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Yield and Chemical Composition of Ginger Essential Oils as Affected by Inter-Varietal Variation and Drying Treatments of Rhizome

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Abstract: Ginger (*Zingiber officinale Rosc*; *Zingiberaceae family*) is an herb commonly used as a spice and remedy for a broad spectrum of diseases. The essential oil extracted from ginger is an effective antioxidant, anti-inflammatory, and antifungal agent. The present study has investigated the variations in yield and chemical composition of essential oils of two cultivars (Chinese and Thailand) of ginger locally available in Pakistan. Two different drying pretreatments were employed to observe the changes in compositional variations of the essential oils of ginger. The essential oil extracted from fresh, oven-dried, and sun-dried samples of two different cultivars of ginger was analyzed using gas chromatography-mass spectrometry (GC-MS). The essential oil yield was found to be highest for the sun-dried sample of each variety. The major compounds (>4%) overall in the essential oil of fresh, oven-dried, and sun-dried ginger samples from Thailand origin were camphene, 3-carene, o-cymene, caryophyllene, α-curcumene, sabinol trans, citral, and santalol. Major compounds overall in the essential oil of fresh, oven-dried, and sun-dried ginger samples of Chinese origin were α-pinene, Camphene, limonene, longicyclene, copaene, longifolene, β-sesquiphellandrene, alloaromadendrene, γ-muurolene, α-curcumene, α-farnesene, and citral. The inter-varietal variations and pretreatment methods considerably affected yield and chemical composition. Cluster analysis was performed to validate the results further. Significantly varying compounds responsible for the significant variation among varieties and treatments of the ginger were identifies by using the heat map. There was clear differentiation among Chinese and Thailand varieties due to the variation in the concentrations of the volatile compounds. The results obtained can be helpful for the ginger growers and end users to choose the ginger variety and the way of use that is more beneficial.

Keywords: *Zingiber officinale Rosc*; GC-MS; ginger; essential oil; drying methods; volatile constituents

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1. Introduction

Zingiber officinale is an herbaceous perennial plant belonging to the family *Zingiberaceae*. This plant owes importance due to its rhizome, which is laterally flattened and grows horizontally. Botanically termed as *Zingiber officinale* and commonly called adrak in Pakistan, it is an important plant with a long history of uses for different purposes. It

has grown and been used worldwide since ancient times [1]. The most popular use of ginger is in spices and as a flavoring agent. It is considered as one of the most important culinary agents worldwide [2]. In cuisines, ginger is added in its fresh and in dried form. Both the powder and sliced form of ginger is used for taste and aroma. Tea flavored with ginger is also famous, especially in the sub-continent [3]. Due to its unique flavor, it is an important ingredient in many biscuits, bread, pickles, and other confectionaries [4]. Ginger has been used as a remedy for various ailments far before its benefits were scientifically analyzed. Its aroma makes it important for aromatherapy for coughs and colds [5]. It is used as a traditional remedy and an important component of various medicines. Together with honey, ginger is curative for asthmatic bronchitis [6]. Several studies reveal the medicinal importance of ginger as an effective antioxidant, anticancer, antimicrobial, anti-inflammatory, and antipyretic agent [7,8].

Ginger oil is extracted from *Z. officinale* rhizomes, whose chemical composition is influenced by geographical origin, extraction methods, and the freshness or dryness of rhizomes. The essential oil of ginger has been reported to be effective in massage for low back pain [9]. Tropical, subtropical, and temperate zones are best for ginger cultivation. It requires a warm and humid climate. The soil must be rich in organic matter, having a pH of about 5.5–6.6 [1,2,10,11]. Several countries, such as Nigeria, Thailand, New Zealand, Nepal, Uganda, Trinidad, Sri Lanka, Taiwan, Philippines, Indonesia, Jamaica, Malaysia, India, Guatemala, Hawaii, China, Brazil, Bangladesh, and Australia, are major producers [12]. India has been ranked as the number one country in the production of ginger. In 2019, Pakistan was found to be the 34th country in the production of ginger, according to a survey. The yield of ginger in Pakistan in the year 2019 was reported to be 52 metric tons, far greater than that achieved in 2018 [13]. Production of ginger in Pakistan is not so common as its commercial cultivation is limited to only ten districts of Sindh province [2].

Different cultivars of ginger exist, which differ in essential oil's yield and chemical composition. These varieties also vary in appearance from each other. Yellow and white ginger cultivars are primarily found throughout the world. Efforts are being made to cultivate ginger in Pakistan, but Pakistan still imports a major portion of ginger every year. Pakistan's annual import of ginger was recorded as 83 million US Dollars in 2019 [14,15].

Ginger mostly contains two types of components, i.e., volatile and non-volatile. Volatile components comprise 1–3% of the total weight of ginger. The importance of ginger is attributed explicitly to its oils, which contain sesquiterpenes, namely bisabolene, zingiberene, zingiberol, sesquiphellandrene, curcurmene, and other compounds, such as 6-dehydrogingerdione, galano lactone, ginger sulfonic acid, zingerone, geraniol, neral, monoacyldigalactosyl glycerols, and ginger glycolipids. Gingerols and shogaols are the most important phenolic compounds found in ginger oils. The unique pungent odor of ginger is due to the gingerols [16]. Among these, the most abundant is 6-gingerol. The specific odor in fresh ginger is due to gingerols. In the dried form of ginger, gingerols, after dehydration, are transformed into compounds called shogaols. These shogaols give a pungent odor to the dried form of ginger [17].

Various methods are adopted for the improvements in yield and composition of essential oils. One of these methods is drying pretreatment, which can be useful in preventing the microbial growth, slowing down the enzyme activity, and many moisture-controlled reactions [18]. Along with this, the drying pretreatments can alter the aroma and prevent loss of nutrient, changes in color, and the development of oxidation products [19,20]. Different drying pretreatments including temperature, time, environment, and special equipment usage can cause decrease or increase in concentration of various volatile compounds specially in food and medicinal materials [21]. The reason of the change in concentration of volatiles is not well known and majorly depends on the treatment and spice type under test. Shade drying, sun drying (SD), and oven-drying at 20 °C and 40 °C, respectively, were compared, and it was observed that there is no significant effect on qualitative and quantitative properties of the essential oil of the *Lippia graveolens* essential oil [22,23].

Variations in the chemical composition of ginger oils have been observed due to various factors, including drying treatments, temperature, geographical conditions, and distillation methods [24,25]. Numerous reports in the literature describe the effect of drying or inter-varietal variation individually on ginger essential oil composition. However, to our knowledge, reports on the simultaneous effect of drying pretreatment and inter-varietal variation, specifically on essential oil from Chinese and Thai cultivars of ginger, have rarely been reported. In the present work, we analyzed the effects of different drying pretreatment methods, including sun-drying and oven-drying methods, and inter-varietal variations on the yield and chemical composition of essential oil of ginger cultivars, which are available in the local market in Pakistan.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh ginger samples of two different origins, i.e., China and Thailand, were bought from an authentic ginger importer local market in Pakistan. It was also assured from the seller that the time of the import and storage conditions of both the Thai and Chinese ginger were almost the same. Both varieties differed in color; the ginger from Thailand was relatively white, while that from China was relatively yellow. The ginger samples were checked and authenticated by Dr. Muhammad Arfan, Botanist, Department of Life Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan. The ginger of the two origins was divided into three parts. One part of each was used for extraction and analysis in its fresh form. The second and third parts were separated for subjecting to an electric oven (Model UN55, Memmert, Germany) drying at 90 °C for 5 h, and sun-drying for five days before extracting the essential oil, respectively. The sun drying was done during the month of April when the temperature ranges from 20–39 °C and the average humidity is 20%. The sun drying was done in full sunshine of five hours during five consecutive days.

2.2. Extraction of Essential Oils

Samples of fresh, oven-dried, and sun-dried ginger cultivars were subjected to hydro distillation for ≅3 h using a Clevenger-type apparatus. Essential oil from each sample was extracted and kept at −4 °C until analyzed. The extraction parameters were optimized as per the already available literature [26].

2.3. Physical Analysis of Essential Oils

Physical properties, including the refractive index and density of ginger essential oil, were determined following the standard method [27]. A digital refractometer RX-7000α (Atago Co. Ltd., Tokyo, Japan) was used to calculate the physical properties of ginger essential oil. Digital refractometer is an automatic device. The operator only needs to clean the sensor and put a drop of the sample on it. It automatically gives the result of specific gravity and refractive index.

2.4. GC-MS Analysis of Essential Oils

GC-MS analysis of the ginger essential oils from two varieties was done in the TTI Testing Laboratory, Lahore, using the GC-7890B (Agilent, CA, USA) interface equipped with triple quadrupole MSD (Mass-selective detector). The compounds separation and identification were made on DB 5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). A sample of essential oil (1.0 µL) was injected in the split mode. The split ratio was 100:1. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The ionization energy was 70 eV, with mass scanning range varying over 50–550 m/z, while injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The initial column temperature was set at 70 °C with 0 min hold time. The temperature was allowed to increase at 10 °C min^{-1} with a maximum temperature of up to 300 °C. The total run time of sample analysis was set at 24 min.

2.5. Identification of Compounds

The identification of the ginger oil constituents was based on comparing their retention indices relative to (C9–C24) *n*-alkanes either with those of published data or with authentic compounds. Compounds were also identified using their mass spectral data compared to those from the NIST mass spectral library and published mass spectra [28].

2.6. Statistical/Cluster Analysis

All determinations were made in triplicates, and the data is reported as mean ± SD for ($n = 1 \times 3$). Cluster analysis was conducted using R-software (MetaboAnalystR package fourth edition) to evaluate the difference in the presence of various volatile compounds in essential oils among two varieties of ginger, i.e., Chinese and Thailand [29].

3. Results and Discussion

3.1. Yield and Physical Analysis

A slight variation in the essential oil yield from both varieties was observed. The yield of essential oil from fresh Chinese ginger (0.09%) was found to be greater than the ginger from Thailand (0.04%) (Table 1). The overall increase in the essential oil yield of both varieties was observed with drying. The yield of essential oil from oven-dried Thailand ginger was found to be 0.09%, whereas in Chinese ginger, it was 0.14%. Among sun-dried (SD) samples, Thailand ginger yielded a higher amount (0.2%) of essential oil than the Chinese variety, which was found to be 0.15%. Drying pretreatments considerably affected the yield of essential oil, and the fresh forms of both varieties yielded the least quantity of essential oil. However, the yield increased for the oven-dried (OD) ginger samples, but was highest for the SD forms of ginger from Thailand and China, as shown in Table 1. This increase in SD samples yield follows the previously reported work on ginger [19,21].

Table 1. Physical properties of Thailand and Chinese ginger essential oils.

Variety	Treatment	Yield (g 100 g^{-1})	Density (g/cm^3) (25 °C)	Refractive Index (25 °C)
Thailand variety	Fresh	0.04 ± 0.02	0.873 ± 0.03	1.4885 ± 0.03
	Oven-dried	0.09 ± 0.03	0.880 ± 0.05	1.4881 ± 0.03
	Sun-Dried	0.2 ± 0.02	0.877 ± 0.03	1.4888 ± 0.04
Chinese variety	Fresh	0.09 ± 0.04	0.871 ± 0.04	1.4884 ± 0.04
	Oven-dried	0.14 ± 0.03	0.875 ± 0.02	1.4910 ± 0.03
	Sun-Dried	0.15 ± 0.04	0.880 ± 0.02	1.4885 ± 0.03

Values are mean ± standard deviation (SD), $n = 3$.

Refractive indices and densities for all the essential oil samples were similar and not affected by the drying treatments. Thailand's fresh, OD, and SD ginger oils density was 0.873 g/cm^3, 0.880 g/cm^3, and 0.877 g/cm^3, respectively. The refractive index of fresh, OD, and SD Thailand ginger oil was 1.4885, 1.4881, and 1.4888, respectively. The density of fresh, OD, and SD ginger oil from China was 0.871 g/cm^3, 0.875 g/cm^3, and 0.880 g/cm^3, respectively. While refractive index values for Chinese fresh, OD, SD ginger oil were 1.4884, 1.4910, and 1.4885, respectively. This range was in accordance with the one reported previously [30].

3.2. GC-MS Analysis of Ginger Essential Oils

The essential oil extracted from both Chinese and Thailand ginger varieties was analyzed by using GC-MS. As the yield of essential oil extracted from Chinese ginger differs from that obtained from ginger of Thailand origin, the chemical composition of essential oils also shows significant variations. Several compounds were detected in both varieties, i.e., Chinese and Thailand ginger varieties (Tables 2 and 3). Certain differences have also been observed, which may arise due to geographical factors. A more significant number of compounds were detected in the Chinese ginger essential oil than in ginger from Thailand.

The results in Tables 2 and 3 show that the essential oil of Chinese ginger contains monoterpene hydrocarbons (limonene) and sesquiterpene hydrocarbons (sesquiphellandrene) as the major constituents. Oxygenated monoterpene and sesquiterpene hydrocarbons also constitute the chemical composition of Chinese ginger essential oil (Figure 1). Camphene, carene, curcumene, *cis*-thujopsene, and elemene were commonly detected in all three ginger forms. Curcumene gives ginger its particular taste [31]. Both varieties differ in the main constituent of essential oil as Thailand ginger samples contained curcumene and caryophyllene as the major chemical compounds, and Chinese ginger samples contained limonene and sesquiphellandrene as the major compounds, respectively. This difference in main chemical constituents is supported by the previously published research work on the different cultivars of ginger [32].

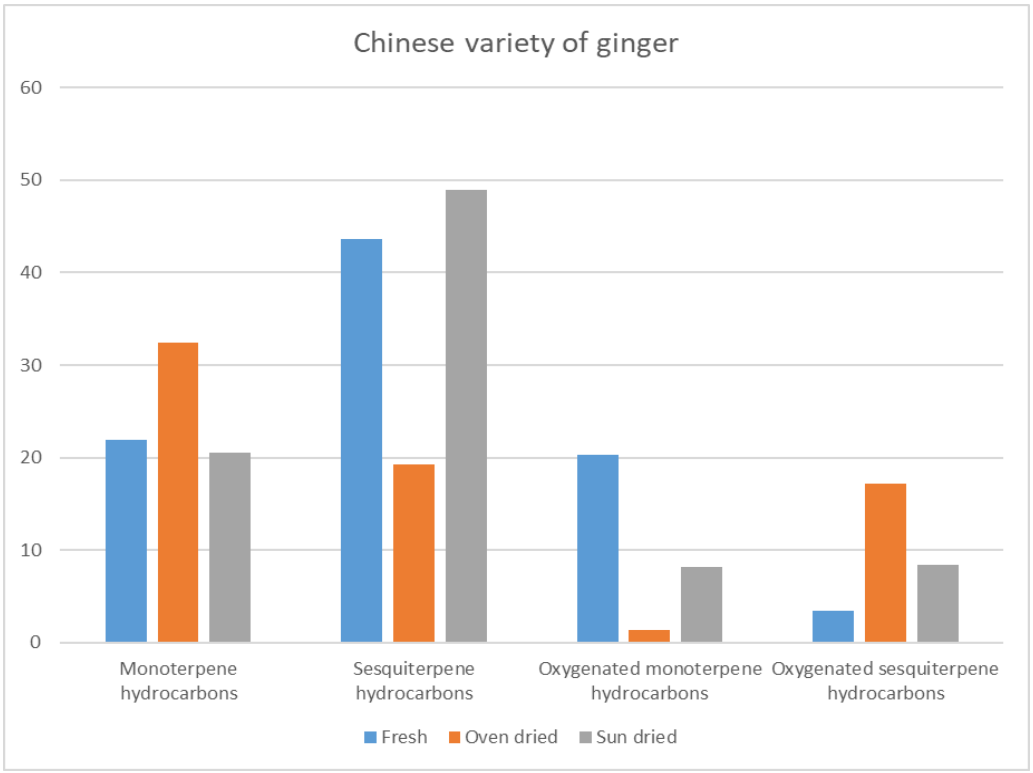

Figure 1. Variations in major classes of compounds in essential oils of fresh, OD, and SD pretreatment of China ginger.

3.2.1. Fresh Ginger

In the fresh ginger from Thailand, a total of 19 compounds were detected, constituting 82.61% of the essential oil. The different compounds were found to be as follows: monoterpenes, 12.38%; sesquiterpenes, 34.97%; oxygenated monoterpenes. 18.86%; and oxygenated sesquiterpenes, 18.69%, respectively. On the other hand, 34 compounds were identified in the fresh ginger from China, constituting 89.38% of the essential oil. Different classes of compounds were as follows: monoterpenes, 35.41%; sesquiterpenes, 43.64%; oxygenated monoterpenes, 19.83%; and oxygenated sesquiterpenes, 4.00%. A significant variation was observed among the essential oils of the two varieties concerning the inter-varietal differences and drying pretreatment effects.

Major compounds identified in the essential oils from the fresh form of ginger from Thailand were eucalyptol (9.06%), citronellyl butyrate (4.02%), 3-carene (8.63%), 2-bornene (3.31%), Ar-curcumene (15.57%), beta-bisabolene (5.96%), β-sesquiphellandrene (7.30%), and nerolidol (14.32%). The identification of these compounds in the essential oil of fresh ginger sample is in accordance with the published work on two varieties of ginger from Guinea and China [26], where sesquiterpene hydrocarbons constituted 32.68% of

the total composition of essential oil extracted from fresh Thailand ginger, whereas Ar-curcumene (15.57%) was the most prevalent compound. Citral (5.43%), longicyclene (4.98%), α-cedrene (3.27), β-eudesmol (2.53%), zingiberene (0.42%), camphene (7.68%), 3-carene (3.79%), limonene (8.58%), isoborneol (4.31%), bornyl acetate (2.82%), cis-thujopsene (2.53%), α-curcumene (8.69%), and α-farnesene (5.68%) were dominantly identified in the essential oil of fresh Chinese ginger. This result is in accordance with the published data [33,34].

Fresh ginger oil was composed mainly of sesquiterpenes (43.64%), and the most prevalent compound was β-sesquiphellandrene (9.48%). Both varieties contained sesquiterpene hydrocarbons as the major group of compounds and differed in the total number of compounds constituting the essential oil. This result is in accordance with a previously published work on the fresh forms of ginger [28]. The concentration of β-bisabolene was highest in the fresh ginger sample and least in the SD sample. Linalool, β-sesquiphellandrene, and nerolidol were found predominantly in the essential oil of fresh ginger samples and traced in other forms. 3-carene concentration in the essential oil of the fresh ginger sample was higher than the OD Chinese ginger sample. This trend is the same as observed in the forms of Thailand ginger. Isoborneol was in higher concentration in the fresh ginger sample than in the SD ginger sample. The level of α-curcumene was highest in the fresh ginger sample and considerably decreased in SD and OD ginger samples.

Isoborneol is another common compound whose concentration is higher in the fresh sample than in SD sample. The essential oil of the fresh ginger sample showed the highest concentration of bornyl acetate and α-farnesene.

3.2.2. Oven-Dried Ginger

The total number of compounds detected and identified in ginger from Thailand and China were 39 and 27 for the OD form of ginger, respectively (Table 3). The percent peak area of each detected compound shows information about the relative concentration of the compound. The major compound in the essential oil of Thailand OD was found to be α-Curcumene (9.44%). This result is in accordance with a previously published work on dried forms of ginger [28]. Results reveal that the ginger essential oil from Thailand origin comprises of the most sesquiterpene hydrocarbons, monoterpene hydrocarbons, oxygenated monoterpene hydrocarbons, and oxygenated sesquiterpenes hydrocarbons (Figure 2).

Similarly, oven-dried essential oil of Chinese variety was comprised mainly of α-pinene (8.87%), limonene (16.11%), cis-thujopsene (3.50%), isoaromadendrene (3.47%), isolongifolol (6.35%), and phytol (3.52%) (Table 3).

Essential oil of OD ginger was characterized by camphene (5.85%), 3-carene (3.94%), 2-bornene (3.31%), α-curcumene (9.44%), citral (4.96%), farnesene (4.36%), zingiberene (1.84%), elemol (3.44%), and isobornyl acetate (2.12%), whereas these compounds not detected in fresh and sun-dried forms. Other dominantly detected compounds were 3-carene (3.94%), γ-muurolene (4.22%), α-farnesene (4.36%), β-bisabolene (3.66%), 2-bornene (3.31%), β-curcumene (2.24%), and ledol (4.67%). The increased levels of farnesene and β-curcumene in the OD samples are consistent with the research work reported previously [35]. The presence of these compounds increases the pungency of ginger. The ginger essential oil of OD form mainly comprises sesquiterpenes hydrocarbons (34.97%) and α-curcumene (9.44%), the most prevalent compound. Monoterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes hydrocarbons constituted 20.32%, 12.74% and 12.57%, respectively.

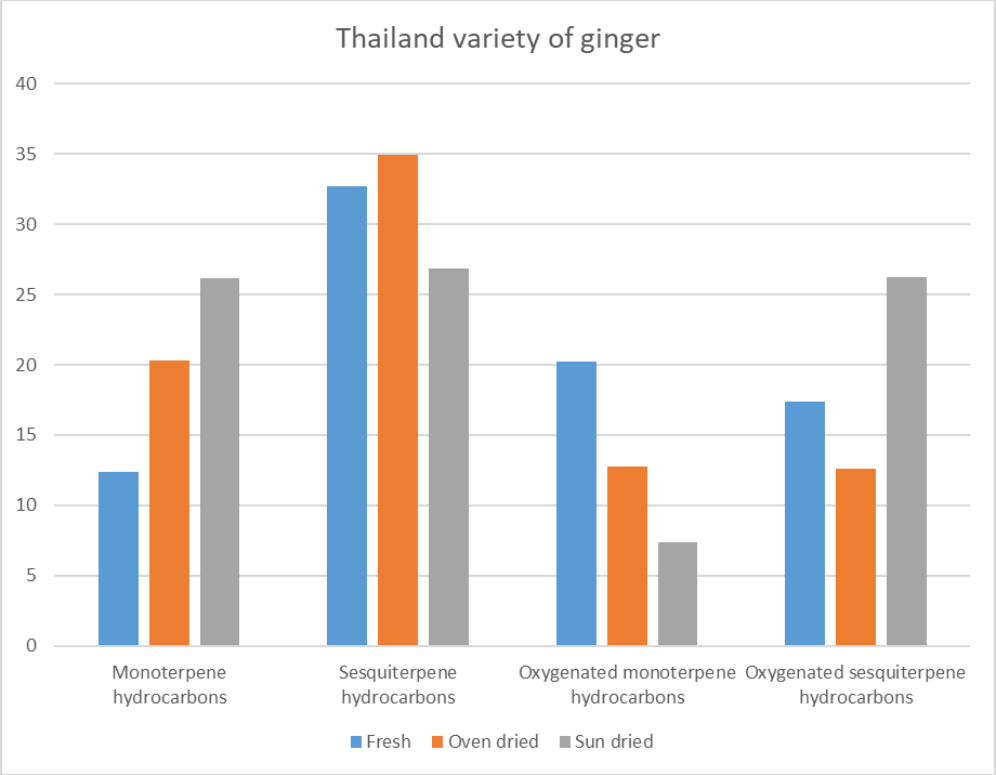

Figure 2. Variations in major classes of compounds in essential oils of fresh, OD, and SD pretreatment Thailand ginger.

Oven-dried ginger oil showed the highest quantity of γ-muurolene, and the fresh sample showed the least amount. Ledol, β-curcumene, and 2-bornene were found in the greater amounts in the OD ginger sample and in traces in other forms. Isolongifolol (6.53%) and phytol (3.52%) were found only in the essential oil of the oven-dried form and were not detected in the fresh and SD forms of Chinese ginger. Other dominantly detected compounds included isoaromadendrene epoxide (3.47%), α-pinene (8.87%), 3-carene (2.19%), cedrene (3.77%), and *cis*-thujopsene (3.50%). The major group composing this type of oil was monoterpenes hydrocarbons (32.38%); the most prevalent compound was limonene (16.11%). Sesquiterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes hydrocarbons constituted 19.23%, 1.41%, and 17.24% of the total composition, respectively. Isoaromadendrene epoxide also existed commonly and showed the highest concentration in the OD ginger sample.

Limonene is responsible for the distinct flavor of ginger, which is famous and is a monoterpene. Limonene concentration was highest in the OD and lowest in the fresh ginger sample. α-pinene concentration was the highest in the OD ginger essential oil, which is in accordance with that observed in the case of the Thailand variety.

Table 2. Chemical composition of essential oil of ginger from Thailand samples.

Sr. #	Compounds	RI	%Composition			Identification Mode
			Fresh	Oven-Dried	Sun-Dried	
	Monoterpene hydrocarbons					
1	α-Pinene	934	ND	0.33 ± 0.03	ND	a, b, c
2	Norbornane	937	ND	1.57 ± 0.01	ND	a, b, c
3	Camphene	947	1.81 ± 0.02	5.85 ± 0.02	ND	a, b, c
4	α-Fenchene	957	ND	ND	0.86 ± 0.03	a, b, c
5	β-Pinene	973	ND	0.48 ± 0.03	ND	a, b, c

Table 2. *Cont.*

Sr. #	Compounds	RI	%Composition			Identification Mode
			Fresh	Oven-Dried	Sun-Dried	
6	3-Carene	1007.2	8.63 ± 0.05	3.94 ± 0.03	13.5 ± 0.04	a, b, c
7	m-Cymene	1012	1.11 ± 0.01	0.40 ± 0.07	ND	c
8	o-Cymene	1032	ND	ND	5.58 ± 0.04	a
9	Thujone	1124	ND	1.88 ± 0.01	ND	a, b, c
10	Menthone	1150	0.83 ± 0.03	ND	ND	a, b, c
11	β-Myrcene	1173	ND	1.85 ± 0.05	ND	a, b, c
12	2-Bornene	1508	ND	3.31 ± 0.01	ND	a, b, c
13	Acorenone 1	1632	ND	0.71 ± 0.04	4.14 ± 0.06	c
14	Geranyl-α-terpinene	2001	ND	ND	2.10 ± 0.05	a, b, c
	Sesquiterpene hydrocarbons					
15	Longipinene	1350	ND	1.10 ± 0.04	ND	a, b, c
16	α-Copaene	1375	ND	0.41 ± 0.03	ND	a, b, c
17	β-Elemene	1388	ND	0.95 ± 0.01	ND	a, b, c
18	β-Ylangene	1416	ND	0.40 ± 0.03	ND	a
19	Caryophyllene	1419	ND	ND	18.89 ± 0.03	a
20	Cis-thujopsene	1433	ND	1.86 ± 0.03	ND	a, b, c
21	γ-Elemene	1449	ND	1.51 ± 0.03	ND	a, b, c
22	α-Patchoulene	1451	ND	1.60 ± 0.07	ND	a, b, c
23	Alloaromadendrene	1459	ND	0.44 ± 0.07	ND	a, b, c
24	Ar-Curcumene	1471	15.57 ± 0.07	ND	ND	a
25	γ-Himachalene	1471	ND	ND	3.94 ± 0.08	b
26	α-Curcumene	1471	ND	9.44 ± 0.05	ND	a, b, c
27	γ-Muurolene	1473	0.92 ± 0.01	4.22 ± 0.08	2.81 ± 0.03	a, b, c
28	α-Zingiberene	1482	ND	1.84 ± 0.06	ND	a, b, c
29	Valencene	1483	ND	0.50 ± 0.03	ND	c
30	Epi-Bicyclosesquiphellandrene	1488	ND	0.44 ± 0.03	ND	a, b, c
31	α-Farnesene	1496	ND	4.36 ± 0.01	ND	a
32	β-Bisabolene	1499	5.96 ± 0.03	3.66 ± 0.02	1.18 ± 0.01	a, b, c
33	β-Curcumene	1512	1.55 ± 0.05	2.24 ± 0.03	ND	a, b, c
34	β-Sesquiphellandrene	1513	7.30 ± 0.04	ND	ND	a, b, c
35	α-Cadinene	1526	1.38 ± 0.04	ND	ND	a, b, c
	Oxygenated monoterpene hydrocarbon					
36	Eucalyptol	1025	9.06 ± 0.01	ND	ND	a
37	Linalool	1086	2.08 ± 0.05	1.30 ± 0.04	ND	a, b, c
38	Sabinol, trans	1130	ND	ND	6.44 ± 0.01	a, b, c
39	Isopulegol	1148	ND	ND	0.92 ± 0.04	a, b, c
40	Citronellal	1153	ND	0.98 ± 0.04	ND	a, b, c
41	Citral	1165	ND	4.96 ± 0.03	ND	a, b, c
42	α-Terpineol	1175	ND	1.94 ± 0.03	ND	a, b, c
43	p-Cymen-7-ol	1270	1.43 ± 0.01	ND	ND	c, a
44	Isobornyl acetate	1271	ND	2.12 ± 0.06	ND	b
45	Bornyl acetate	1283	2.27 ± 0.04	ND	ND	b
46	Geranyl acetate	1361	ND	1.44 ± 0.04	ND	a, b, c
47	Citronellyl butyrate	1529	4.02 ± 0.04	ND	ND	a, b, c
	Oxygenated sesquiterpenes hydrocarbons					
48	Elemol	1536	ND	3.55 ± 0.03	ND	a, b, c
49	Nerolidol 2	1550	ND	1.50 ± 0.07	ND	c
50	Nerolidol	1550	14.32 ± 0.04	ND	ND	a, b, c
51	Caryophyllene oxide	1570	ND	0.30 ± 0.07	2.19 ± 0.04	a, b, c
52	Globulol	1578	ND	ND	1.45 ± 0.03	a, b, c
53	Ledol	1582	1.19 ± 0.04	4.67 ± 0.04	ND	a, b, c
54	Cedrol	1597	ND	ND	15 ± 0.03	a, b, c
55	γ-Eudesmol	1616	ND	1.17 ± 0.03	ND	a, b, c
56	τ-Cadinol	1640	ND	0.56 ± 0.05	ND	a, b, c
57	α-Bisabolol	1668	ND	ND	1.02 ± 0.06	a
58	Alloaromadendrene oxide	1672	ND	ND	1.31 ± 0.05	c
59	Farnesol	1691	1.83 ± 0.04	ND	ND	a, b, c

Table 2. *Cont.*

Sr. #	Compounds	RI	%Composition			Identification Mode
			Fresh	Oven-Dried	Sun-Dried	
60	β-Santalol	1703	ND	ND	5.23 ± 0.05	a, b, c
61	Trans-Z-alpha-Bisabolene epoxide	1820	ND	0.82 ± 0.05	ND	a, b, c
	Oxygenated Diterpenes					
62	Trans-geranylgeraniol	2201	1.35 ± 0.02	ND	ND	a, b, c
	Total		84.90	80.6	86.56	

a = identification based on retention indices; b = identification based on comparison of mass spectra; c = identification based on co-injection with authentic compounds compounds are listed in order of retention times. Values are mean ± standard deviation (SD), $n = 3$.

3.2.3. Sun-Dried Ginger

The total number of compounds detected and identified in ginger from Thailand and China were 20 and 27 for SD forms of ginger, respectively (Table 2). The percent area of each detected compound peak shows information about the composition and relative concentration of the ginger essential oils. The major compounds in Thailand SD essential oil were 3-carene (13.50%), acorenone 1 (4.14%), caryophyllene (18.89%), o-Cymene (5.58%), sabinol (6.44%), γ-himachalene (3.94%), β-santalol (5.23%), and geranyl-α-terpinene (2.10%) were the compounds identified explicitly in the essential oil of the SD ginger from Thailand. We detected a variation in concentrations of 3-carene (13.5%), γ-muurolene (2.81%), caryophyllene oxide (2.19%), and acorenone (4.14%). Ginger oil from the SD form was the most prevalently composed of sesquiterpenes hydrocarbons and caryophyllene (18.89%). Monoterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes hydrocarbons constituted 26.18%, 7.36%, and 26.2%, respectively.

An elevated amount of 3-carene was found in the essential oil of SD ginger sample, and low levels were found in the OD ginger sample. Caryophyllene oxide and acorenone were detected in greater concentrations in the SD ginger sample oil while the other two forms showed traces of these compounds. 4-Carene (2.14%) and γ-muurolene (6.27%) were specifically identified in the essential oil of SD form of Chinese ginger and not detected in any other form. Other predominant compounds included α-pinene (3.31%), isoborneol (2.97%), isobornyl acetate (3.43%), α-copaene (6.03%), longifolene (5.96%), α-cedrene (5.42%), *cis*-thujopsene (4.54%), alloaromadendrene (7.82%), α-curcumene (5.69%), caryophyllene oxide (3.99%), and isoaromadendrene epoxide (3.19%). The major group identified was sesquiterpenes, and the most prevalent compound was limonene (12.12%). *Cis*-thujopsene concentration was highest in the SD ginger oil and lowest least in the fresh ginger oil. Cedrene concentration was higher in SD ginger than in the OD ginger sample.

The SD ginger sample was rich in isobornyl acetate and alloaromadendrene. Our results confirm the changes in yield and chemical composition of essential oils as a result of different drying pretreatments for the same cultivar and verify the effect of inter-varietal variations. The fresh forms of both varieties differed in the chemical composition of essential oils; hence the trend for changes in OD and SD forms of both varieties also showed considerable differences. These factors have also affected the number of chemical compounds detected in the two cultivars. Results obtained through GC-MS analysis are in accordance with the literature survey, which verifies our research methodology's validity. These results confirmed that ginger essential oil's yield and chemical composition had been considerably affected by the inter-varietal variations and different drying pretreatment methods.

Table 3. Chemical composition of essential oil of ginger from Chinese samples.

Sr. #	Compounds	RI	%Composition			Identification Mode
			Fresh	Oven-Dried	Sun-Dried	
	Monoterpene Hydrocarbons					
1	α-pinene	934.6	ND	8.87 ± 0.07	3.31 ± 0.04	a, b, c
2	Camphene	947	7.68 ± 0.03	0.92 ± 0.04	0.43 ± 0.03	a, b, c
3	2-Norpinene	948	ND	ND	1.74 ± 0.05	b
4	β-Pinene	973	1.50 ± 0.03	ND	ND	a, b, c
5	2-Carene	1002	ND	1.91 ± 0.03	ND	a, b, c
6	3-Carene	1007.2	3.79 ± 0.03	2.19 ± 0.03	0.38 ± 0.04	a, b, c
7	p-Cymene	1015	ND	1.11 ± 0.07	ND	a, b, c
8	4-Carene	1017	ND	ND	2.14 ± 0.05	a, b, c
9	Limonene	1023	8.58 ± 0.04	16.11 ± 0.06	12.12 ± 0.06	a, b, c
10	o-Cymene	1032	ND	1.27 ± 0.03	ND	a, b, c
11	γ-Terpinene	1050	ND	ND	0.46 ± 0.02	a, b, c
12	Camphor	1125	0.36 ± 0.02	ND	ND	c
	Sesquiterpene hydrocarbons					
13	α-Phellandrene	999	0.68 ± 0.05	ND	1.95 ± 0.02	a, b, c
14	α-Longipinene	1350	ND	1.07 ± 0.05	ND	a, b, c
15	Longicyclene	1371	4.98 ± 0.04	ND	ND	a, b, c
16	α-Copaene	1375	0.86 ± 0.04	ND	6.03 ± 0.03	a
17	β-Elemene	1388	1.53 ± 0.04	1.71 ± 0.05	0.86 ± 0.05	a
18	Longifolene	1404	0.45 ± 0.04		5.96 ± 0.05	b
19	α-Gurjunene	1405		1.53 ± 0.05		b
20	Cedrene	1410	ND	3.77 ± 0.03	ND	a, b, c
21	β-bourbonene	1411	0.30 ± 0.03	ND	ND	a, b, c
22	α-Cedrene	1415	3.27 ± 0.07	ND	5.42 ± 0.05	a, b, c
23	β-Ylangene	1416	0.70 ± 0.05	ND	ND	a, b, c
24	β-Sesquiphellandrene	1417	9.48 ± 0.03	ND	1.60 ± 0.02	a, b, c
25	α-Cedrene	1433	ND	1.22 ± 0.03	ND	a, b, c
26	Cis-thujopsene	1433	2.53 ± 0.05	3.50 ± 0.05	4.54 ± 0.03	a, b, c
27	β-Gurjunene	1435	0.87 ± 0.08	ND	ND	a, b, c
28	Aromadendrene	1439	ND	1.16 ± 0.03	ND	a, b, c
29	γ-Elemene	1449	1.84 ± 0.01	ND	1.33 ± 0.02	a, b, c
30	β-Farnesene	1449	0.97 ± 0.02	ND	1.04 ± 0.03	a, b, c
31	Alloaromadendrene	1459	ND	1.06 ± 0.03	7.82 ± 0.03	a, b, c
32	γ-Muurolene	1473	ND	ND	6.27 ± 0.02	a, b, c
33	α-Curcumene	1480	8.69 ± 0.05	1.70 ± 0.03	5.69 ± 0.07	a, b, c
34	Zingiberene	1490	0.42 ± 0.08	ND	ND	a, b, c
35	Valencene	1491	ND	ND	0.49 ± 0.09	a, b, c
36	α-Farnesene	1496	5.68 ± 0.05	0.94 ± 0.05	ND	b
37	α-Bulnesene	1500.1	ND	0.94 ± 0.09	ND	b
38	β-Curcumene	1503	ND	0.63 ± 0.04	ND	a, b, c
39	δ-Cadinene	1513	0.39 ± 0.03	ND	ND	a, b, c
	Oxygenated monoterpene hydrocarbons					
40	Linalool	1086	0.79 ± 0.04	ND	ND	a, b, c
41	Isoborneol	1147	4.31 ± 0.05	ND	2.97 ± 0.03	a, b, c
42	α-Terpineol	1175	1.45 ± 0.06	ND	ND	a, b, c
43	Cis-Carveol	1206	ND	ND	0.41 ± 0.04	a, b, c
44	Citronellal	1212	1.19 ± 0.04	ND	ND	a, b, c
45	Geraniol	1238	0.85 ± 0.04	ND	ND	a, b, c
46	Citral	1244	5.43 ± 0.06	ND	ND	a, b, c
47	Bornyl acetate	1270	2.82 ± 0.05	ND	1.42 ± 0.04	a
48	Isobornyl acetate	1271	0.59 ± 0.06	ND	3.43 ± 0.04	a, b, c
49	Citronellyl acetate	1335	0.51 ± 0.04	ND	ND	a, b, c
50	Geranyl acetate	1361	1.89 ± 0.05	ND	ND	a, b, c
51	Citronellyl isobutyrate	1488	ND	0.92 ± 0.05	ND	a, b, c
	Oxygenated sesquiterpene hydrocarbons					
52	Caryophyllene oxide	1570	ND	1.61 ± 0.02	3.99 ± 0.01	a, b, c
53	Ledol	1582	0.96 ± 0.03	ND	ND	a, b, c
54	Isoaromadendrene epoxide	1590	ND	3.47 ± 0.03	3.19 ± 0.04	a, b, c
55	Longiborneol	1592	ND	1.10 ± 0.05	ND	a, b, c
56	β-Eudesmol	1649	2.53 ± 0.03	ND	ND	a, b, c
57	α-Bisabolol	1668	ND	ND	1.21 ± 0.03	a, b, c
58	Isolongifolol	1695	ND	6.35 ± 0.03	ND	a, b, c
59	β-Santalol	1720	ND	1.19 ± 0.03	ND	c
	Oxygenated Diterpenes					
60	Phytol	2112	ND	3.52 ± 0.03	ND	a, b, c

Table 3. *Cont.*

Sr. #	Compounds	RI	%Composition			Identification Mode
			Fresh	Oven-Dried	Sun-Dried	
61	Trans-Geranylgeraniol	2201	ND	0.49 ± 0.04	ND	a, b, c
	Others					
62	3,4-Dimethylanisole	1147	0.51 ± 0.03	ND	ND	a, b, c
	Total		89.38	70.26	86.6	

a = identification based on retention indices; b = identification based on comparison of mass spectra; c = identification based on co-injection with authentic compounds compounds are listed in order of retention times. Values are mean ± standard deviation (SD), $n = 3$.

3.3. Cluster Analysis

Cluster analysis was conducted using F-software in order to evaluate the differences of presence of essential oils among two varieties of the ginger, i.e., Chinese and Thailand.

Cluster analysis is a statistical tool which is used to classify samples into groups where samples in one group are more similar to each other and different from those in other groups. It is normally used for exploratory data analysis, and as a method of discovery by solving classification issues. Here, it was used for exploring the majorly varying volatiles in essential oils responsible for the separation among different varieties and pretreatments of ginger rhizome.

The dendrogram (Figure 3) shows different varieties of ginger. Chinese and Thailand varieties are different from each other. This variation may be due to various cultivation procedure, climate, and storage conditions. The average distance method was used for cluster analysis—the higher the average distance, the lower the similarity of the samples. The dendrogram indicates that the data set could be regrouped into two groups. The cluster has an average distance of 70, indicating the volatiles of fresh samples of Chinese were greatly different from the volatiles of fresh samples of Thailand variety. The difference is based on volatile compounds in the essential oil extracted from each ginger variety.

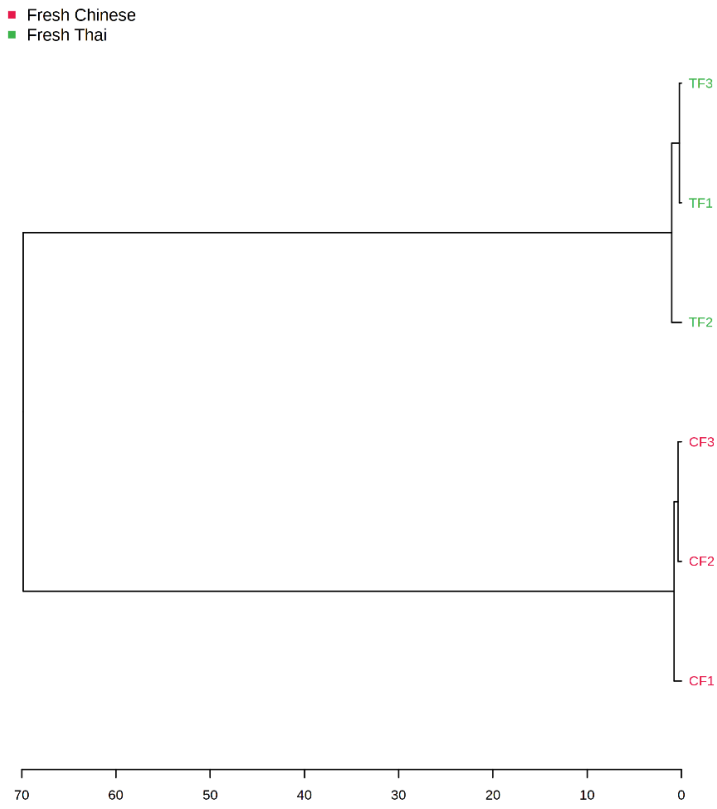

Figure 3. Cluster analysis of ginger varieties, i.e., Chinese and Thailand.

Heat maps were generated to determine the significantly varying compounds of each variety. In the heat map, two clusters in rows and columns were generated and highlighted by the hierarchical clustering; different clusters of samples indicate significant differences in two ginger varieties, i.e., Chinese and Thailand. The color difference showed the abundance of different volatile compounds extracted from ginger varieties (Figure 4). The results show that many compounds abundant in the Chinese cultivar shown in red color are rare in the Thailand cultivar, while compounds abundant in the Thailand cultivar are rare in the Chinese cultivar (Figure 4). The fresh ginger sample of the Chinese variety is shown in the dark maroon color for the camphor compound indicating its abundance.

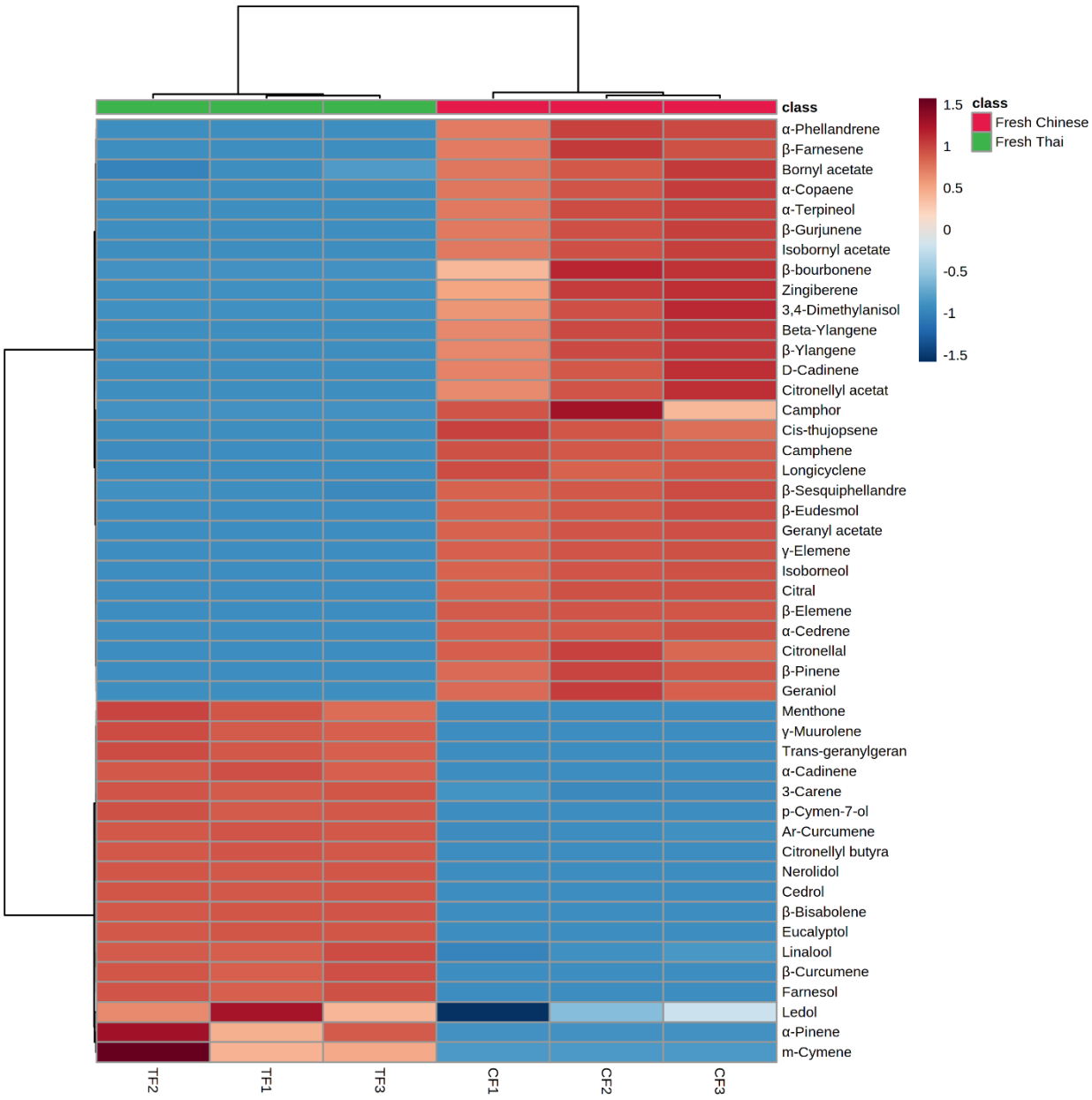

Figure 4. Heat maps showing the major class of compounds in both fresh Chinese and fresh Thai variety.

Similarly, α-phellandrene, β-farnesene, and β-bourbonene are abundant in Chinese and significantly rare in Thailand. The other abundant Chinese essential oils include bornylacetate, α-copaene, α-terpineol, β-gurjunene, isobornyl acetate, zingiberene, β-ylangene, δ-cadinene, and citronellyl acetate. In Thailand, m-cymene is most abundant colored in maroon, followed by α- pinene and Ledol, representing higher contents (Figure 4).

Figure 5 shows the cluster analysis of extracted volatile oils from the Chinese ginger variety. Three groups are observed in the dendrogram with significant average distance among them. The groups represent the Chinese ginger variety's fresh ginger oils (CF), oven-dried (COD), and sun-dried (CSD) volatile oils. The dendrogram indicates the formation of clusters, the first group (fresh Chinese essential oil variety) with the highest average distance of 80 shows a significant difference between the other two clusters. The second cluster is between the Chinese oven dried 90 °C for 5 h ginger variety and the Chinese sun-dried for five days. Here, the average distance of 56 is observed. It indicates that heat treatment affects the essential oil composition of the two treatments. It is further proved in the heat map showing the presence of extracted oils in different concentrations in three heat treatment groups, i.e., Chinese fresh, sun-dried, and oven-dried.

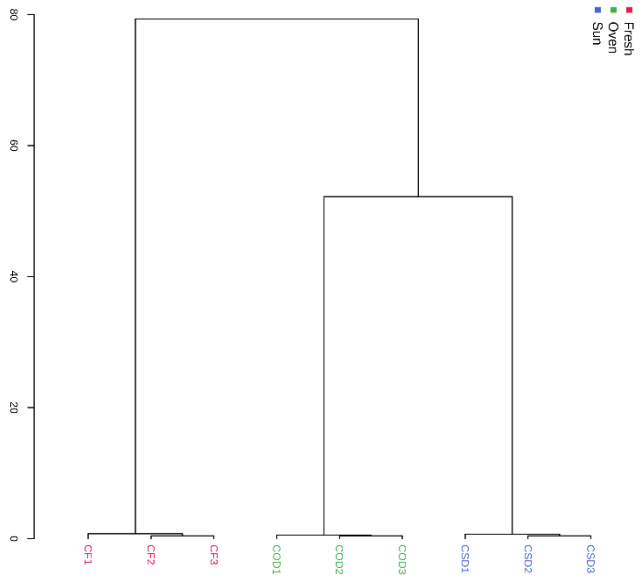

Figure 5. Cluster analysis of Chinese variety of volatile oils after heat treatment.

The volatiles responsible for this clustering were identified by corresponding heat-map (Figure 6). Twenty-five major essential oil compounds are significantly abundant in the Chinese fresh ginger variety, considerably lower in concentration in CSD, and least in COD. The abundant compounds in Chinese fresh are indicated in red/maroon color, white (CSD) indicates less presence, and blue of COD column indicates the least presence of various compounds in essential oils, such as γ-elememe, isoborneol, α-curcumene, and bornyl acetate. The most abundant compound in fresh Chinese essential oil is camphor, and this was also identified in the heat map. Linalool, ledol, β-gurjunene, α-terpineol, β-bourbonene, β-yiangene, δ-cadinene, citronellyl acetate, zingiberene, 3,4-dimethylanisol, and camphene are also present in the greater amount in CF sample than in COD and CSD.

The compounds abundant in CSD samples include valencene, cis-carveol, γ terpinene, and 15 other compounds shown in red in sun-dried column (Figure 6). All compounds have significantly less in concentration in fresh and oven-dried samples. Abundant compounds in oven-dried Chinese variety shown in red were 19 comtotal, among which trans-teranylgeraniol, β-curcumene, α-longipinene, α –pinene, and isolongifolol are the most abundant. The presence of limonene is significantly less observed in the fresh Chinese sample; similarly, 3-Carene and β-Elemene are the least prevalent t in sun-dried samples.

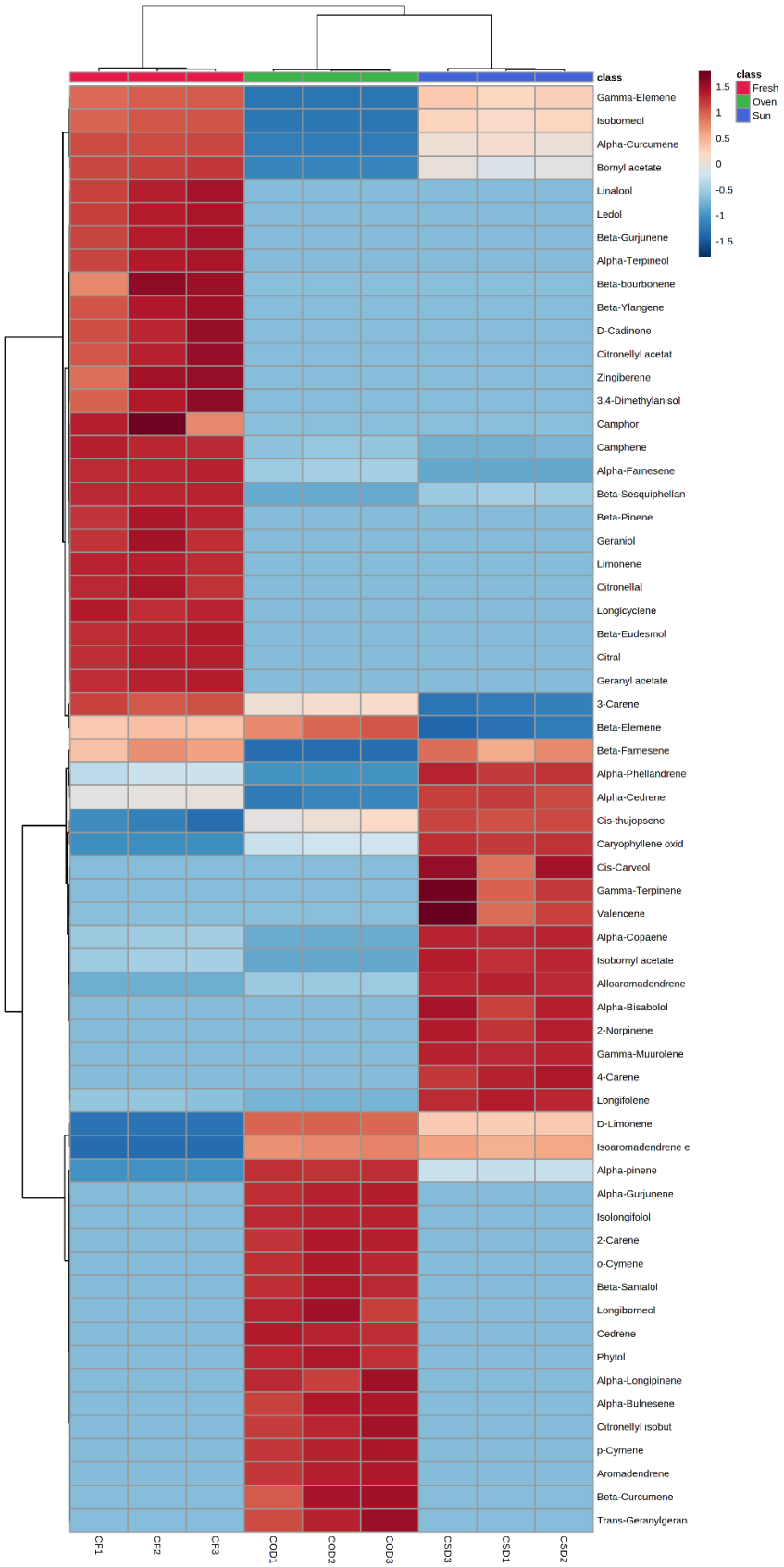

Figure 6. Heat maps showing major class of compounds in fresh, oven-dried, and sun-dried Chinese ginger variety.

The dendrogram in Figure 7 represents the cluster analysis after drying treatment of the Thailand ginger variety. The presence of three groups indicates the three-pre-treatments, i.e., Thailand fresh (TF), Thailand oven-dried (TOD), and Thailand sun-dried (TSD). The most considerable average difference is observed in Thailand's fresh cluster and the other two pre-treated clusters. The Thailand fresh indicated an average distance of more than 80, showing a significant difference in the composition of extracted oils than of TOD and TSD-treated ginger essential oil. An average distance of 65 is observed in Thailand oven-dried and Thailand sun-dried ginger essential oil, which indicated the difference in their essential oil composition. This difference is further verified by heat map (c), showing the variation in concentration of compounds in TF, TOD, and TSD.

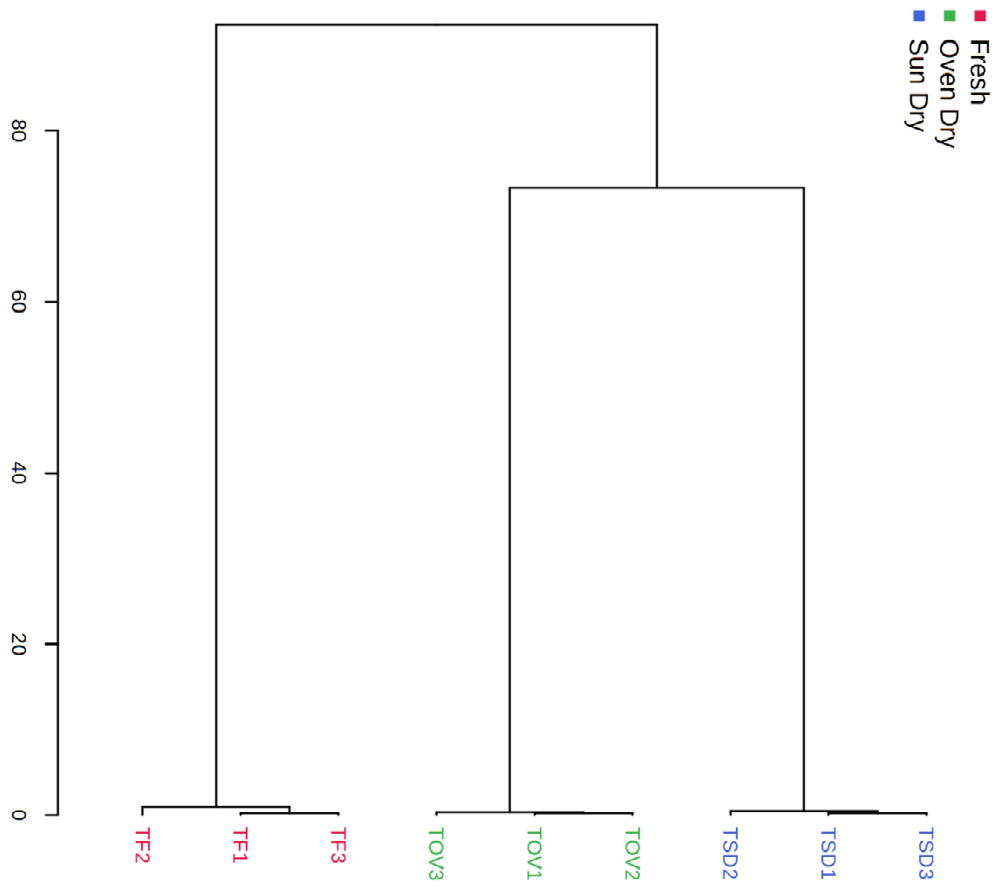

Figure 7. The dendrogram represents the cluster analysis after heat treatment done on the Thailand ginger variety.

Most of the compounds are abundant in Thailand oven-dried varieties (Figure 8). The drying pre-treatment enhances their concentration in TOD and TSD samples. The major abundant compounds in TSD are m-cymene, camphene, ledol, cadinol, trans-z-alpha-bisa, acorenone 1, α-copene, longipinene, valencene, β-ylangene, β-pinene, thujone, y-elemene, α-patchoulene, 2-bornene, α-terpineol, and cis-thujopsene. y-muurolene is significantly less in TF than in TSD and TOD. After the Thailand oven-dried variety, TF has more abundant compounds. Here, α-pinene is more in concentration. Nerolidol, menthone, trans-geranylgeraniol, p–cymen-7-ol, farnesol, α-cardinene, eucalyptol, cedrol, and citronellyl butyrate were also abundant in Thailand fresh variety. Linalool and βbisabolene are significantly abundant in TF, less in TOD, and least in TSD. The sun-dried samples of Thailand verity have only 13 major compounds, including caryophyllene oxid, alloaromadendrene, isopulegol, α-bisabolol, glubolol, β-santalol, o-cymene, Sabinol, and yhHimachalene. 3-carene is abundant in TSD, less in TF, and less in TOD.

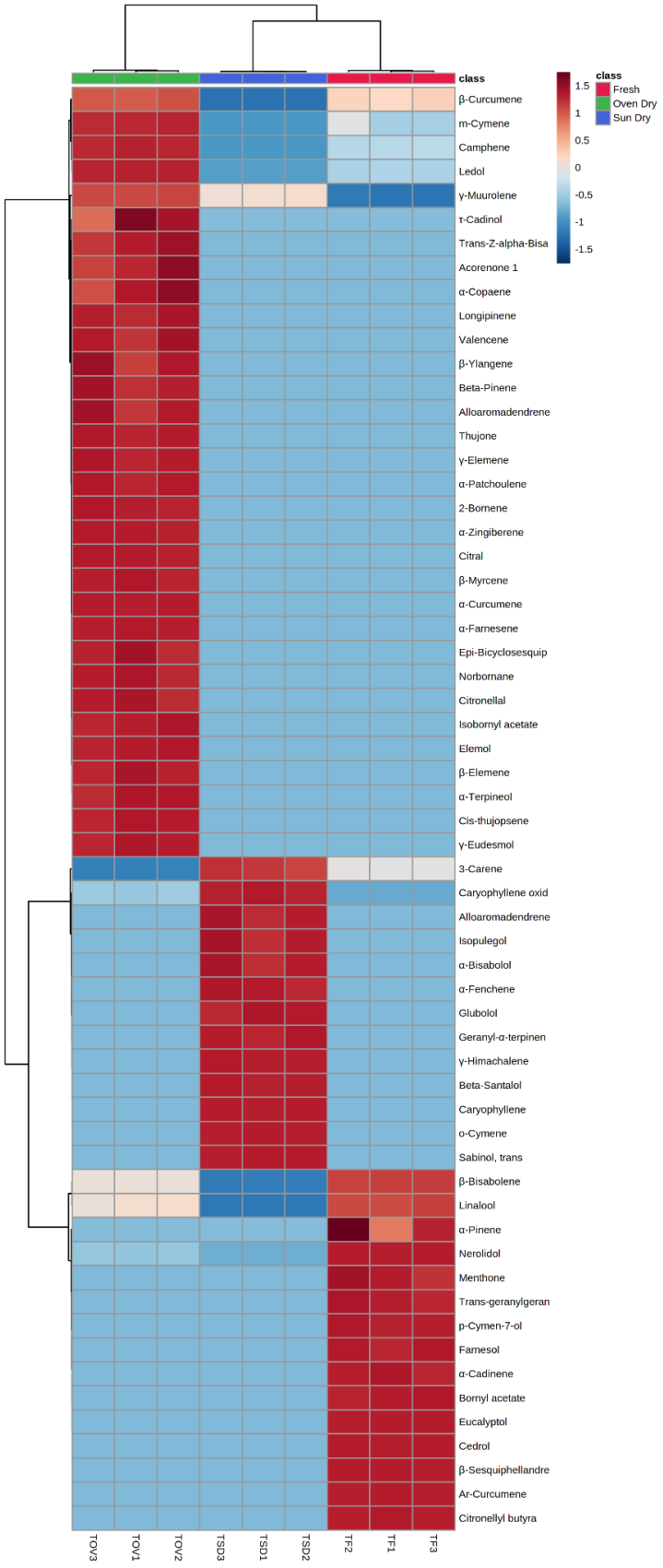

Figure 8. Heat maps showing the major class of compounds in fresh, oven-dried, and sun-dried Thailand ginger variety.

The presence of high content of camphor adds aroma and taste to the spice. α-Phellandrene and β–bourbonene essential oils show basic antimicrobial activity. Phellandrene and zingiberene show antioxidant properties, which are abundant in fresh Chinese varieties [36,37]. These are used in nano-emulsion, which are further used for making edible coatings for food products as the application of antibacterial and antioxidant activity [38]. Antioxidants are used to increase food's shelf life by decreasing lipid molecules' oxidation. Ginger is a source of natural oxidants; it serves as an alternative replacing the commercial antioxidants in fish and meat products [39].

Curcumene, zingberene, and zingereone are ginger bioactive compounds that show antibacterial activity against human pathogenic bacteria and catalytic activity as a reducing agent [40].

It is found that α-pinene, camphene, and cineol act as antimicrobial agents. These have been suggested to be used to reduce the population of microorganisms in green papaya, and other fresh and processed products [41]. Curcumene, β-bisabolene, α-zingiberene, and β-sesquiphelladrene are found to inhibit seed germination of certain weeds. Ginger essential oils can effectively be used as bio-herbicide against weeds without phytotoxicity to crops [42]. *Ar*-cucumene, β-bisabolene, linalool, caryophyllene, and eucalyptol are known for their antifungal activity. This relates to using ginger spice to develop natural pesticides fighting against root-rot disease [43,44]. A -bisabolol containing essential oil has the potential for pest control and can be used as an insecticide [45]. Pinene, limonene, and δ-3-carene are incorporated into the polymer and used as films, promising alternatives to food packaging and wound dressing materials [46]. Moreover, ginger essential oil microspheres are prepared and applied in the food and medical industries [47,48]. Zingiberene, arcurcumene, citral, β-bisabolene, geranial, and camphene are the major active compounds in ginger essential oil. Ginger oil also has high therapeutic values. It possesses anti-inflammatory, analgesic, anticancer, antioxidant, antinociceptive, and antitussive properties. Terpenes have been reported to possess phytoestrogenic activity [49]. Ginger essential oil with zingiberene, *δ*-amorphene, α-curcuminand α-bisabolene had significant effects against the *A. flavus* growth and aflatoxin B_1 and B_2 production [50]. Citral, camphor, and eucalyptol act as a bronchodilator. Studies on rat models showed a relaxing effect on the airway system. The ginger essential oil is used in modern medicine to treat cough [51]. α-zingiberene, ar-curcumene, and β-sesquiphellandrene caused nucleosomal DNA fragmentation and cell apoptosis to treat cancer [52]. 1,8-cineole, *β*-phellandrene, neral, and geranial cause cell death by apoptosis [53].

4. Conclusions

Different ginger varieties available in Pakistan's local markets are imported from different countries. These varieties are used in fresh and dried forms, prepared by different drying methods, for culinary and remedial purposes. Our results obtained after analyzing extracted essential oils of different forms of ginger belonging to Thailand and China using the GC-MS technique revealed that yield and chemical composition of essential oil is significantly affected by the different drying pretreatments and inter-varietal variations. The major compounds constituting the essence of ginger varied greatly based on drying methods and inter-varietal changes. Major compounds of ginger from Thailand were found to be sesquiterpenes (curcumene and caryophyllene), while those of the Chinese variety were monoterpenes (limonene) and sesquiterpenes (sesquiphellandrene). Due to differences in the concentration of these essential compounds, the flavor and odor of ginger forms vary greatly.

Moreover, cluster analysis has been performed to strengthen the obtained results. The obtained results were further exploited to differentiate among essential oils from varieties and pretreatments of ginger. Cluster analysis and heatmaps provided very clear differentiation among both the varieties and pretreatments of ginger rhizome. In the Pakistani market, the most sold ginger comes from China and Thailand. The user is mostly conscious about the fragrant profile of the ginger. The scientific evidence for this was

limited to the Pakistani perspective, and thus the current study can help the growers, ginger essential oil industry and end users to choose the best form of ginger, including variety or pretreatment method. The medicinal importance of ginger essential oil is recommended to be explored by testing and analyzing the antioxidant, anti-inflammatory, anti-tumor, and antimicrobial activities.

Author Contributions: G.M.K. and J.U.: conceptualization, methodology, writing original draft; N.N. and A.N.: performed the experimental studies; A.S., M.S. and J.K.: revised and refined the original draft; prepared the figures of manuscript; G.M.K., S.M. and X.Z.: reviewed and edited the manuscript; G.M.K. and B.J.: wrote the materials and methods of the manuscript; J.U. and X.Z.: reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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