. 4). In these two sets of observation, 14 and 13 lipophilic compounds were observed after the maintenance of the *zisha* and *zhuni* teapots, respectively. However, these compounds were also primarily fatty acids and linear hydrocarbons that were particularly rich in palmitic acid (C-16:0) and stearic acid (C-18:0). Similar lipophilic compounds were at a substantially low level, particularly for palmitic acid and stearic acid, in the control teapots (i.e., they were filled with boiling water without tea granules in the maintenance process) because these teapots were used previously.

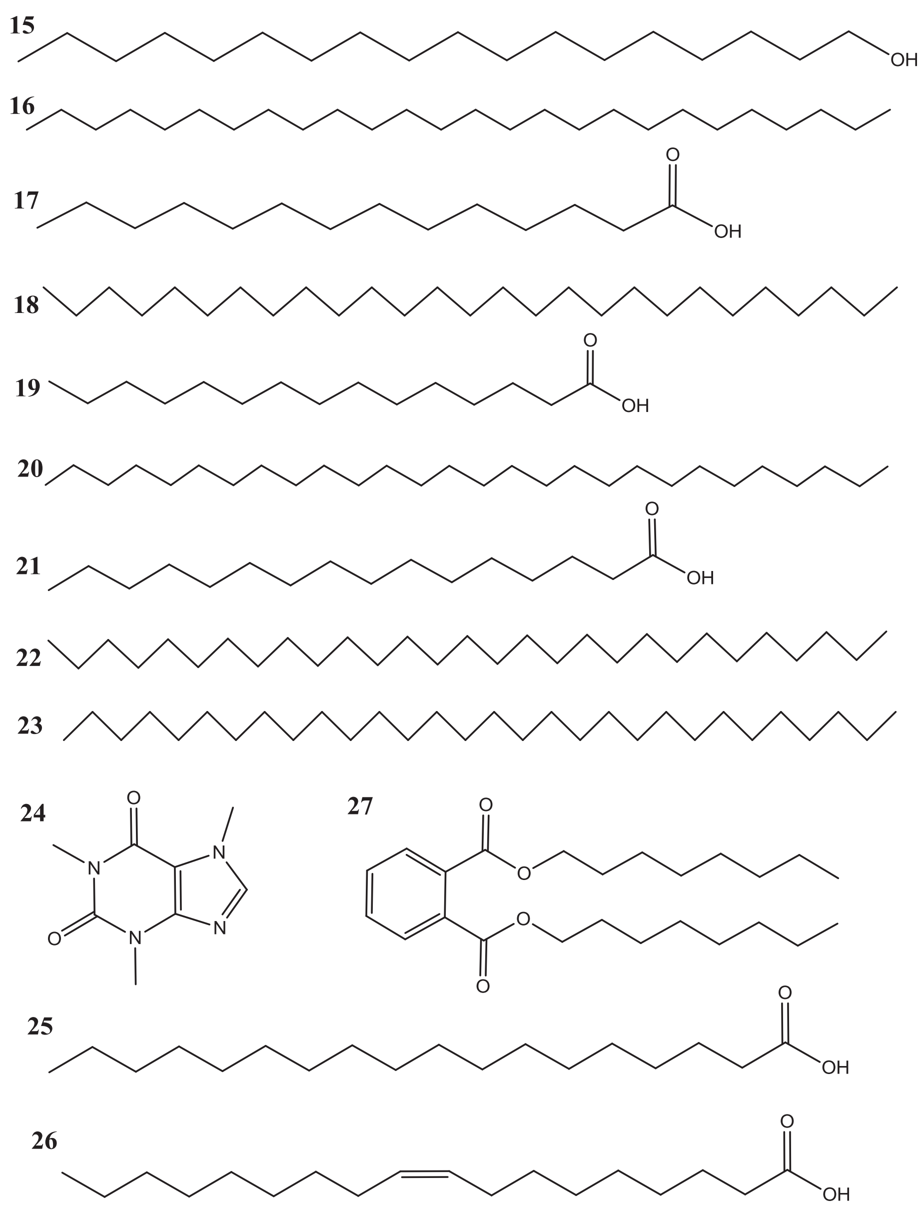

Fig. 3 — (continued).

3.3. *Cell morphology of tea leaves at different stages of oolong tea manufacture*

To explore the possible source of the two abundant fatty acids (i.e., palmitic acid and stearic acid) coated on the teapot surface, the morphology of fresh tea leaves and the morphology of tea leaves at three major stages of oolong tea manufacture (i.e., wilting, heating, and shaping) were observed by microscopy (Fig. 5). Compared to the morphology of fresh tea leaves (Fig. 5A), the wilting process did not alter the morphological structure of tea leaf, although dehydration with plasmolysis occurred (Fig. 5B). After the process of heating, the cells of the palisade and spongy tissues were broken (Fig. 5C). Furthermore, the tea leaves became wrinkled on the upper and lower epidermis, which led to morphological changes after the shaping stage (Fig. 5D). The integrity of oolong tea leaves was apparently destroyed in the heating stage, and its cell membranes were decomposed. Free fatty acids possibly resulted from the degradation of membrane phospholipids in the process of oolong tea manufacture.

3.4. *Analysis of fatty acids in fresh tea leaves, granules, infusion, and vapor of infusion*

Fatty acids of fresh tea leaves and those in the granules, infusion, and infusion vapor of oolong tea and old oolong tea were analyzed and compared (Fig. 6), and their amounts were estimated (Table 2). Fresh tea leaves were rich in palmitic acid (C-16:0), unsaturated linolenic acid (C-18:3),

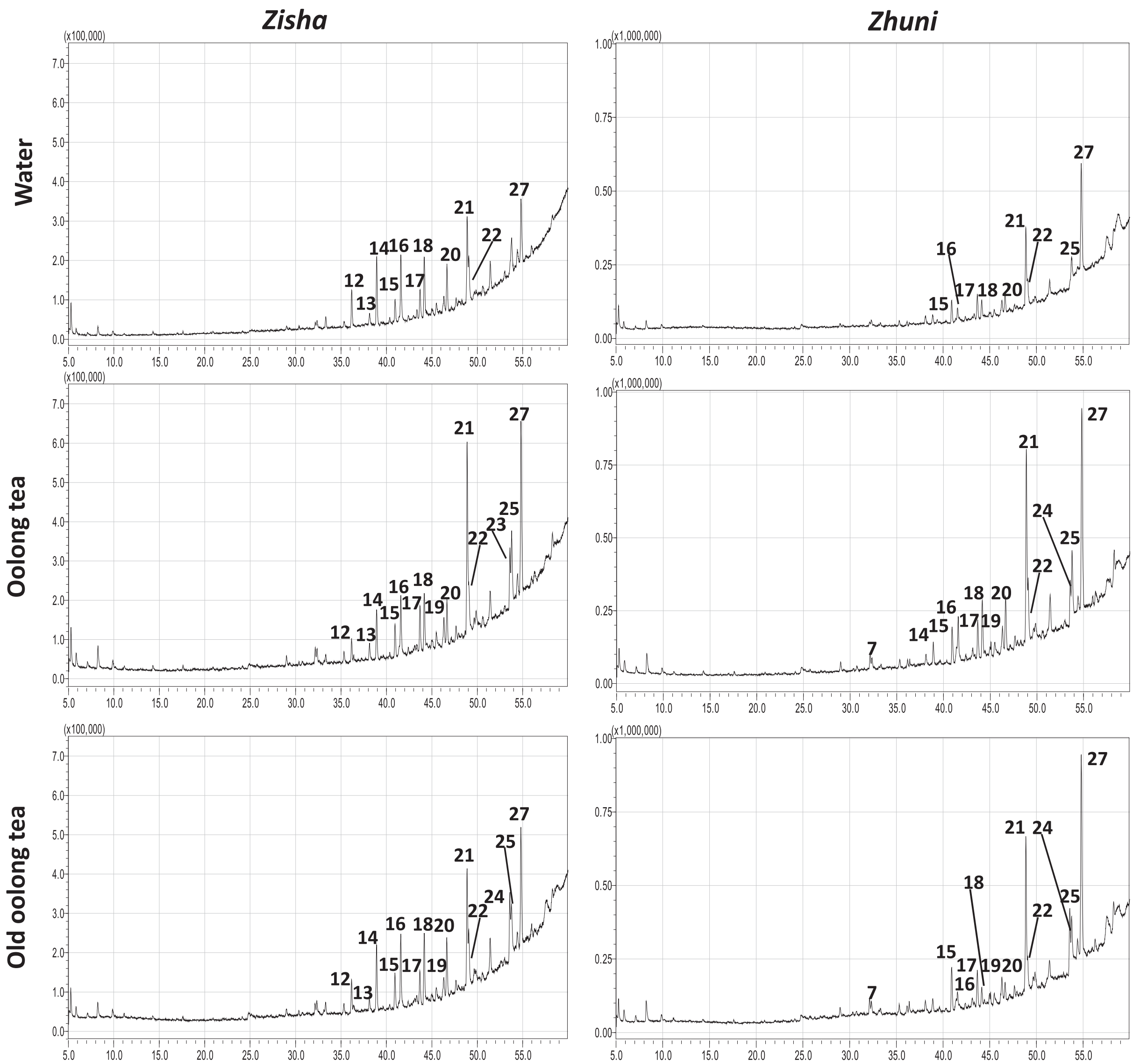

Fig. 4 – Gas chromatography–mass spectroscopy chromatograms of lipophilic compounds coated on the surface of *zisha* and *zhuni* teapots under a well-controlled process of teapot maintenance. The compound numbers in these chromatograms are the same as those in Fig. 2 and Table 1.

linoleic acid (C-18:2), and oleic acid (C-18:1). The levels of the three unsaturated fatty acids were substantially reduced, whereas the level of the saturated fatty acids plamitic acid and stearic acid (C-18:0) were enriched in the tea granules and in the infusion of oolong tea or old oolong tea. The infusion vapor of oolong tea or old oolong tea was interestingly composed primarily of palmitic acid and stearic acid.

4. Discussion

Regardless of whether oolong tea or old oolong tea is used, the waterproof films coated on the surface of *zisha* and *zhuni* teapots is predominantly composed of palmitic acid and stearic acid, accompanied by minor linear hydrocarbons and shorter length saturated fatty acids (i.e., compounds 5, 7, 13, 17, and 19) that are presumably derived from the degradation of the two abundant saturated fatty acids. The possible origin of the two abundant saturated fatty acids (i.e., palmitic acid and stearic acid) coated on the surface of teapots is hypothesized later. According to this study, fresh tea leaves were rich in palmitic acid (C-16:0) and unsaturated linolenic acid (C-18:3), linoleic acid (C-18:2), and oleic acid (C-18:1), which are presumably in membrane phospholipids. During the heating and shaping processes of manufacturing oolong tea, the cellular structures of tea leaves collapse. As a consequence, membrane

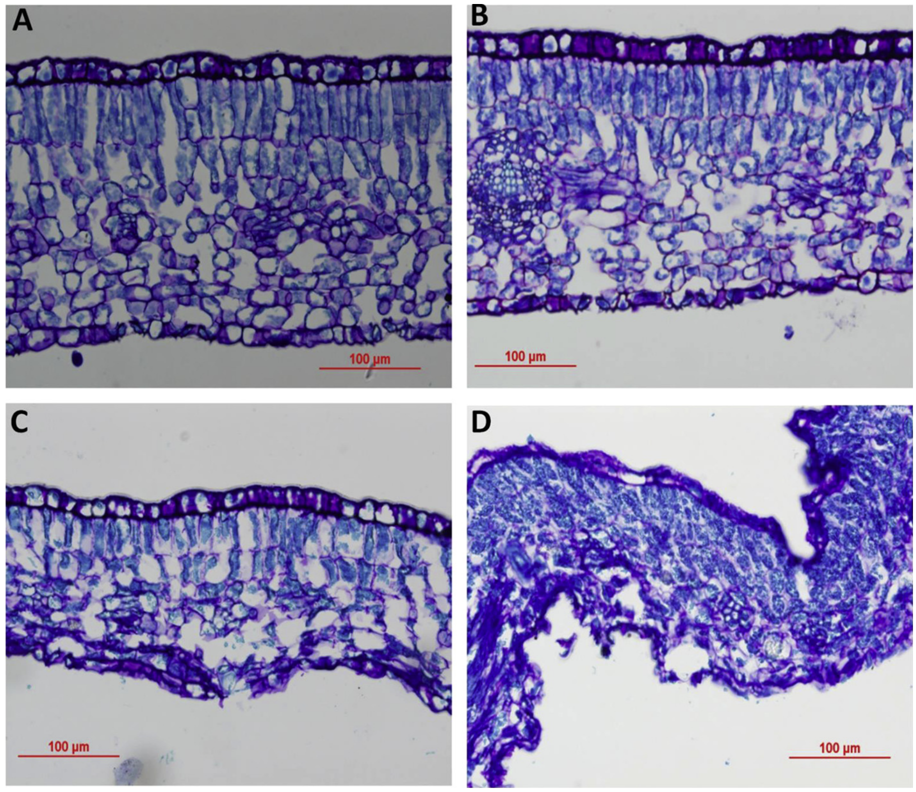

Fig. 5 – Morphological changes in tea leaf cells in oolong tea manufacture. (A) Fresh tea leaves. Oolong tea leaves after undergoing (B) wilting, (C) heating, and (D) shaping processes. The leaves were observed under a light microscope.

phospholipids decompose. The released linolenic acid, linoleic acid, and oleic acid may be substantially degraded or oxidized to stearic acid (C-18:0), which was enriched with palmitic acid (C-16:0) in the tea granules and infusion. The vapor of tea infusion was primarily composed of palmitic acid and stearic acid, which is in agreement with the observation of the lipophilic compounds coated on the teapots.

It has been documented that the volatile compounds of oolong tea are drastically different from those of old oolong tea [10]. In addition to the long straight-chains of alcohols and acids in the volatile compounds of oolong tea, the characteristic aroma in old oolong teas is primarily produced by nitrogen-containing compounds (e.g., N-ethylsuccinimide, 2-acetylpyrrole, 2-formylpyrrole, and 3-pyridinol) that were converted from amino acids during the baking process. However, the nitrogen-containing compounds responsible for the baking smell of old oolong tea are water-soluble and tend to be washed away during the conventional cleaning process of teapots by rinsing with running water. This explains why oolong tea and old oolong tea smell so distinct, whereas their lipophilic compounds coated on the surface of clay teapots are almost indistinguishable.

It is intriguing that only two saturated fatty acids—palmitic acid and stearic acid—were detected in the vapor of the tea infusion whereas substantial amounts of unsaturated fatty acids (i.e., linolenic acid, linoleic acid, and oleic acid) were coexistent with these two saturated fatty acids in the tea infusion (Fig. 6). The drastic difference did not seem to result from their boiling points (227°C, 225°C, 224°C, and 223°C for stearic acid, linolenic acid, linoleic acid, and oleic acid, respectively) [20]. It may be because of the different interactions of saturated fatty acids and unsaturated fatty acids with water molecules; the polar property of water tends to interact with the double bonds of unsaturated fatty acids via dipole-induced dipole force. Therefore, in contrast to the evaporation of saturated fatty acids, unsaturated fatty acids seemed to be trapped by water molecules in the tea infusion.

In the analysis of constituents coated on the teapots after well-controlled maintenance (Fig. 4), di-*n*-octyl phthalate (i.e., compound 27), a non-tea constituent, was detected as a major peak in all six samples, which include the control samples. It presumably resulted from the contamination of the plastic beaker used to fill the teapots with boiling water. The speculative contamination resulting from the plastic container seems to be reasonable because di-*n*-octyl phthalate was detected at a substantially low level in the waterproof films of the two *zisha* teapots at the initiation of this research (Fig. 2).

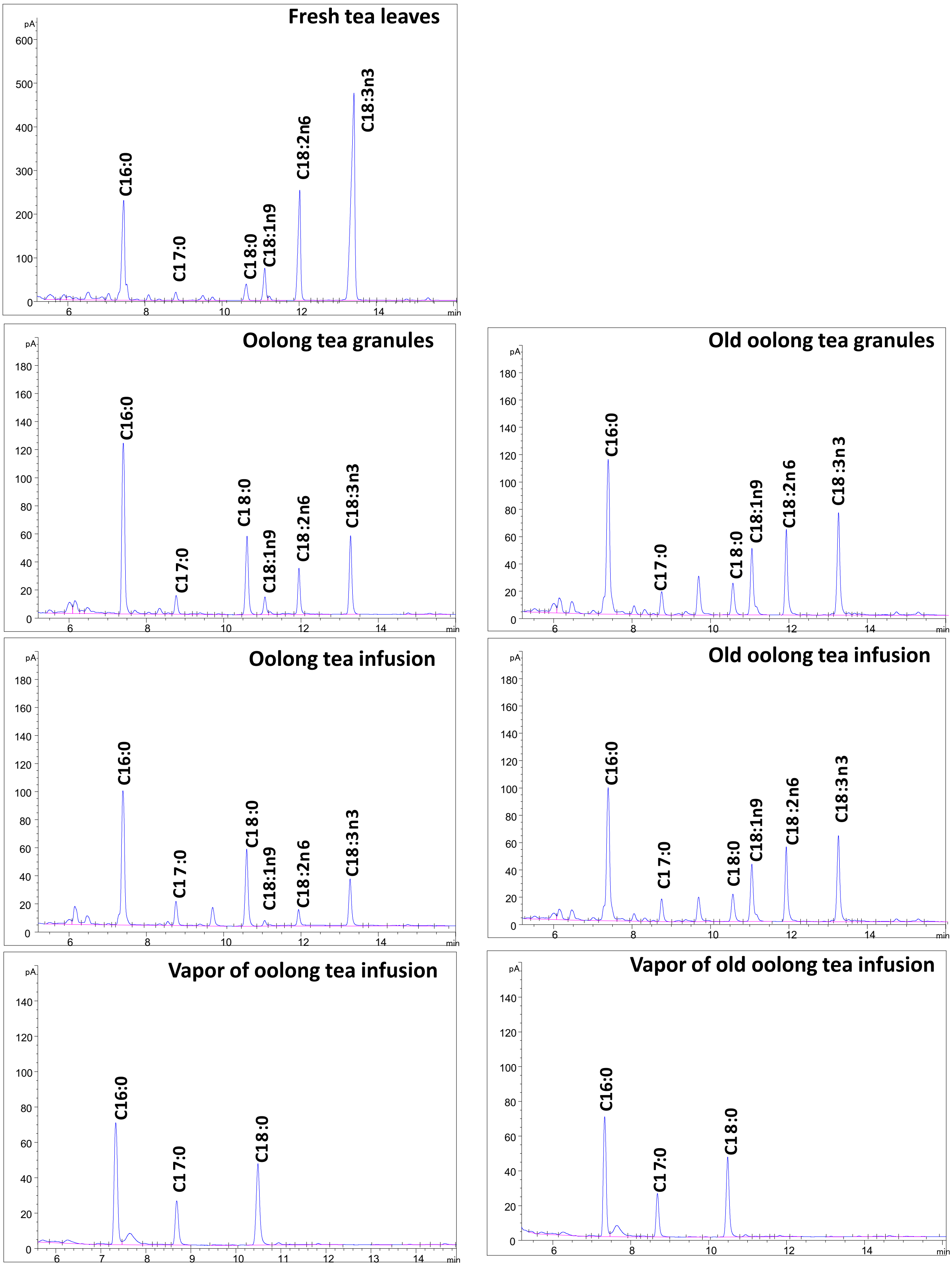

Fig. 6 — Gas chromatography chromatograms of fatty acids extracted from fresh tea leaves, granules, infusion, and infusion vapor of oolong tea and old oolong tea. Margaric acid (C17:0) has been added in each sample as an internal standard.

Table 2 – The amount of fatty acids in tea leaves, granules, infusion, and the vapor of tea infusion. Fig. 6 shows the chromatographic profiles of these fatty acids.

Sample	The amount of fatty acid (μg)				
	C16:0	C18:0	C18:1n9	C18:2n6	C18:3n3
Fresh tea leaves	1779.80	256.03	432.81	976.25	2693.05
Oolong tea granules	1308.06	576.55	125.05	290.87	548.91
Oolong tea infusion	690.69	345.16	25.31	81.21	218.79
Vapor of oolong tea infusion	283.47	180.07	12.27	9.46	4.19
Old oolong tea granules	961.29	174.29	362.85	394.43	526.49
Old oolong tea infusion	882.14	162.11	330.92	367.35	474.15
Vapor of old oolong tea infusion	215.11	148.63	5.41	3.47	1.46

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

This work was supported by a grant (NSC 100-3114-B-005-001 to JTC Tzen) from the National Science Council in Taipei, Taiwan, R.O.C.

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