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Influence of Cultivar and Maturity at Harvest on the Essential Oil Composition, Oleoresin and [6]-Gingerol Contents in Fresh Ginger from Northeast India

Challa Ravi Kiran, Ashok Kumar Chakka, K. P. Padmakumari Amma, A. Nirmala Menon, M. M. Sree Kumar, and V. V. Venugopalan*

Agroprocessing and Natural Products Division, National Institute for Interdisciplinary Science and Technology (NIIST), Council of Scientific and Industrial Research (CSIR), Trivandrum 695 015, India

Supporting Information

ABSTRACT: Severe flooding of the Brahmaputra River during the monsoon season and continuous rainfall in the northeast region (NER) of India cause an enormous loss of ginger crop every year. In this context, the present study investigates the variation in the essential oil composition and oleoresin and [6]-gingerol contents in 10 different fresh ginger cultivars harvested at 6- and 9-month maturity from five different states of NER. Monoterpenes, sesquiterpenes, and citral composition in the essential oil were evaluated to ascertain their dependence upon the maturity of ginger. Except Mizoram Thinglaidum, Mizoram Thingria, Nagaland Nadia, and Tripura I ginger cultivars, all other cultivars showed an increase in the citral content during the maturity that was observed for the first time. At 6-month maturity, a higher undecanone level was found in Nagaland Nadia (7.36 ± 0.61%), Tripura I (6.23 ± 0.61%), and Tripura III (9.17 ± 0.76%) cultivars, and these data can be used as a benchmark to identify those immature varieties. Interestingly, the Nagaland Nadia cultivar showed higher *ar*-curcumene (9.57 ± 0.58%) content than zingiberene (5.84 ± 0.24%), which was unique among all cultivars. Ginger harvested at 9-month maturity from the Tripura II cultivar had the highest citral content (22.03 ± 0.49%), and the Meghalaya Mahima cultivar had the highest zingiberene content (29.89 ± 2.92%). The oleoresin content was found to decrease with maturity in all cultivars, except Assam Fibreless and Manipur I. Moreover, the highest oleoresin (11.43 ± 0.58 and 9.42 ± 0.63%) and [6]-gingerol (1.67 ± 0.03 and 1.67 ± 0.05 g) contents were observed for Tripura II and Nagaland Nadia, respectively. This study suggests that Tripura and Nagaland are the most ideal locations in NER for ginger cultivation to obtain high yields of oleoresin and [6]-gingerol contents and harvesting at the 6-month maturation will compensate for the loss of ginger crop caused by the Brahmaputra River flooding in NER every year.

KEYWORDS: *Ginger, maturity of ginger, essential oil, citral, oleoresin, [6]-gingerol*

INTRODUCTION

The northeast region (NER) of India is best known for its biodiversity, with wide variations in ecology, topography, and soil characteristics.[1] Agriculture is the major economic activity in NER, where the Brahmaputra River is the major water supply for irrigation. According to agricultural data, ginger is a main crop in NER, contributing 49% of India's total area under ginger cultivation and 72% of India's ginger crop production.[1,2] Every year during monsoon season (August−October), severe floods of the Brahmaputra River cause enormous loss of the ginger crop at the 6-month maturity period. Further, because of continuous rainfall, 9-month-matured cultivars have a high moisture content, which results in a low yield upon drying and a high cost for the drying operation. Upon drying, it gives a highly shriveled and unattractive dry ginger; hence, the crop is marketed largely as fresh green ginger.[2,3]

Ginger (*Zingiber officinale* Roscoe; family Zingiberaceae) is an underground rhizome, widely used as a spice in the food and beverage industry.[4] Ginger is also used as a valuable traditional medicine and ayurvedic formulations from the time immemorial. The chemical constituents of ginger act as potent antioxidant and pharmacological agents to several health discomforts.[4,5] Extensive applications of ginger were found in the perfumery and cosmetic industries because of its unique flavor and fragrance. Ginger essential oil is a mixture of monoterpenic and sesquiterpenic compounds that include zingiberene, β-bisabolene, γ-cadinene, β-sesquiphellandrene, neral, and geranial.[5,6] Ginger oleoresin is a mixture of gingerols and shogaols, among which [6]-gingerol is a major pungent compound.[7] The essential oil contributing to the characteristic flavor of ginger varies from 1.0 to 3.0%, and oleoresin varies from 4.0 to 7.5%.[8,9] Characterization of ginger essential oil and oleoresin from ginger cultivars from different geographical locations is one of the important areas of research.[10−13] Govindarajan et al.[3] demonstrated the effect of climatic conditions on ginger rhizome growth and composition and showed that moist climatic conditions are favorable for the growth. Wohlmuth et al.[14] studied the effect of genetic variability on the essential oil and the gingerol content of diploid and tetrapliod clones of Australian ginger cultivars. They showed that essential oil and gingerol compositions are

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genetically determined.[14,15] Bailey-Shaw et al.[16] studied the changes in contents of oleoresin and pungent compounds of Jamaican ginger during 6−8 months maturity and found that oleoresin and gingerol contents vary with maturation and locality. However, no comprehensive studies on the essential oil composition and oleoresin yield of fresh ginger cultivars of NER at 6- and 9-month maturity periods were reported. The aim of the present study is to investigate the variation in essential oil composition as well as oleoresin and [6]-gingerol contents from NER ginger cultivars harvested at 6- and 9-month maturation stages to identify superior varieties of ginger and an appropriate time to harvest ginger with higher yields of oleoresin.

MATERIALS AND METHODS

Plant Materials and Chemicals. Fresh ginger rhizomes of 10 ginger (*Z. officinale* Roscoe) cultivars (Table 1) harvested at 6-month maturity (September 2012) and 9-month maturity (December 2012) periods were collected from six different states of NER, namely, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura, via field stations of Spices Board, Indian Council of Agriculture Research (ICAR), and North Eastern Regional Agricultural Marketing Corporation (NERAMAC).

Table 1. Ginger Cultivars Studied and Their Origin

cultivar name	origin	ID[a]	ID[b]
Assam Fibreless	Assam	NES1	NED1
Manipur I	Manipur	NES2	NED2
Meghalaya Mahima	Meghalaya	NES3	NED3
Mizoram Thingpui	Mizoram	NES4	NED4
Mizoram Thinglaidum	Mizoram	NES5	NED5
Mizoram Thingria	Mizoram	NES6	NED6
Nagaland Nadia	Nagaland	NES7	NED7
Tripura I	Tripura	NES8	NED8
Tripura II	Tripura	NES9	NED9
Tripura III	Tripura	NES10	NED10

[a]Harvested in September 2012 at 6-month maturity. [b]Harvested in December 2012 at 9-month maturity.

Citral (neral + geranial) was purchased from Alfa Aeser, Germany. Nonanoic acid vanillylamide (NVA) was purchased from Sigma Chemical Co. (St. Louis, MO), and high-performance liquid chromatography (HPLC)-grade ethylene dichloride, hexane, methanol, acetonitrile, and acetic acid were purchased from Merck (Darmstadt, Germany).

Essential Oil Isolation. Unpeeled rhizomes were washed, chopped, and hydrodistilled in a Clevenger distillation[14] apparatus for 6 h to isolate the essential oil. The essential oil was collected in the sample tubes, stored in a freezer at −20 °C, and further subjected to gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS) analyses.

Oleoresin Extraction. Oleoresin from all ginger cultivars was extracted by a Soxhlet apparatus using ethylene dichloride as a solvent. Ethylene dichloride has been recommended as a solvent in view of water immiscibility, selectivity in extracting flavor components (by not extracting non-flavor polar components as sugars, oligosaccharide, etc.), and no flammability; no refrigeration is required for its recovery.[3] The extract was evaporated to dryness in a rotary evaporator (Buchi, Switzerland) under a nitrogen stream. A total of 1% of oleoresin was prepared by dissolving in methanol and purified through polytetrafluoroethylene (PTFE) filters for further HPLC analysis.

GC Analysis. Essential oils were analyzed on a Shimadzu GC 2010 model gas chromatograph. Helium was used as the carrier gas at a flow rate of 1 mL/min. A capillary column (60 m × 0.22 mm inner diameter × 1.00 μm d_f) was used for the analysis (BPX-5, SGE, Ltd., Melbourne, Victoria, Australia), and a flame ionization detector (FID) was used. The split ratio was 1:50. The oven temperature was programmed from 80 °C (hold time of 1 min) to 220 °C at 5 °C/min and held for 10 min. The injector temperature was 250 °C, and the detector temperature was 300 °C. Each compound was identified by retention indices. Retention indices were determined using a C_8−C_{22} *n*-alkane mixture.[17]

GC−MS Analysis. Essential oils were analyzed on a Shimadzu GC−MS model GC-17A equipped with mass spectrophotometer GC−MS QP 5050A. Helium was used as the carrier gas at a flow rate of 1 mL/min. A capillary column (60 m × 0.22 mm inner diameter × 1.00 μm d_f) was used for the analysis (BPX-5, SGE, Ltd., Melbourne, Victoria, Australia). The split ratio was 1:50. The oven temperature was programmed from 80 °C (hold time of 1 min) to 220 °C at 5 °C/min and held for 10 min. The injector temperature was 250 °C, and the interface temperature was 270 °C, with an ionization voltage of 70 eV. Compound identification was based on comparisons to mass spectra and retention indices of authentic reference compounds where possible and by reference to the National Institute of Standards and Technology (NIST) library, flavors and fragrance of natural and synthetic compounds (FFNSC) library, and published data.[18,19]

HPLC Analysis of Ginger Oleoresin. HPLC analysis was carried out using the ISO 13685:1997(E) method for HPLC analysis of the pungent principles of ginger.[20] A Waters HPLC system (Waters/Millipore, Milford, MA) consisting of a model 515 pump, and a model 2487 dual wavelength absorbance detector was used. The separation of the oleoresin extract was conducted in a C_{18} column (Waters), 250 mm, 4.6 mm inner diameter, and 5 μm. A mixture of acetonitrile with Millipore water (65:35 volume ratio) containing 1% acetic acid was used as the mobile phase. Samples were eluted isocratically at a flow rate of 1 mL/min for 30 min and detected at a wavelength of 280 nm. NVA was used as the external standard. A volume of 20 μL of all samples and standards was injected in triplicate.

Statistical Analysis. Statistical analysis, including cluster analysis, was performed by Wessa's statistical software.[21] All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD) values. The oil composition of the 10 cultivars was the subject of hierarchical cluster analysis based on the 17 most abundant constituents.

RESULTS AND DISCUSSION

Influence of Cultivar and Maturity on the Essential Oil Composition. The essential oil composition is one of the important parameters to understand the variation among different ginger cultivars. A total of 30 volatile compounds were confirmed in the essential oil of 10 NER ginger cultivars through GC and GC−MS analyses. The percentage compositions of the 17 most abundant constituents in the essential oil from ginger cultivars harvested at 6- and 9-month maturity were described in Table 2. These data clearly show a wide variation in essential oil composition among all cultivars. For instance, the Assam Fibreless cultivar showed an increased camphene content (NES1 with 8.45 ± 0.99% and NED1 with 18.22 ± 0.56%) during maturity, while Manipur I (NES2 with 19.95 ± 1.23% and NED2 with 2.91 ± 0.02%) and Mizoram Thinglaidum (NES5 with 18.99 ± 1.05% and NED5 with 7.50 ± 0.29%) cultivars showed significant reduction in the camphene content during maturity. Furthermore, the Tripura I cultivar showed the highest citronellal content (NES8 with 6.43 ± 0.27%) among 6-month-matured ginger cultivars, while it showed the lowest level of citronellal (NED8 with 1.18 ± 0.08%) among 9-month-matured ginger cultivars. These results clearly explain the influence of different cultivars on essential oil composition and also their variation during maturity. Higher contents of α-pinene, camphene, and β-pinene were found in NES5 and NES6 cultivars in comparison to the NES4 cultivar from the same region (Mizoram). Similarly, NES8 (6.23 ±

Table 2. Essential Oil Composition of NER Ginger Cultivars Harvested in (a) September 2012 at 6-Month Maturity and (b) December 2012 at 9-Month Maturity[a]

compound	NES1	NES2	NES3	NES4	NES5	NES6	NES7	NES8	NES9	NES10	mean ± SD (range)
(a) Essential Oil Composition of NER Ginger Cultivars Harvested in September 2012 at 6-Month Maturity											
α-pinene	2.65	6.41	1.48	1.89	6.16	6.05	1.99	1.63	3.77	2.31	3.43 ± 0.24 (0.18−0.34)
camphene	8.45	19.95	4.76	4.93	18.99	18.12	5.41	5.18	9.65	6.05	10.15 ± 1.14 (0.97−1.23)
β-pinene	1.31	3.49	1.01	0.98	2.54	2.67	1.10	1.18	1.75	1.36	1.74 ± 0.27 (0.13−0.48)
limonene	1.39	3.40	0.91	1.05	2.65	2.75	1.12	0.97	1.65	1.01	1.69 ± 0.18 (0.11−0.26)
β-phellandrene	2.73	10.57	2.58	3.76	7.68	9.06	1.74	1.65	6.23	1.66	4.77 ± 0.39 (0.26−0.68)
cineole	1.56	3.44	0.93	1.58	2.45	2.40	3.17	1.78	3.77	1.33	2.24 ± 0.41 (0.14−0.67)
neral	7.97	8.01	5.02	5.20	5.67	4.27	4.85	4.37	4.17	3.92	5.35 ± 0.96 (0.87−1.13)
citronellal	1.13	0.74	3.73	1.37	1.91	2.80	4.53	6.43	1.39	4.37	2.84 ± 0.15 (0.09−0.27)
geranial	12.35	12.00	9.06	7.86	8.85	7.00	12.79	7.19	13.01	9.70	9.98 ± 0.39 (0.12−0.67)
undecanone	1.26	0.16	3.28	0.59	0.75	0.81	7.36	6.23	0.51	9.17	3.01 ± 0.46 (0.04−0.76)
ar-curcumene	9.01	3.32	6.65	9.79	5.75	7.01	9.57	6.55	5.86	5.26	6.88 ± 0.34 (0.12−0.65)
zingiberene	12.85	7.16	24.00	20.52	11.12	11.41	5.84	20.69	17.37	19.97	15.09 ± 1.16 (0.24−1.89)
β-bisabolene	5.07	2.12	4.51	5.86	3.44	4.06	4.40	4.90	4.16	4.53	4.31 ± 0.26 (0.07−0.65)
γ-cadinene	2.31	1.01	2.05	4.29	1.62	1.92	3.72	2.80	3.12	1.99	2.48 ± 0.25 (0.13−0.46)
β-sesquiphellandrene	7.16	3.69	8.37	8.57	6.44	6.07	3.78	8.30	10.85	7.55	7.08 ± 0.87 (0.56−1.18)
eudesmane	0.21	0.06	0.17	0.26	0.10	0.10	0.46	0.19	0.12	0.17	0.18 ± 0.03 (0.01−0.04)
(*E*)-nerolidol	1.18	0.18	0.96	0.78	0.39	0.44	0.99	1.42	0.60	1.60	0.85 ± 0.11 (0.07−0.18)
(b) Essential Oil Composition of NER Ginger Cultivars Harvested in December 2012 at 9-Month Maturity											
α-pinene	5.97	0.70	1.50	1.50	2.30	4.93	3.11	5.40	1.56	3.63	3.06 ± 0.16 (0.06−0.36)
camphene	18.22	2.91	5.00	3.50	7.50	16.00	9.96	16.50	5.06	10.57	9.52 ± 0.31 (0.02−0.56)
β-pinene	3.38	1.60	1.37	1.00	1.50	2.30	1.95	2.68	1.37	2.09	1.92 ± 0.14 (0.08−0.24)
limonene	2.81	1.34	1.35	1.00	1.70	2.46	1.68	2.39	1.37	1.97	1.81 ± 0.14 (0.12−0.19)
β-phellandrene	7.63	4.54	4.19	5.70	6.50	8.30	4.66	5.12	3.66	3.04	5.33 ± 0.23 (0.03−0.41)
cineole	3.59	2.14	1.42	2.60	1.57	4.40	3.48	2.75	1.41	2.61	2.60 ± 0.28 (0.08−0.38)
neral	6.17	5.08	4.80	5.72	4.14	3.68	4.93	1.76	3.62	7.51	4.74 ± 0.34 (0.12−0.42)
citronellal	2.82	1.89	1.40	1.30	3.94	2.68	2.90	1.18	2.84	3.18	2.41 ± 0.17 (0.08−0.29)
geranial	13.44	15.00	12.60	12.80	6.75	6.43	10.45	5.90	14.25	14.52	11.21 ± 0.46 (0.20−0.62)
undecanone	0.78	1.26	1.02	0.63	0.64	0.16	1.12	1.60	2.39	1.28	1.09 ± 0.11(0.01−0.19)
ar-curcumene	2.81	5.49	4.65	7.06	6.35	6.37	3.05	3.80	4.46	4.31	4.84 ± 0.26 (0.08−0.36)
zingiberene	12.39	23.90	29.89	29.20	25.70	9.76	22.30	21.10	24.50	21.88	22.06 ± 2.34 (0.28−2.92)
β-bisabolene	2.79	5.10	5.28	5.70	4.90	4.30	4.50	6.20	6.20	4.16	4.91 ± 0.33 (0.17−0.49)
γ-cadinene	1.50	3.00	2.49	4.50	2.10	2.60	1.70	3.50	4.42	1.72	2.75 ± 0.44 (0.27−0.74)
β-sesquiphellandrene	4.66	9.20	10.10	8.92	7.90	6.30	6.10	9.10	9.81	6.92	7.90 ± 0.66 (0.03−0.96)
eudesmane	0.06	1.10	0.18	0.30	0.17	1.40	0.06	3.00	2.61	0.20	0.91 ± 0.20 (0.01−0.36)
(*E*)-nerolidol	0.47	0.60	1.32	0.84	0.84	0.76	0.50	1.20	1.11	0.54	0.82 ± 0.12 (0.08−0.19)

[a]Values are percentage content.

0.61%) and NES10 (9.17 ± 0.76%) cultivars had a higher undecanone content (found to possess insecticidal activity) than the NES9 (0.51 ± 0.09%) cultivar of the same region (Tripura). These observations clearly demonstrate that there was a significant variation in essential oil composition among the cultivars from the same region and also different regions. It can also be inferred that most of the cultivars had a different constituent in higher proportions, apart from the zingiberene content. NES2, NES5, NES6, and NED1 cultivars had a higher camphene content (camphor aroma). The NES2 cultivar had a higher β-phellandrene content (peppery minty aroma). NES8 and NES10 cultivars had higher cital and citronellal contents (lemony aroma), which imparts a particular aroma to the essential oil of these cultivars. Moreover, use of these cultivars exerts a great effect on the food and beverage industry because of their peculiar aroma profile. The GC profile of the essential oil of 10 NER ginger cultivars at 6- and 9-month maturity periods was represented in Figure 1. There was a huge variation observed in both monoterpene and sesquiterpene compositions during maturity. Interestingly, the NES7 cultivar showed a higher *ar*-curcumene content (9.57 ± 0.58%) than the zingiberene content (5.84 ± 0.24%) at 6-month maturity. Similarly, it showed a higher undecanone concentration at 6-month maturity (NES7 cultivar with 7.36 ± 0.61%) compared to 9-month maturity (NED7 cultivar with 1.12 ± 0.14%), where zingiberene was the major compound (NED7 cultivar with 22.30 ± 2.14%). These values suggest that volatile composition of each cultivar depends upon the maturity and also the location, and it is in agreement with previous findings.[3]

Variation in the Citral Content during Maturity. Neral (*cis*-citral) and geranial (*trans*-citral) are the stereoisomers occurring in many plants, often referred to as citral. The total citral content influences the lemony aroma of ginger and has wide applications in the food and cosmetic industries.[22] The comparison of the citral content among NER ginger cultivars at 6- and 9-month maturity stages was shown in Figure 2. At 6-month maturity, the citral content ranged from 11.27 ± 0.31% (NES6) to 20.01 ± 0.35% (NES2), while it ranged from 7.66 ± 0.35% (NED8) to 20.08 ± 0.38% (NED2) at 9-month maturity. Except Mizoram Thingpui and Tripura III cultivars, the citral content in all other cultivars did not vary with maturity. Interestingly, the citral content decreased among the

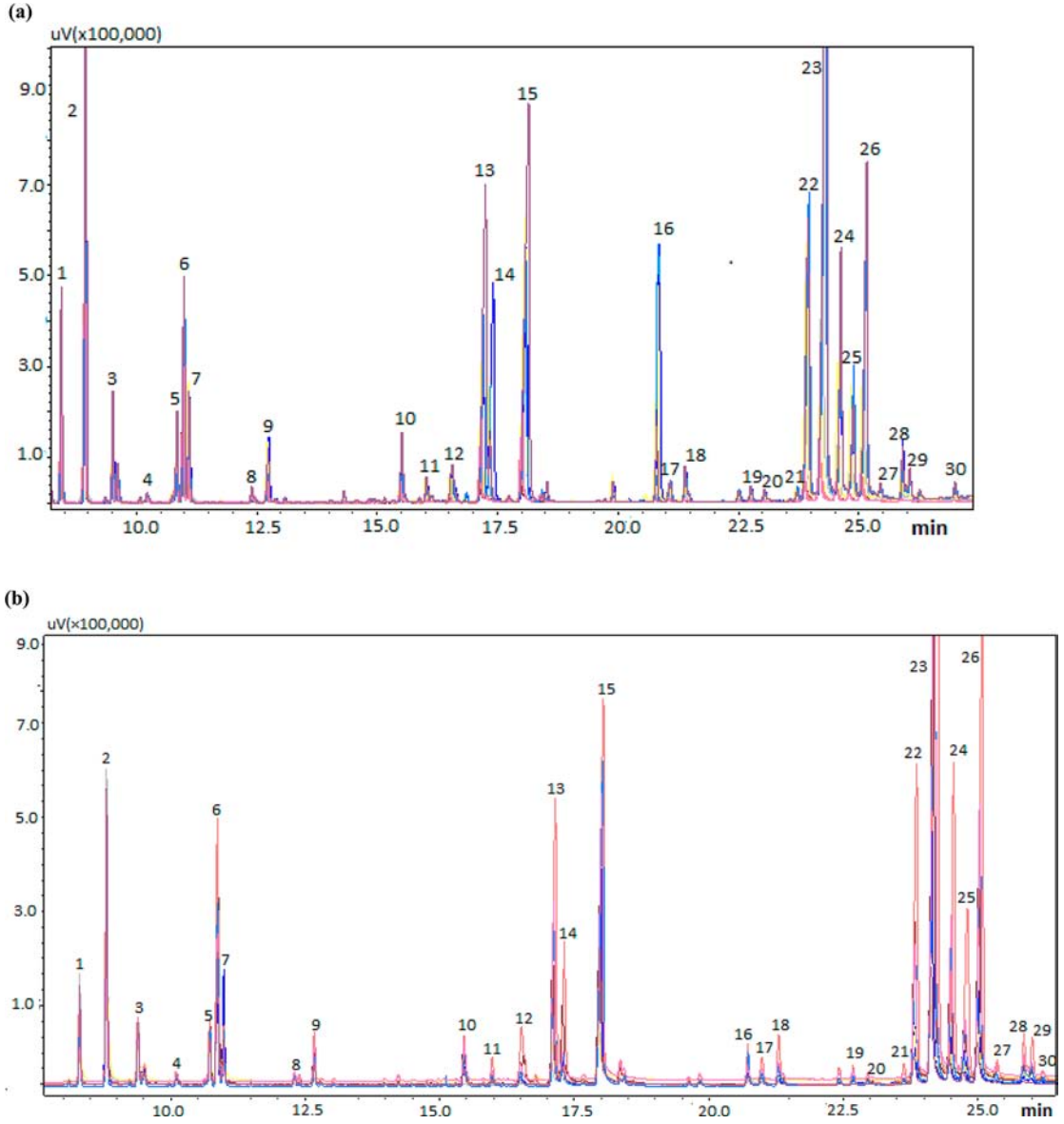

Figure 1. GC profile of essential oil composition of NER ginger cultivars harvested in (a) September 2012 at 6-month maturity and (b) December 2012 at 9-month maturity: 1, α-pinene; 2, camphene; 3, β-pinene; 4, *p*-cymene; 5, limonene; 6, β-phellandrene; 7, cineole; 8, linalool oxide; 9, linalool; 10, citronellol; 11, borneol; 12, α-terpeniol; 13, neral; 14, citronellal; 15, geranial; 16, undecanone; 17, copaene; 18, β-elemene; 19, thujopsene; 20, caryophyllene; 21, alloaromadendrene; 22, *ar*-curcumene; 23, zingiberene; 24, β-bisabolene; 25, γ-cadinene; 26, β-sesquiphellandrene; 27, eudesmane; 28, (*E*)-nerolidol; 29, caryophyllene oxide; and 30, elemol.

other two Mizoram cultivars, Nagaland Nadia and Tripura I, which is not reported earlier. The mean value of the citral content in ginger cultivars at 6-month maturity was 15.25 ± 0.36%, while at 9-month maturation, it was about 15.96 ± 0.39%. These values clearly indicate that the citral content was consistent during 6- and 9-month maturation, and overall results demonstrate that NER ginger cultivars have a moderate citral content, which is similar to that of Sri Lankan ginger.[23,24] Bhaisa and Majulay ginger varieties from Sikkim (another state of NER) were found to have 9.4 and 18.7% of the citral content, respectively.[19] Sub-Himalayan region ginger varieties were found to have 8.50−29.51% of the citral content,[25] whereas south Indian ginger varieties were found to have 6.73−17.55% of the citral content.[11] These results explain that the citral content of NER ginger varieties is comparable to other ginger varieties from India. However, Fiji and Australian ginger cultivars are known to have a rich source of citral, while Jamaican ginger is low in citral content.[14,26]

Generally, total cital composes of more geranial content than neral content. Sekiwa-Iijima et al.[27] studied the geraniol dehydrogenase activity related to the formation of geranial during maturation of ginger and found that its activity is higher in matured ginger and decreased upon storage. Sakamura et al.[28] found the inverse relationship between geranial and geranyl acetate during storage and cultivation of ginger rhizome. In agreement with these studies, our results clearly show that the geranial content in all ginger cultivars is higher at the 9-month maturation than the 6-month maturation. In this study, the neral/geranial ratio ranged from 0.32 (NES9) to 0.67 (NES2) at 6-month maturity, while it ranged from 0.25 (NES9) to 0.61 (NES4) at 9-month maturity, showing that geranial formation was higher than neral formation during maturation.

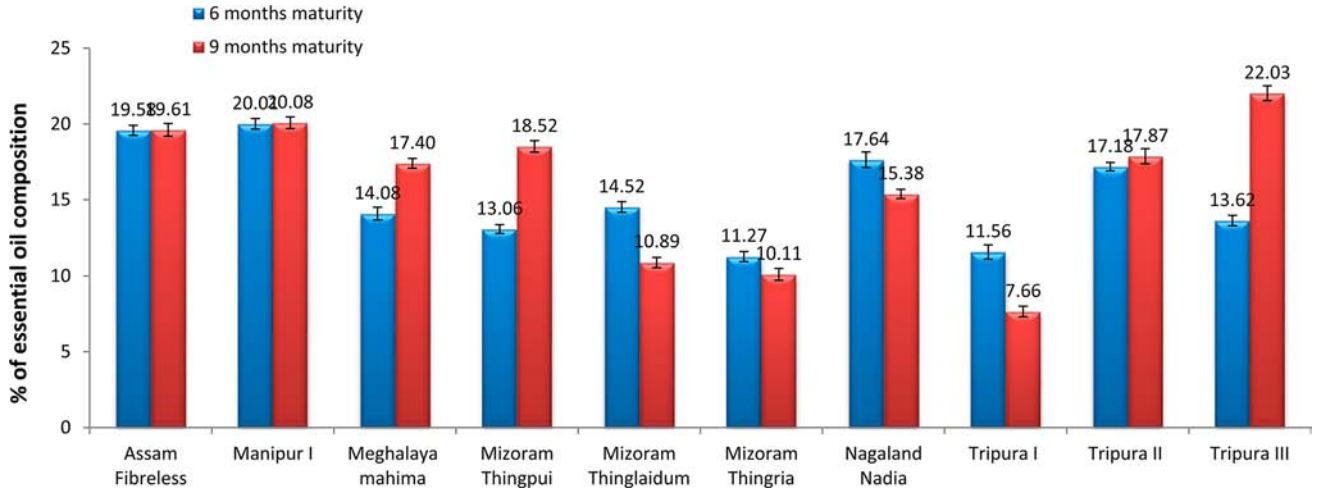

Figure 2. Variation of the citral content in the NER ginger cultivars harvested in September 2012 at 6-month maturity and December 2012 at 9-month maturity.

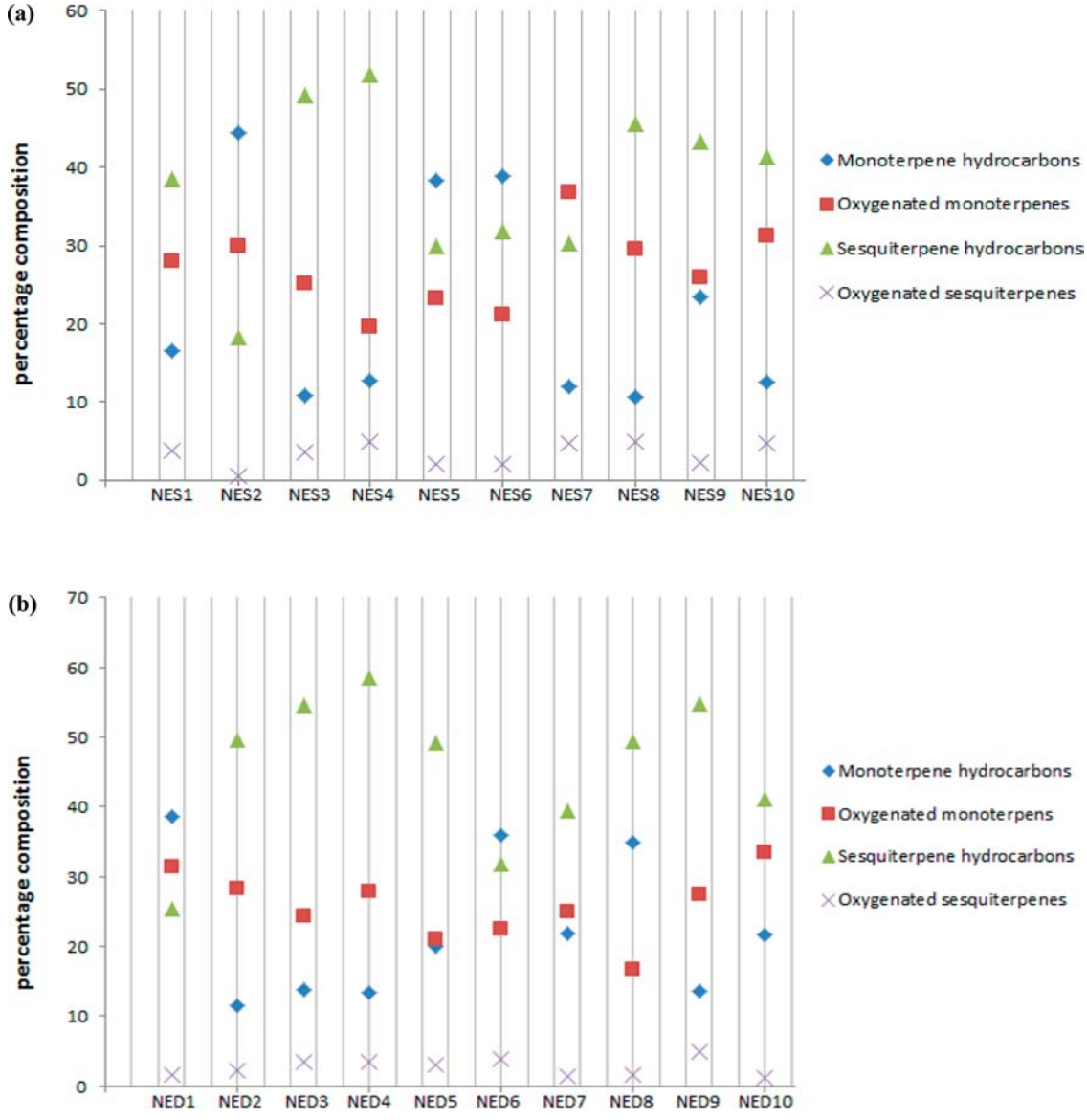

Figure 3. Percentage composition of monoterpenes and sesquiterpenes in the NER ginger cultivars harvested in (a) September 2012 at 6-month maturity and (b) December 2012 at 9-month maturity.

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The citral/zingiberene ratio was between 0.56 (NES8) and 3.02 (NES7) among the ginger cultivars harvested at 6-month maturation and was between 0.36 (NED8) and 1.58 (NED1) among the ginger cultivars harvested at 9-month maturation. In summary, the citral content among NER ginger cultivars was increased with maturity but zingiberene is higher than citral in all cultivars (except NED6) at 9-month maturity. The inverse relationship between citral and zingiberene contents can be explained by their common biosynthetic origin.[14]

Variation in Monoterpene and Sesquiterpene Contents. Variation in the monoterpene and sesquiterpene levels in all ginger cultivars during maturity was shown in Figure 3. Among ginger cultivars harvested at 6-month maturity, NES2, NES5, and NES6 cultivars showed the highest monoterpene content compared to sesquiterpenes. The NES7 cultivar showed a higher oxygenated monoterpene content than sesquiterpenes, and that was highest among all cultivars. At 9-month maturity, the NED1 cultivar showed a higher monoterpene concentration over sesquiterpenes and the NED6 cultivar was consistent with its higher monoterpene content. Overall results showed that the monoterpene hydrocarbon content decreased during maturation of 6 months (22.06 ± 1.98%) to 9 months (22.47 ± 1.57%). In the 6-month-maturated cultivars, the zingiberene content ranged from 5.84 ± 0.24% (NES7) to 24.00 ± 1.89% (NES3), with the mean value of 15.09 ± 1.16%. However, in the 9-month-matured cultivars, it ranged from 9.76 ± 0.28% (NED6) to 29.89 ± 2.92% (NED3), with the mean value of 22.06 ± 2.34%. These results clearly show that the zingiberene content was increased with maturity, which is in accordance with the literature.[3,5] Assam Fibreless, Mizoram Thingpui, Mizoram Thingria, and Nagaland Nadia cultivars showed a higher *ar*-curcumene content than *β*-sesquiphellandrene at 6-month maturity, and that was decreased at 9-month maturity. Usually, higher *ar*-curcumene levels indicate the aging of oil or oil recovered from stored ginger, and it was also explained that *ar*-curcumene can be formed at the expense of zingiberene and *β*-sesquiphellandrene during the storage.[1] However, our results reflect the possibility of conversion of *ar*-curcumene to zingiberene and *β*-sesquiphellandrene during the maturity of ginger rhizome. Rani[29] postulated that the bisabolyl cation that may be derived from farnesyl pyrophosphate is the penultimate precursor of *ar*-curcumene, zingiberene, and *β*-bisabolene. Further, two 1,2-hydrogen shifts in the bisabolyl cation lead to the formation of zingiberene, whereas only one 1,2-hydrogen shift leads to the formation of *ar*-curcumene. This can be one of the factors that affects the composition of *ar*-curcumene and zingiberene in the ginger oil, and there are no reports regarding the change in their composition during maturity. However, an inverse relationship between monoterpenes and sesquiterpenes can be explained by their biosynthetic phenomena. The farnesyl cation (which forms through ionization of farnesyl diphospahte, a fundamental sesquiterpene precursor), which can further isomerize to the nerolidyl cation and the geranyl cation (which forms through ionization of geranial diphosphate, a fundamental monoterpene precursor), makes monoterpenes and sesquiterpenes compete for common precursors, which eventually lead to their compositional variations, and it is genetically determined.[14,30,31]

Cluster Analysis. To examine the change in similarity of oil composition displayed by the 10 cultivars during maturity, a hierarchical cluster analysis based on the composition of major constituents was performed. This is a multivariate procedure that allows for the classification of cases (or variables) into groups based on Euclidean distances between cases.[21] The dendogram of the 10 cultivars of 6-month maturity was shown in Figure 4a. Two major clusters, viz., clusters 1 and 2, can be seen. Cluster 1 was composed of six cultivars, while cluster 2 was composed of four cultivars. Cluster 1 formed two subclusters, viz., NES4, NES3, and NES1, NES10 and NES8, and NES7 formed an out group within the cluster. Cluster 2 formed two subclusters, viz., NES6, NES5, and NES2 and NES9 that stand alone within the cluster. Another dendogram of the 10 cultivars of 9-month maturity was shown in Figure 4b. Two major clusters, viz., clusters 1 and 2, can be seen. Cluster 1 was composed of six cultivars, while cluster 2 was composed of four cultivars. Cluster 1 formed two subclusters, viz., NED3 and NED1, NED10 and NED9, and NED8 and NED4. Cluster 2 formed two subclusters, viz., NED7 and NED6 and NED5 and NED2, which formed separate out groups within the cluster. From these two dendograms, it can be inferred that the cultivars under the same group showed more similarity in the essential oil composition. NES7 and NES9 cultivars showed more similarity at 6-month maturation, while NED7 and NED9 formed showed more dissimilarity at 9-month maturation. Moreover, these clusters show that similarity mainly exists between different cultivars from different regions than the same regions. In addition, it can be useful for farmers to choose alternate varieties with more similarity in the absence of a desired variety at the time of cultivation and also maturation.

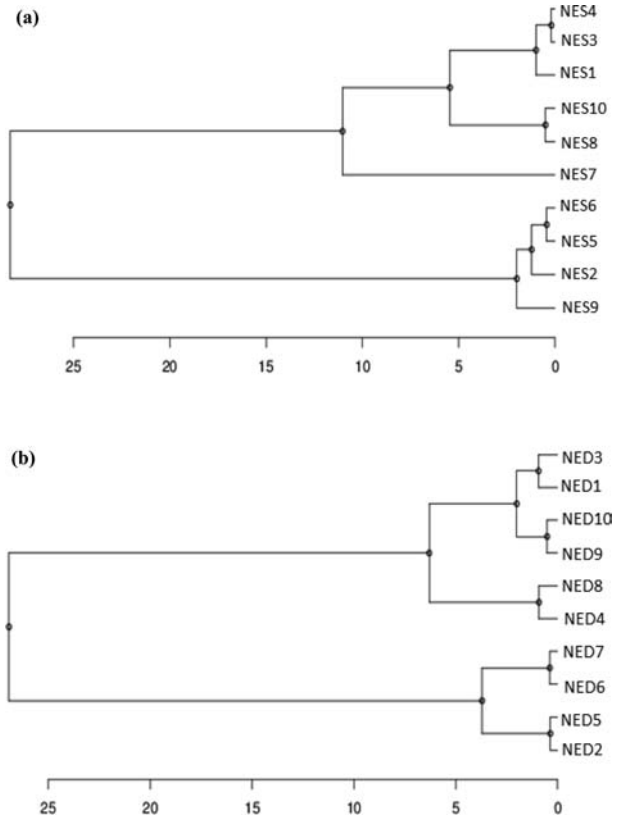

Figure 4. Dendrogram of hierarchical cluster analysis of ginger cultivars harvested in (a) September 2012 at 6-month maturity and (b) December 2012 at 9-month maturity.

Effect of Maturity on the Oleoresin Yield and [6]-Gingerol Content. The comparisons between the oleoresin yields of NER ginger cultivars at both maturation stages are

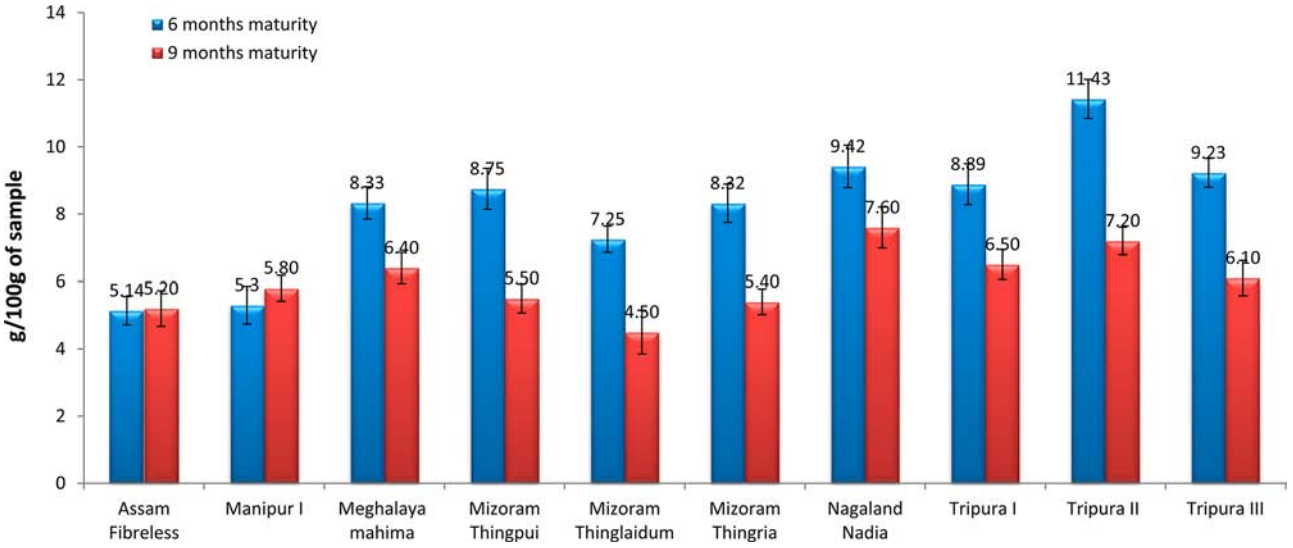

Figure 5. Variation in the oleoresin yield from NER ginger harvested in September 2012 at 6-month maturity and harvested in December 2012 at 9-month maturity.

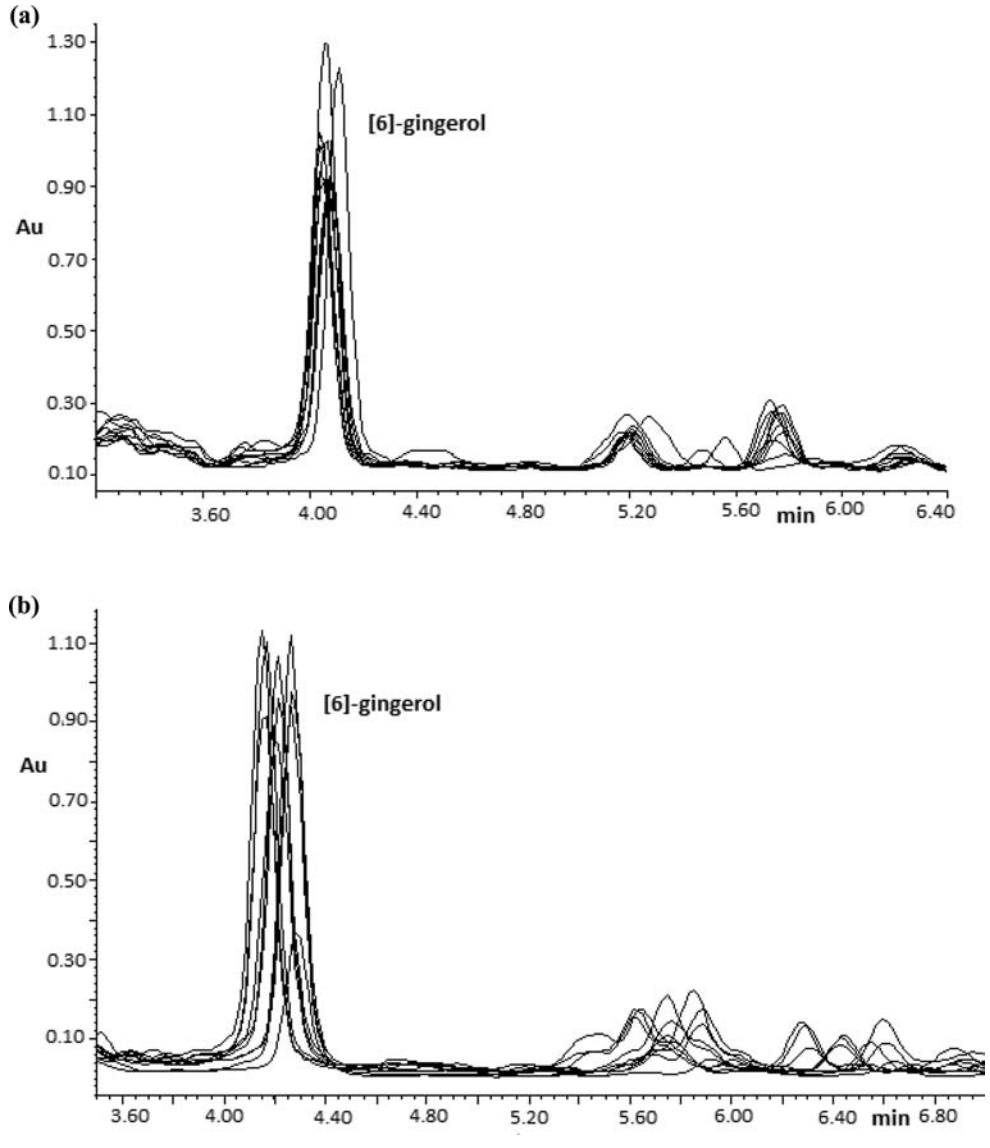

Figure 6. HPLC profile of NER ginger cultivars harvested in (a) September 2012 at 6-month maturity and (b) December 2012 at 9-month maturity.

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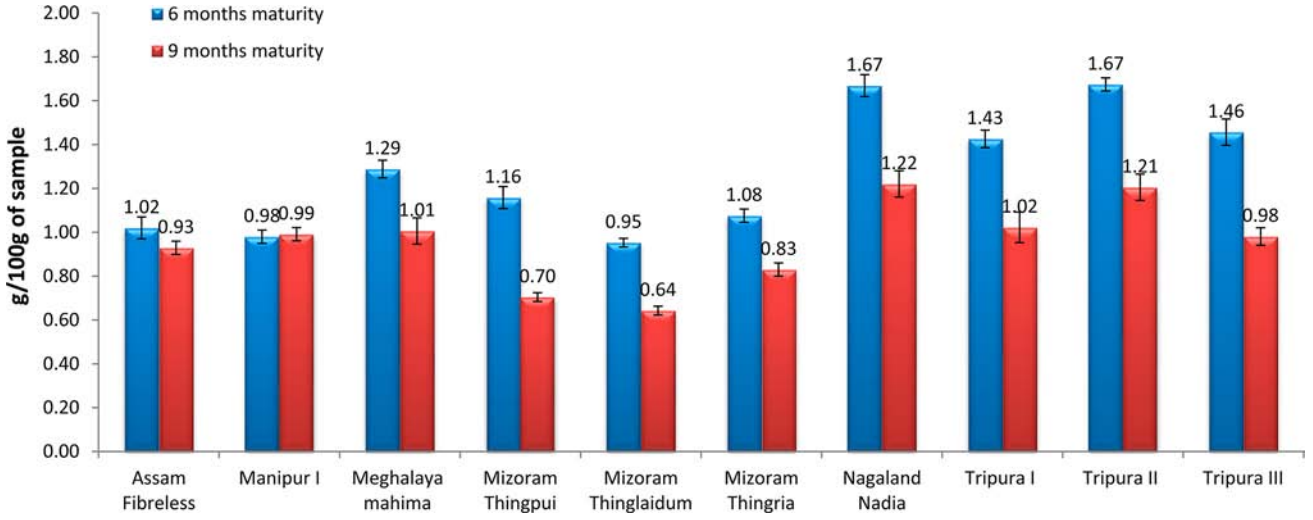

Figure 7. Variation in the [6]-gingerol content in NER ginger harvested in September 2012 at 6-month maturity and harvested in December 2012 at 9-month maturity periods.

shown in Figure 5. The oleoresin yield ranged from 5.14 ± 0.42% (NES9) to 11.43 ± 0.58% (NES1) at 6-month maturity. At 9-month maturity, the oleoresin yield ranged from 4.50 ± 0.65% (NED5) to 7.60 ± 0.61% (NED7). It was observed that the oleoresin yield was higher at 6-month maturity among all cultivars, except for NES1 and NES2 cultivars. Earlier reports concluded that the oleoresin content sharply increases up to about 5−6 months after planting, fell to half of the value in another 2 months of maturation, and gradually fell further.[32] This is mainly because of the rapid growth of rhizome weight and fiber development between 6 and 7 months of maturity. Mathai et al.[33] found a relative reduction in the oleoresin content with maturity concurrent with an increase in the dry matter content during different maturation stages of ginger. However, the oleoresin yield of 9-month-matured NER ginger cultivars in this study was found to be higher than Cochin ginger (4.25%) and closer to ginger from Sierra Leone (7.2%), Nigerian ginger (6.5%), and Ghana ginger (5.7%).[3] Two Jamaican ginger cultivars showed 7.25% (Bourbon and Portland) and 7.18% (Retreat and St. Mary) of the oleoresin yield at 9-month maturity,[16] which are found to be similar to the Tripura II cultivar.

A reverse-phase HPLC method was used to quantify the [6]-gingerol composition in all ginger cultivars at both maturity periods. The HPLC profile of oleoresin extracted from all NER ginger cultivars at 6- and 9-month maturity periods was shown in Figure 6. It was observed that [6]-gingerol was the major pungent compound in the oleoresin extract of all ginger cultivars at both maturity stages. The composition (expressed in grams per 100 g of ginger) of [6]-gingerol in NER ginger cultivars at both maturation periods was shown in Figure 7. The [6]-gingerol concentration varied from 0.95 ± 0.02 g (NES5) to 1.67 ± 0.03 g (NES7 and NES9) at 6-month maturity, while it was in the range of 0.64 ± 0.02 g (NED5) and 1.22 ± 0.06 g (NED7) at 9-month maturity. It has been clearly shown that the [6]-gingerol content is directly proportional to the oleoresin content and decreased with maturity, which are in accordance with previous findings.[16,33,34] The Tripura II cultivar (NES9) showed the highest oleoresin content and also the highest [6]-gingerol content at 6-month maturity, while it was highest in the Nagaland Nadia cultivar (NED7) at 9-month maturity. Moreover, these results show that there was only minor variation in the [6]-gingerol content of all of the ginger cultivars along with maturity. Northwestern Himalayan region ginger varieties were found to have a higher [6]-gingerol content (4.02−8.45 g/100 g of ginger)[35] than NER ginger cultivars, while south Indian ginger varieties were found to have comparable levels of [6]-gingerol content (0.93−1.81 g/100 g of ginger) with NER ginger cultivars. Further, the [6]-gingerol content in NER ginger cultivars was higher in comparison to the Chinese ginger having 1.16−1.38 g/100 g of ginger,[3] Jamaican ginger having 0.86−1.31 g/100 g of ginger,[11] Queensland ginger having 0.13−0.33 g/100 g of ginger,[15] and Nigerian ginger having 0.13−0.21 g/100 g of ginger.[36] These values were in agreement with our results, showing that 6-month-matured NER ginger cultivars have a higher pungency than other cultivars cultivated worldwide. These results can be useful to farmers as well as manufacturers engaged in ginger processing for selecting the appropriate cultivar and maturity for harvesting ginger to obtain the desired aroma profile and also to maximize the yields of oleoresin and [6]-gingerol contents for making value-added products.

In conclusion, this study provided a comprehensive analysis in essential oil composition, oleoresin yield, and [6]-gingerol content in 10 fresh ginger cultivars from NER at 6- and 9-month maturation. Manipur I (NES2), Mizoram Thinglaidum (NES5), and Mizoram Thingria (NES6) cultivars had a higher camphene content, and Nagaland Nadia (NES7), Tripura I (NES8), and Tripura III (NES10) had a high undecanone content. Nagaland Nadia (NES7) had a higher content of *ar*-curcumene than zingiberene, which is unique among NER ginger cultivars and provides valuable information to the flavor industry to choose these cultivars with the desired aroma profile. Tripura II (NES9) with the highest oleoresin yield and also the highest [6]-gingerol content along with the Nagaland Nadia (NES7) can serve as a valuable database for the trading and manufacturing sectors engaged in ginger processing. Because the characteristics of agricultural crops may change year to year, repeated analysis of the same study for two more different crop cycles and also interlaboratory studies on the same ginger cultivars can provide more information. We also suggest that harvesting the ginger at 6-month maturity is beneficial to farmers and the oleoresin industry, and it will help

to avoid the loss of ginger crop caused by the Brahmaputra River flooding in this region every year.

■ ASSOCIATED CONTENT

Supporting Information

Figure denoting the GC profile of essential oil composition of Nagaland Nadia ginger cultivar harvested in (a) September 2012 at 6-month maturity and (b) December 2012 at 9-month maturity. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +91-9847032159. Fax: +91-471-491712. E-mail: venugopalvv@yahoo.com.

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Notes

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