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The interaction of temperature and relative humidity affects the main aromatic components in postharvest *Torreya grandis* nuts

Yuanyuan Hu [a,1], Zuying Zhang [a,1], Bin Hua [c], Liu Tao [a], Wenchao Chen [a], Yadi Gao [a], Jinwei Suo [a], Weiwu Yu [a], Jiasheng Wu [a,*], Lili Song [a,b,*]

[a] State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an 311300, Zhejiang Province, China
[b] Sino-Australia Plant Cell Wall Research Centre, School of Forestry and Biotechnology, Zhejiang A&F University, Lin'an 311300, Zhejiang Province, China
[c] Forestry Station of Agricultural Technology Extension Center in Hangzhou Fuyang District, Hangzhou 311400, Zhejiang, China

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ABSTRACT

The postharvest ripening stage is necessary for *Torreya grandis* (*T. grandis*) nuts to complete aromatic synthesis, which requires appropriate temperature and relative humidity (RH). Currently, scarce information is available regarding the changes in aroma profiles in *T. grandis* nuts and the relationship with their response to different environmental conditions. Therefore, the interaction of temperature (20 °C or 30 °C) and relative humidity (70% RH or 90% RH) was investigated on aromatic substances after harvest. The results showed that 56 aromatic components were detected by a gas chromatography-mass spectrometer (GC–MS) and mainly divided into five categories, among which terpenes were the most abundant (56.2–86.7%). Principal component analysis (PCA) showed that both temperature and humidity can affect the aroma composition, and terpenes were mainly influenced by humidity. Specifically, D-limonene occupied the largest proportion of terpenes (63.0–90.8%) and was significantly upregulated by high humidity.

1. Introduction

Torreya grandis (*T. grandis*) is in the yew family (Taxaceae), subgenus *Torreya*, and is an evergreen coniferous tree. China lists this species as a national secondary key protected wild plant species (Yu, 1999). Within this genus, *Torreya grandis* cv. 'Merrillii,' a popular nut tree, is endemic in South China (Li & Dai, 2007). Owing to its economic and medicinal value, the growing area of *T. grandis* has rapidly expanded with an increase of 7.5-fold from 2005 to 2020, and the output value of nuts exceeded 1.5 billion RMB in 2019 (Yu, 2020). Roasted *T. grandis* nuts are popular in China because of their unique flavor, attractive aroma, and composition of healthy bioactive compounds, such as sciadonic acid, squalene, β-sitosterol, and tocopherol (Lou et al., 2019; Suo et al., 2019; Wu et al., 2018). Notably, aroma, which is synthesized during *T. grandis* nut development and ripening, is an important feature for *T. grandis* nut quality evaluation and accumulates at the postharvest stage. Unlike other tree nuts, such as walnuts and hickory, *T. grandis* nuts are in need of a highly coordinated and sophisticated postharvest ripening stage for nutrient conversion and aromatic synthesis (Zhou et al., 2019; Zhang et al., 2020). However, the main aromatic components in the postharvest ripening process are unknown, and the effect of environmental factors on their composition requires further research. Consequently, it is of great significance to reveal the aroma profile and features during the postharvest ripening stage of *T. grandis* nut.

Aroma plays a well-established role in determining the final sensory quality and is also a major determinant of consumer preference (Yan et al., 2020). The aroma profile and contribution of key aromatic compounds are different among various species. Volatile hexanal, nonanal, and benzaldehyde were reported as the main volatile components of raw almonds (Mexis et al., 2009; Xiao et al., 2014). Shi et al. (2020) found that the most volatile substances in raw walnut were aldehydes and alkanes. Kim and Chung (2007) identified 142 compounds in raw adzuki bean, and hexanal volatiles mainly contributed to the 'beany' flavor. However, the volatile profile in raw *T. grandis* nuts during the postharvest stage is still unknown.

Numerous studies have reported that the content and composition of aroma are affected by postharvest treatments, such as different hormones (Obenland et al., 2009; Ortiz et al., 2010) and temperature

* Corresponding authors at: State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an 311300, Zhejiang Province, China.
E-mail addresses: wujs@zafu.edu.cn (J. Wu), lilisong@zafu.edu.cn (L. Song).
[1] These authors have contributed equally to this work.

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treatments (Lado et al., 2019; Obenland et al., 2013). For example, the contents of monoterpenes, aldehydes, and alcohols in grapefruit significantly increased at 2 °C, whereas the emission of cyclic sesquiterpenes and aliphatic esters were stimulated at 12 °C (Lado et al., 2019). In mandarins, the warm temperature (20 °C) promoted an increase in the concentration of aromatic volatiles compared with cold temperatures (5–10 °C) (Obenland et al., 2013). Ethylene-treated fruit had lower concentrations of esters and higher aldehydes than the untreated control fruit (Makkumrai et al., 2014). Our previous studies demonstrated that postharvest treatments with different temperatures or humidity levels were important factors for nutrient conversion in *T. grandis* nuts (Zhang et al., 2020; Zhou et al., 2019), but there are few reports on other important quality attributes in this process, such as the formation of aromatic components.

Therefore, an interaction experiment using two temperatures (20 ± 2 °C or 30 ± 2 °C) and two relative humidity levels (70% ± 2% and 90% ± 2% RH) was performed to elucidate the effect on the aroma profile of *T. grandis* nuts after harvest. The overall objectives of this study were (i) to characterize and determine the aroma profile of *T. grandis* nuts during the postharvest stage and (ii) to clarify the interaction between temperature and relative humidity on aroma formation. This study provides theoretical and technical guidance to further improve the quality of *T. grandis* nuts during the ripening process.

2. Materials and methods

2.1. Plant materials

The cracked *T. grandis* cv. Merrillii (later written only as *'T. grandis'*) nuts were harvested from a commercial orchard in Xinchang, Zhejiang province, China, on September 10th in 2018 (about 525 days after full bloom). The *T. grandis* nuts with arils were transported to the laboratory within 4 h on the same day, and then the arils of *T. grandis* nuts were removed by hand. The nuts with hard shell of uniform size (about 27.11 ± 2.1 mm length and 13.63 ± 0.5 mm width) and free of visible damage were selected for subsequent experiments. Before the treatment, the nuts were washed with clean water, and then dried in the air until there was no excess water outside the shell. The *T. grandis* nuts were randomised and divided into four groups, each comprising approximately 10 kg nuts. Each group was further randomly divided into three subgroups as biological replicates.

2.2. Postharvest treatments with different temperature and humidity

The *T. grandis* nuts are in need of a postharvest ripening stage for nutrient conversion before roasting in September within the range of 20 ℃–30 ℃. In practice, the harvested nuts were placed and covered by wet straw (in order to maintain high humidity) in a room. Therefore, the quality of *T. grandis* nut during the postharvest ripening stage were influenced by humidity and temperature. In our previous experiment, we found that 70% RH and 90% RH had significant effect on the nutrient conversion (Zhang et al., 2020). Here, the nut samples with shell were treated with two temperature conditions (20 ± 2 °C or 30 ± 2 °C) and two RH levels (70% ± 2% RH or 90% ± 2% RH) for four treatment combinations: T20-LH (20 ± 2 °C and 70% ± 2% RH), T20-HH (20 ± 2 °C and 90% ± 2% RH), T30-LH (30 ± 2 °C and 70% ± 2% RH), and T30-HH (30 ± 2 °C and 90% ± 2% RH). To avoid creating anoxic conditions, the nut samples were manually stirred and flushed with fresh air for 10 min every day. Our previous work found that nutrient conversion is completed within 16 days, which could be used as a sign that the postmaturity stage had been completed (Zhang et al., 2020). Here, the nuts were sampled at 0 day, 5 day, 10 day, and 15 day after postharvest treatment with three biological replicates and were frozen in liquid nitrogen instantly and stored at − 80 °C for further analysis.

2.3. Aroma extraction using solid-phase microextraction (SPME)

Fresh nuts (2.0 g) were ground to a fine particle size and transferred into a 15 mL headspace bottle (ANPEL Laboratory Technologies Inc., Shanghai, China). To reach sample headspace equilibrium, the headspace bottle was incubated at 80 °C for 15 min in a water bath before volatile collection. Then, the volatile compounds were collected for 30 min at the same temperature using a SPME fiber coated with polydimethylsiloxane-divinylbenzene (65-μm PDMS/DVB, Sigma-Aldrich, Shanghai, China) by placing the fiber in the headspace of a volatile collection bottle.

2.4. Gas chromatography-mass spectrometer (GC–MS) analysis

The SPME fiber was placed in the GC injector to desorb the concentrated volatile compounds at 250 °C for 3 min and then the volatiles were further separated by GC–MS. The GC–MS system included an Agilent 7697A gas chromatograph coupled to an Agilent 7890 mass spectrometer (Agilent, USA) and was equipped with a capillary column HP-5MS (30 m × 250 μm, 0.25 μm phase film thickness, Agilent, USA). The initial oven temperature was held at 60 °C for 1 min, and then increased from 60 °C to 280 °C at a rate of 5 °C·min^{-1} and held for 2 min at 280 °C. The mass spectrometer was operated in electron impact (EI) ionization mode, with an energy voltage of 70 eV, and the ion source temperature was set at 230 °C. After ionization, the molecules were subsequently sorted according to their report mass/charge (*m/z*) by a quadrupole analyzer maintained at 150 °C with a mass range of 33 to 350 *m/z* in SCAN mode.

2.5. Identification and quantitation of aromatic compounds

Volatile compounds were identified by comparison of their retention indices (RIs) and mass spectra with library entities (NIST 12). The RIs were determined with a set of C_7–C_{30} *n*-alkanes under the same GC conditions. D-limonene, 1-dodecano, nonanal, *n*-heptadecane, and isopropyl myristate were used as an external standard for terpenes, alcohol, aldehyde, alkane, and ester compounds, respectively. The aroma concentration was calculated according to the peak area ratio of the external standard as a reference, and the calculation formula was as follows:

$$m_s = \frac{m_i \times A_s}{A_i \times m_0} \times 1000000$$

where m_s is the concentration of identified volatiles, expressed as μg g^{-1}; m_i is the weight of the external standard, expressed as μg; m_0 is the fresh weight of *T. grandis* nuts, expressed as g; A_s is the peak area of identified volatiles; A_i is the peak area of the internal standard. Considering the different water content of nuts treated with different postharvest conditions, all aromatic compounds were expressed as μg g^{-1} dry weight (DW).

2.6. Statistical analyses

Statistical analysis was performed with SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Means within different treatments at different ripening time were compared using least significant difference (LSD) test at $P < 0.05$. The significance level of the main effect analysis was evaluated by a three-way analysis of variance (ANOVA) for RH, temperature, and postharvest ripening stage without initial samples (0 day), and their interaction terms were indicated: ns, $P > 0.05$; ***, $P < 0.001$. In addition, principal component analysis (PCA) was used to comprehensively evaluate the impact of different postharvest treatments on the aromatic compounds of *T. grandis* nuts. Figures were created with Origin 8.0 (MicroCal Software) and SigmaPlot 12.5. Data are presented as the mean ± standard deviation (SD).

3. Results and discussion

3.1. Analysis of the aroma profile of T. grandis nuts during the postharvest ripening stage

A few studies provide aroma profiles for *T. grandis* arils and oil, and>50 volatile molecules have been reported (Niu et al., 2011; Wang et al., 2016). Little research has been done on the aroma of *T. grandis* nuts, which requires a postharvest ripening stage. Here, aromatic compounds of raw *T. grandis* nuts during the postharvest ripening stage were first characterized by GC–MS. The chromatograms obtained at different temperature and humidity treatments during ripening time were presented in Fig. 1 and Fig. S1-S3. A combined total of 56 aroma-active volatile compounds were detected in raw *T. grandis* nuts, including terpenes (19), alcohols (19), aldehydes (8), esters (5), and alkanes (5) (Table 1).

The aroma profile changed as the nuts progressed through maturation, harvest, and subsequent postharvest treatment and storage (Strojnik et al., 2019). In the present study, 43 volatile compounds were detected before the postharvest ripening began (0 day). About 40–46, 42–45, 43–48, and 44–51 volatile compounds were identified in nuts under T20-LH, T20-HH, T30-LH, and T30-HH treatments, respectively, during the ripening stage (Table 1 and Table S1). It had been previously reported that the volatile compounds in coffee beans increased significantly as the temperature increased, ranging from 31 ℃ to 62 ℃ (Steen et al., 2017). Possibly, the higher volatile compounds emission under higher temperature treatments were influenced by their greater evaporation. Consistent with a previous report, that the saturated vapor pressure of aroma compounds increased with increasing temperature (Covarrubias-Cervantes et al., 2004). Similarly, our results revealed that the total volatile production in *T. grandis* nuts under higher temperature treatments (T30-HH and T30-LH) was significantly higher than that in

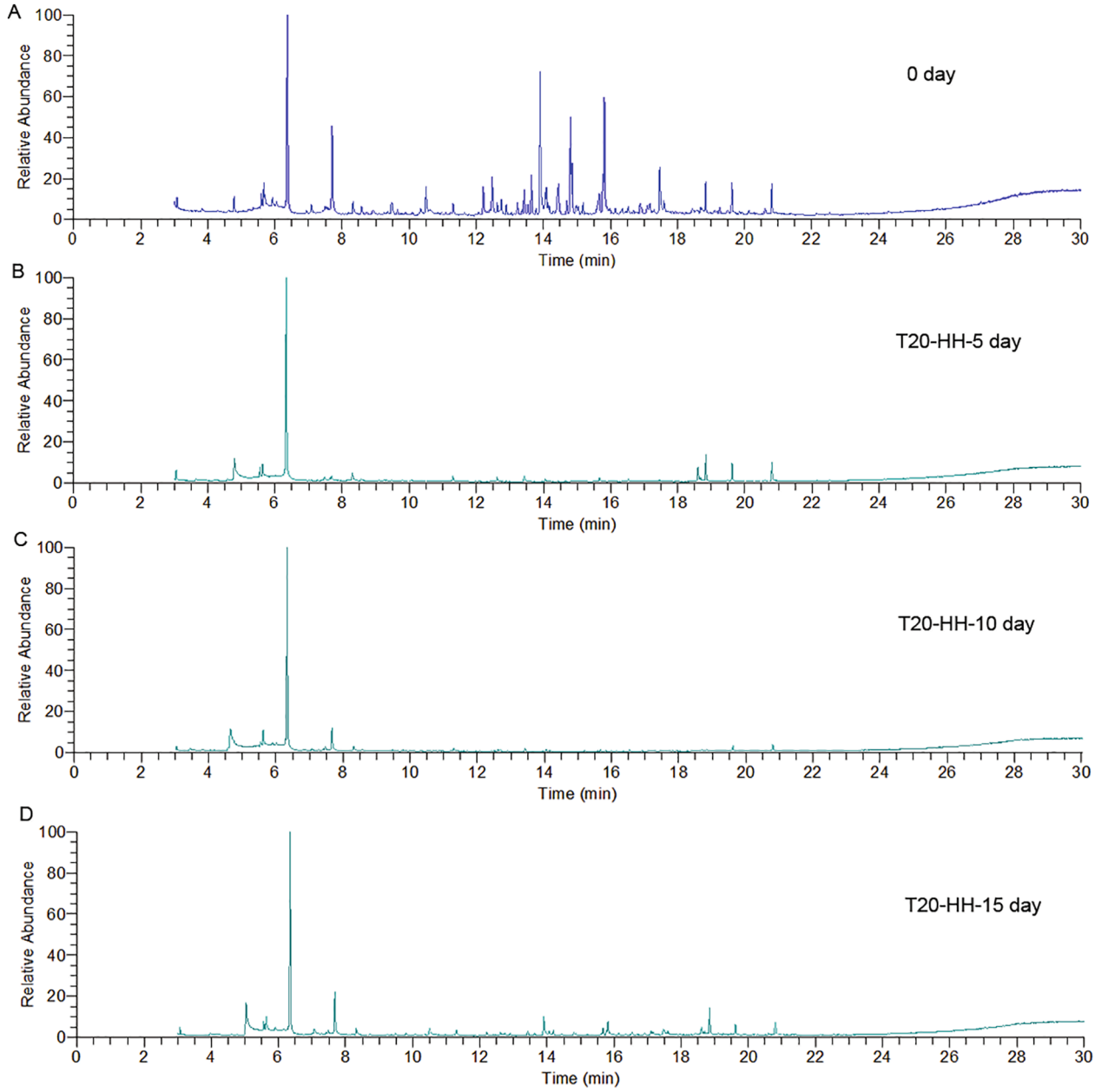

Fig. 1. GC–MS chromatogram related to the aroma in *Torreya grandis* cv. 'Merrillii' nuts at T20-HH (20 °C and 90% RH) during ripening time on a HP-5MS column. Note: A, 0 day of ripening time; B, 5 day of ripening time at 20 °C and 90% RH; C, 10 day of ripening time at at 20 °C and 90% RH; D, 15 day of ripening time at at 20 °C and 90% RH.

Table 1
Main volatile compounds and their content in *Torreya grandis* 'Merrillii' nuts during the postharvest ripening process at different temperatures and relative humidity conditions.

Volatile compounds	RI	Aroma substance contents (μg·g^{-1} DW)											
		0 day	T_{20}-LH			T_{20}-HH			T_{30}-LH			T_{30}-HH		
			5 day	10 day	15 day	5 day	10 day	15 day	5 day	10 day	15 day	5 day	10 day	15 day
Terpenes compounds														
3-Carene	899	1.48a	0.41c	nd	nd	nd	nd	0.68c	0.48c	0.11d	0.42c	1.17b	0.08d	0.03d
(1S)-(1)-β-Pinene	913	0.55cd	1.27a	0.87b	0.36d	0.82bc	1.22a	1.16ab	0.03e	0.57cd	nd	0.9b	0.02e	1.22a
α-Pinene	921	2.8d	0.52ef	0.04f	nd	10.33b	13.99a	0.96e	7.23c	6.82c	0.06f	2.84d	1.23e	0.37ef
DL-limonene	929	nd	nd	0.01c	0.02c	0.03c	0.02c	nd	0.04c	nd	nd	0.29b	0.01c	0.53a
Camphene	933	0.24b	2.98a	0.06b	3.04a	0.07b	0.07b	0.06b	0.22b	0.16b	0.06b	0.05b	nd	0.04b
Myrcene	962	6.08de	10.96b	19.03a	3.51e	13.22b	5.77de	6.59de	17.16a	17.39a	17.11a	10.18bc	19.52a	7.44cd
Pseudolimonene	981	nd	0.45de	0.32e	0.05f	nd	nd	0.55d	1.56b	1.82a	0.3e	nd	0.87c	nd
D-limonene	994	35.79f	56.94cd	63.39c	73.78b	50.85de	72.34b	85.16a	46.28e	50.79de	59.41c	48.6e	74.56b	84.76a
Terpinolene	1036	nd	0.67abcd	0.58bcd	0.49bcd	0.84a	0.6abcd	0.72abc	0.44d	0.54bcd	0.47cd	nd	0.42d	0.74ab
Longifolene	1247	0.72cd	nd	0.08e	0.09e	0.09e	0.02e	0.24e	2.99a	1.86b	0.11e	1c	0.11e	0.37de
(-)-Isocaryophyllene	1254	2.5a	nd	nd	0.04b	nd	0.11b	0.02b	nd	0.31b	0.01b	0.01b	nd	0.04b
Aromadendrene	1265	1.52a	0.26c	0.03c	nd	0.03c	nd	0.12c	0.06c	0.02c	0.78b	1.38a	0.02c	0.06c
Trans-β-farnesene	1271	7.21a	0.47d	0.05d	0.42d	nd	0.04d	nd	1.17c	1.46c	0.23d	4.33b	0.32d	nd
α-Caryophyllene	1274	0.93a	0.2b	nd	nd	0.07b	nd	nd	nd	0.02b	nd	0.3b	0.04b	0.09b
β-Phellandrene	1289	nd	0.05c	0.06c	nd	nd	0.01c	0.11c	0.5b	nd	0.77a	0.05c	0.04c	0.08c
Muurolene	1299	10.4a	0.31de	0.05e	0.12de	0.49d	0.25de	0.05e	0.21de	0.29de	0.07e	5.6b	0.86c	nd
r-Cadinene	1308	2.33a	0.1de	0.03e	nd	0.23de	nd	0.11d	nd	0.07e	0.69c	1.05b	nd	0.15de
δ-Cadinene	1313	30.18a	nd	0.03b	0.06b	0.28b	0.38b	0.16b	0.13b	nd	0.15b	1.16b	0.53b	0.35b
Cedrol	1362	1.14c	0.04d	0.17d	nd	nd	0.01d	nd	0.24d	0.36d	nd	1.65b	2.21a	nd
Total		103.87a	75.61cd	84.82c	81.98cd	77.36cd	94.81b	96.7ab	78.75cd	82.59cd	80.65cd	80.54cd	100.82ab	96.29ab
Alcohol compounds														
6-Octanol	890	2.33c	0.27ef	0.2ef	2.59bc	0.17f	0.26ef	0.22ef	0.59de	0.9d	nd	2.85b	3.55a	0.68d
1-Undecanol	940	0.46a	0.02ab	0.02ab	nd	0.05ab	0.04ab	0.03ab	nd	0.05ab	0.11ab	0.01b	nd	0.04ab
1-Dodecanol	941	28.04a	1.98b	0.14c	2.5b	nd	nd	nd	0.32c	0.39c	0.45c	nd	0.72c	0
3–4-Terpinen-4-ol	954	nd	0.12b	0.22ab	0.08b	0.31ab	0.32ab	0.12b	0.09b	0.14	0.17b	0.61a	0.18b	0.21ab
Borneol	958	nd	0.28bc	0.38b	0.07ef	0.34b	0.17cde	0.16cde	0.13def	0.02f	0.19cde	0.21cd	0.53a	0.2cde
Dihydrocarveol	974	1.97a	0.66cd	0.52d	0.86c	0.5d	0.39d	0.38d	0.06e	nd	0.46d	1.28b	1.76a	0.55cd
Trans-2-decen-1-ol	1087	0.92a	nd	0.09cd	0.38bc	0.01d	nd	nd	0.01d	0.04d	0.03b	0.49b	0.99a	nd
α-terpineol	1111	0.29b	0.1cd	nd	nd	0.31b	0.63a	0.22cd	0.22cd	0.32b	0.02c	0.03c	nd	0.07c
Cedrol	1200	10.35a	0.95d	1.12cd	1.26bcd	1.49bc	1.5bc	0.14e	1.44bc	1.27bcd	1.53b	0.01e	0.05e	nd
2-Hexadecanol	1236	nd	1.28a	0.21de	0.79b	nd	0.06e	nd	0.34cd	0.41c	0.35cd	0.05e	nd	0.49c
Nerolidol	1269	0.8b	0.74bc	0.3de	0.49cd	0.42d	0.25de	0.28de	1.21a	1.42a	0.11e	0.3de	nd	0.35de
2-Methyl alcohol	1345	7.8a	1.9de	1.36ef	1.2f	2.18cd	1.35ef	1.21f	1.8def	1.6def	0.51g	2.62bc	3.16b	1.95de
3-Tetradecanol	1349	nd	0.1b	0.04	nd	nd	0.28a	nd	nd	nd	nd	0.21ab	0.33a	nd
1-Tetradecanol	1351	5.34a	0.83bcd	0.95bc	0.41def	0.14ef	nd	0.06f	1.1b	0.59cde	nd	0.3ef	0.38def	0.06f
2-Tetradecanol	1358	27.94a	nd	nd	2.11bc	0.2cd	0.12d	0.03d	0.06d	0.27cd	nd	2.64b	0.41cd	0.08d
Alfacalcidol	1384	0.72b	0.07c	nd	nd	0.02c	nd	nd	nd	nd	0.04c	1.36a	0.74b	0.07c
Heptsdecanol	1392	2.92a	nd	0.09d	0.47cd	nd	0.59cd	0.1d	1.26bc	1.02bcd	0.01d	1.74b	2.71a	nd
Tert-hexadecanethiol	1403	3.58a	0.52de	0.34de	0.71de	0.65de	0.12e	0.31e	1.08cd	1.4c	0.34de	2.75b	2.68b	0.64de
1-Hexadecanol	1461	6.36a	0.76def	nd	0.81de	1.0d	0.11ef	0.06f	0.12ef	0.67def	0.05f	4.37b	2.66c	0.04f
Total		99.82a	10.59d	5.99ef	14.73c	7.78e	6.2ef	3.32g	9.83d	10.52d	4.38fg	21.82b	20.83b	5.45f
Aldehyde compounds														
Trans-2-nonenal	1021	0.88d	0.03e	nd	0.2e	0.15e	0.02e	nd	1.45c	nd	0.33e	6.11a	1.96b	0.04e
Nonanal	1047	17.3a	7.13cd	2.66f	9.03c	12.17b	4.03ef	2.48f	18.06a	18.21a	6.04de	13.8b	18.44a	5.85de
Trans-oct-2-enal	1047	nd	0.02c	0.01c	nd	nd	nd	0.07c	0.18c	0.06c	nd	1.51b	4.95a	0.06c
Decanal	1115	4.79a	0.99d	0.76de	0.65de	0.21e	0.39e	0.34e	1.55c	1.69c	0.53de	2.4b	2.23b	0.46de
10-Octadecylen	1180	0.38a	0.06b	nd	nd	nd	0.07b	nd	0.26a	nd	nd	nd	nd	0.06b
2-Undecenal	1216	7.39a	nd	nd	0.26c	0.11c	0.02c	0.08c	0.03c	0.55c	0.07c	4.78b	0.06c	nd
3-Phenyl-2-butenal	1435	nd	1.24a	nd	nd	0.79ab	nd	1.02ab	1.12a	0.77ab	3a	0.6b	0.03c	nd
Dodecyl aldehyde	1477	2.36b	0.38cd	0.23cd	0.34cd	0.04d	0.08d	nd	0.23cd	0.65c	nd	0.04d	3.02a	0.15d

(*continued on next page*)

Table 1 (*continued*)

Volatile compounds	RI	Aroma substance contents (μg·g^{-1} DW)									
		0 day	T_{20}-LH			T_{20}-HH			T_{30}-LH			T_{30}-HH		
			5 day	10 day	15 day	5 day	10 day	15 day	5 day	10 day	15 day	5 day	10 day	15 day
Total		33.09a	9.84f	3.66h	10.48f	13.47d	4.6gh	3.98h	22.88c	21.94c	9.97fe	29.23b	30.69b	6.63eg
Ester compounds														
Methyl oleate	1121	nd	nd	0.03bc	0.003c	0.15a	0.01c	0.003c	nd	0.02c	0.03bc	nd	0.08b	0.003c
Phthalic acid diisobutyl ester	1397	0.54b	0.15c	0.06c	0.07c	0.03c	0.04c	0.47b	0.47b	0.76a	0.06c	0.81a	0.83a	0.12c
Isopropyl myristate	1524	0.25bc	0.06de	0.01e	0.17cd	0.33ab	0.44a	0.01e	0.35ab	0.25bc	0.01e	0.11de	0.14cd	0.07de
Methyl hexadecanoate	1524	0.12a	nd	nd	0.003c	nd	0.01c	0.03bc	nd	0.07abc	nd	0.08ab	0.04bc	0.05bc
Diisooctyl phthalate	1577	0.64ab	0.23def	0.13ef	0.02f	0.36bcde	0.25cdef	0.36bcde	0.11ef	0.73a	0.06ef	0.48abcd	0.55abc	0.08ef
Total		1.55a	0.43cd	0.22d	0.27d	0.86b	0.75bc	0.88b	0.93b	1.82a	0.16d	1.48a	1.62a	0.32d
Alkane compounds														
n-Tetradecane	1231	2.45cd	1.88cd	1.94cd	1.72cd	4.55bc	2.88cd	20.36a	1.8cd	0.77d	1.36cd	2.68cd	6.65b	3.95bcd
n-Pentadecane	1290	nd	1.09cd	0.19e	0.16e	3.27b	0.14e	1.46c	0.52de	0.47de	0.17e	3.78b	10.19a	nd
Nonadecane	1403	nd	0.23e	0.8cd	1.16bc	0.34de	0.77cd	0.4de	0.14e	0.82cd	nd	1.49b	2.13a	0.77cd
n-Heptadecane	1455	2.19b	0.34e	0.22e	1.16cd	nd	0.08e	1.23cd	0.84d	1.43c	0.07e	2.2b	3.32a	0.33e
n-Heptacosane	1558	0.48a	0.05b	nd	nd	nd	nd	nd	0.01b	nd	0.04b	0.04b	0.04b	nd
Total		5.12c	3.58cd	3.15cd	4.19cd	8.15b	3.88cd	23.45a	3.31cd	3.49cd	1.64d	10.17b	22.34a	5.06c

Note: nd indicates that this compound was not detected. Different letters in the same row indicated the significant differences at $P < 0.05$ level. The aroma content was expressed on a dry weight (DW) as μg·g^{-1} DW.

nuts under lower temperature treatments (T20-HH and T20-LH) after 5 day to 10 day of ripening (Table 1 and Fig. S4). The total volatile production significantly decreased in *T. grandis* nuts under higher temperatures from 10 day to 15 day, whereas the emission of total volatiles showed an increase under lower temperature treatments. In addition, the *T. grandis* nuts under HH treatments generally produced higher levels of volatiles than under LH treatments at 5 day to 15 day of ripening; however, there were no significant differences between T20-LH and T20-HH at 10 day of ripening. These results suggested that both temperature and relative humidity had a profound effect on the production of aromatic compounds in *T. grandis* nuts during the postharvest ripening stage.

3.2. Effect of temperature and humidity on accumulation of aromatic compounds

Among the identified volatile compounds, terpenes accounted for 56.2–86.7% and were the most abundant aromatic compound during the postharvest ripening stage, followed by aldehydes (3.1–20.4%), alcohols (2.6–15.2%), alkanes (1.7–18.3%), and esters (0.2–1.5%) (Fig. 2A), indicating that terpenes constituted the most substantial group of aromatic compounds in *T. grandis* nuts during the postharvest stage. Terpenes with a volatile fragrance include monoterpenes and sesquiterpenes, which are strongly linked to flavor perception and consumer acceptance (Ren et al., 2015). Further analysis of the terpene content found that monoterpenes accounted for the vast majority compared with sesquiterpenes (Fig. 2B). Interestingly, at the 0 day postharvest ripening stage, the monoterpene and sesquiterpene content were nearly equivalent, but at 5 day, the sesquiterpene content declined significantly, while the monoterpene content sharply increased (Fig. 2B). Moreover, D-limonene (49.62–85.89%) and myrcene (3.55–20.35%) were the most abundant monoterpenes under the four different treatments (Fig. 2C). These results coincided with previous work in which D-limonene was the main aromatic constituent in *T. grandis* arils and oil (Niu et al., 2011; Wang et al., 2016). Conclusively, the study indicated that terpenes were the main aroma component in *T. grandis* nuts after harvest.

To determine whether there were differences in the *T. grandis* aroma composition based on the temperature and humidity interaction during the postharvest stage, the five aromatic categories with each treatment were analyzed using PCA analysis. The T20-HH treatment and T30-HH treatment at 5 day and 10 day of ripening time were well separated by PC1, and whereas T20-LH treatment and T20-HH treatment were clearly separated by PC2 at 15 day of ripening time (Fig. 3A-3C). PC1 (47.8%) described the difference between the lower temperature treatments versus the higher temperature treatments except for 15 day of ripening time, whereas PC2 (40.8%) mainly described the difference between the higher humidity treatments and the lower humidity treatments except for 5 day of ripening time (Fig. 3D). In addition, the alcohol and aldehyde contents showed the most significant contribution to separate the higher temperature treatments and the lower temperature treatments (Fig. 3D). However, the contents of terpenes and alkanes were the main factor that distinguished higher humidity treatments from lower humidity treatments. A selective emission of volatiles was observed in *T. grandis* nuts that appeared to be a specific response to different temperatures and humidity levels during the postharvest ripening stage. According to the reports, the aromatic compound formation of fruit could be affected by various postharvest environmental conditions. For example, esters could be affected by temperature (Yao et al., 2018); terpenes could be affected by temperature, light, and ethylene (Shen et al., 2018; Zhang et al., 2016), and aldehydes could be affected by temperature and ethylene (Makkumrai et al., 2014). Accordingly, our results suggested the alcohol and aldehyde contents were changed significantly at different temperature treatments during 5 day to 10 day of postharvest ripening ($P < 0.05$), whereas the terpene and alkane content were greatly changed at different humidity treatments during 10 day to 15 day of ripening time ($P < 0.05$).

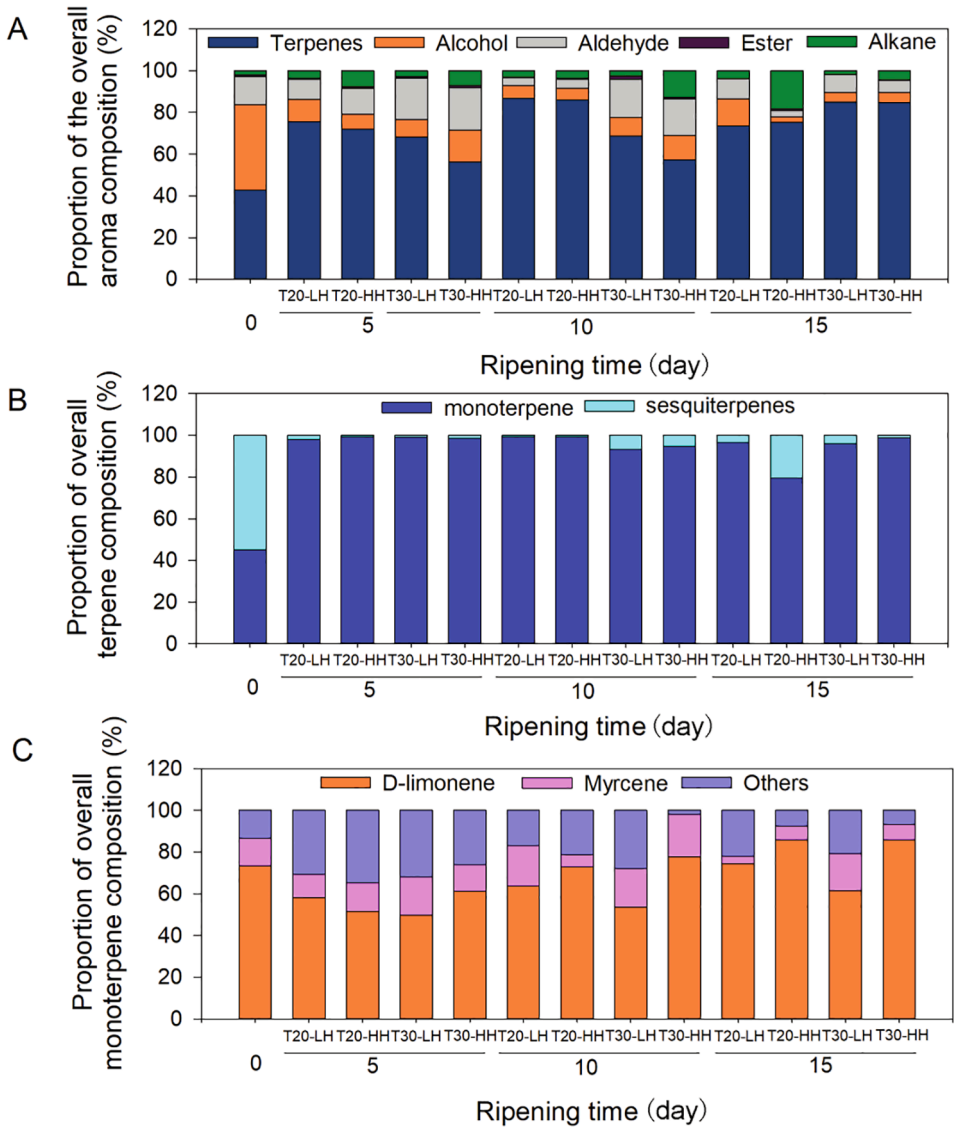

Fig. 2. Relative proportion of different aroma profiles (A), terpenoids (B), and monoterpenoids (C) in *Torreya grandis* cv. 'Merrillii' nuts during the postharvest ripening stage at different temperatures and relative humidity conditions. Note: T20-LH, at 20 °C and 70% RH; T20-HH, at 20 °C and 90% RH; T30-LH, at 30 °C and 70% RH; T30-HH, at 30 °C and 90% RH.

The interaction of temperature and relative humidity showed no obvious effect on the terpenes (Fig. S4). The temperature alone had no obvious effect on terpenes in nuts; the main reason might be that the production of flavor-associated volatiles was sensitive to temperatures below 12 °C, and the loss of tomato volatiles was observed during cold storage (Zhang et al., 2016). Thus, the reason why terpenes were not affected by temperature in our study was due to the higher temperatures used. This was consistent with the result reported by Llusià and Penuelas (1999) that greater emission of terpene content in *Pinus halepensis* under high relative humidity (>60% RH) than under low relative humidity (<60% RH). It can be explained that due to cuticular hydration, plants can increase their permeability to monoterpenes under high humidity environments (Croteau, 1977). Until now, few studies have been involved in high humidity promoting the synthesis of terpenes. Therefore, it can be said that our research provides unique insights into the effect of high humidity on the aroma profile of *T. grandis* nuts after harvest.

3.3. Identification of the main aroma components in T. Grandis nuts in response to different postharvest conditions

In this study, our linear regression analysis revealed that D-limonene was significantly and positively correlated with the total terpene content (Fig. 4A). It indicated that D-limonene was a major constituent which was responsible for the lemon-like odor, and affected the distinctive sensory quality of *T. grandis* nuts (Sun, 2007). As the main terpene constituent of most citrus fruit essential oils, D-limonene is a highly abundant monoterpene and presents a wide spectrum of antioxidant, antimicrobial, therapeutic, and chemotherapeutic properties (Melendez-Rodriguez et al., 2019; Rezaeinia et al., 2019). Furthermore, D-limonene is also widely used in the pharmaceutical, perfume, cosmetic, food, and beverage industries (Zhou et al., 2018). The presence of a large amount of D-limonene in *T. grandis* nuts could facilitate its extensive application in industrial processing.

By analyzing the content of D-limonene under the four different postharvest ripening treatments, its emission significantly increased 137.9 and 136.8% under T20-HH and T30-HH, respectively, from 0 day to 15 day of ripening and 106.1 and 66.0% under T20-LH and T30-LH,

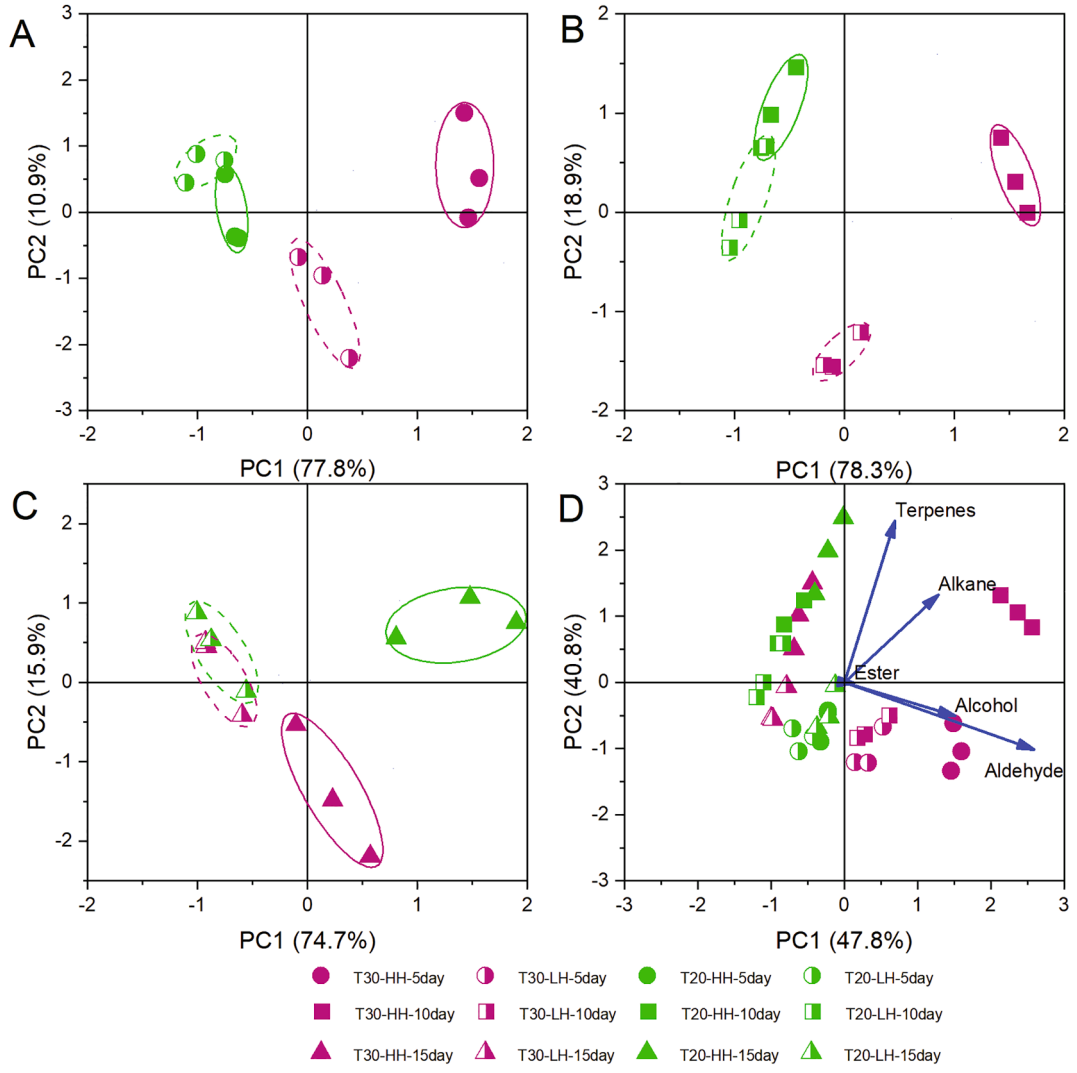

Fig. 3. PCA of the volatile compounds for *Torreya grandis* cv. 'Merrillii' nuts during the postharvest ripening stage at different temperatures and relative humidity conditions. Note: A, score plot at 5 day postharvest ripening; B, score plot at 10 day postharvest ripening time; C, score plot at 15 day postharvest ripening; D, the loading plot for the three different postharvest ripening stages. Note: T20-LH, at 20 °C and 70% RH; T20-HH, at 20 °C and 90% RH; T30-LH, at 30 °C and 70% RH; T30-HH, at 30 °C and 90% RH.

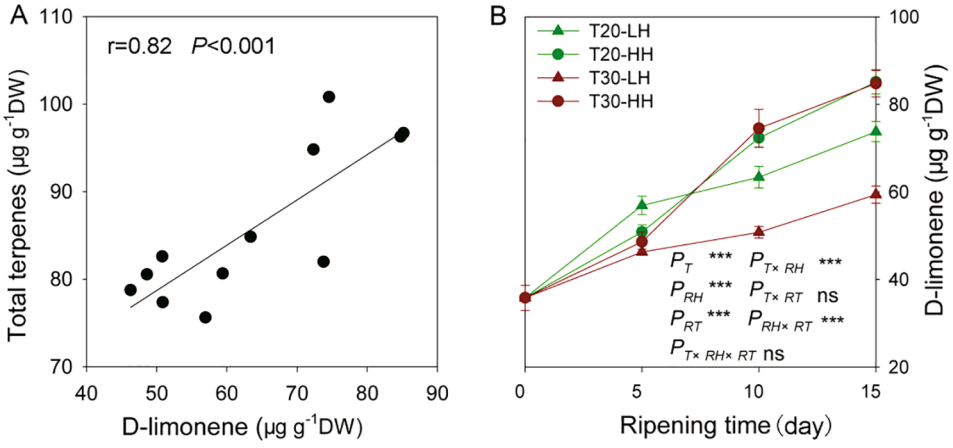

Fig. 4. The changes of D-limonene in *Torreya grandis* cv. 'Merrillii' nuts during the postharvest ripening stage at different temperatures and relative humidity conditions. Note: A, linear regression analysis between the total terpene content and D-limonene content; B, changes in D-limonene content during postharvest ripening stage at different temperatures and relative humidity conditions. P_T, temperature effect; P_{RH}, RH effect; P_{RT}, ripening time; $P_{T\times RH}$, temperature × RH interaction effect; $P_{T\times RT}$, temperature × ripening time interaction effect; $P_{RH\times RT}$, RH × ripening time interaction effect; $P_{T\times RH\times RT}$, temperature × RH × ripening time. Error bars represent standard error based on three biological replicates. Asterisks denote significant differences, $^{***}P < 0.001$. ns stands for not significant. Significant differences were determined by SPSS Statistics 20.0. DW, dry weight.

respectively, indicating the terpenes' formation in *T. grandis* nuts during the ripening stage required a high humidity environment. In addition, the content of D-limonene at T20-LH was obviously higher than that at T30-LH, which suggested that relatively lower temperatures (20 °C) promoted D-limonene accumulation with a low RH. Similarly, the emission of D-limonene in grapefruit sharply increased when stored at a lower temperature than at a higher temperature (Lado et al., 2019), suggesting that the mechanism of environmental temperature on aroma formation of different species are different. Notably, it is unclear whether the relatively low temperature was beneficial for the formation of the terpenes in *T. grandis* nuts after harvest. Consequently, this was considered and a lower temperature environment, such as lower than 20 °C, should be tested to further explore the effect on aromatic terpenes. Overall, this study revealed the main aroma substances and their changing profile during the postharvest ripening process of *T. grandis* nuts, which is of great significance to improve the quality of nuts after harvest and to promote the sustainable development of the nut industry.

4. Conclusions

This study provides the first comprehensive determination of the aromatic components of raw *T. grandis* nuts during the postharvest ripening stage. Our data revealed that terpenes were the predominant aromatic compounds, which were significantly increased by high humidity. The mechanism by which high humidity treatments promote this was through the stimulation of D-limonene synthesis. The research results can enrich the mechanism of the synthesis and regulation of terpenes and provide a scientific basis for improving the postharvest quality of *T. grandis* nuts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130836.

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