Effects of Harvest Time on the Yield, Quality and Active Substance of *Torreya grandis* Nut and Its Oil

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Abstract: *Torreya grandis* is an important economic tree species in China. It provides nutritional value and is important to the health care industry. There are ongoing issues with product quality which are primarily related to improper management and early harvest. This study was carried out during the fruit ripening processes to evaluate the influence of harvesting date on *T. grandis* quality, and to determine the optimal harvest period. The effects of harvest time on the variation of quality and nutritional parameters of *T. grandis* nuts and its oil were evaluated, and the optimal harvest period was determined. The results showed that harvest timing had a strong effect on both oil yield and quality. Prolonged ripening could induce higher levels of kernel rate, fruit inclusions, oil and nutritional quality. When the sample harvested in the mid-September, the kernel rate and oil content were increased by $1.88 \pm 0.31\%$ and $6.65 \pm 0.47\%$, respectively, compared to samples harvested in the beginning of late-August. Similarly, the mid-September harvest resulted in total unsaturated fatty acids content of the oil being increased by $5.3 \pm 0.34\%$, the FFA and peroxide value being decreased by $40.7 \pm 0.15\%$ and $76 \pm 0.08\%$, respectively, and total tocopherols and free amino acids were increased $7.5 \pm 0.24\%$ and $47.3 \pm 0.15\%$, respectively, compared to the samples harvested on Aug. 25. The results indicated that the optimal harvest time of *T. grandis* fruits was mid-September as it was beneficial for improving the quality of *T. grandis* nut and its oil. It was suggested that *T. grandis* fruit should be harvested later.

Key words: *Torreya grandis*, harvest time, yield, quality, active substance

1 Introduction

Torreya grandis (*T. grandis*), also called Chinese torrey, is an evergreen tree of *T. grandis* genus Taxus. It is an important economic tree species in China and has been cultivated for more than 1300 years¹⁾. *T. grandis* nuts are crispy and nutritious, and the oil content of the kernel is about 65%. The primary fatty acids in the oil are linoleic acid and oleic acid, which include 79.41 % unsaturated fatty acids and is a high-level edible oil²⁾. It is also rich in 17 kinds of amino acids and 19 types of essential mineral elements³⁾. The oil of *T. grandis* nut contains a unique fatty acid which is *cis*-5,10,14-eicosenoic acid (also known as cardamaric acid), which has a noticeable effect on regulating blood lipids and significant role in preventing and treating cardiovascular and cerebrovascular diseases⁴⁾.

Since the 1980s, *T. grandis* research mainly focused on plant habitat and growth conditions⁵⁾, cultivation techniques⁶⁾, and pest control⁷⁾, component analysis and nutri-

tional value assessments⁸⁻¹¹⁾, processing of nuts and oil^{12, 13)}, and components analysis and utilization of pseudosperm^{14, 15)}. In recent years, local governments have been paying more attention to the cultivation and management of *T. grandis*. The cultivated area and the yield of *T. grandis* has been growing rapidly, but its industrialization level is still relatively low, and the product quality is still a problem. There are problems with product quality which are largely related to improper management and premature harvest. *T. grandis* fruits are usually picked from early to mid-September when the color of fruit surface changes from emerald green to light yellow and longitudinal cracks appeared on the fruit surface. But in reality, the producers usually harvest earlier because of the difficulties in finding workers, land construction, and the climate conditions during optimal harvest times make harvest difficult. In addition, mixing types also leads to an earlier harvest. There are few unmixed gardens in our country. Therefore, the

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producers prefer to pick the fruits all at once to reduce the harvest costs. In these cases, harvest time is suitable for some varieties but too early for other varieties.

It is known that early harvest induces significant quality and weight losses¹⁶⁾, harvesting too late can make collecting seeds difficult, and mildew creates conditions whereby the nuts easily crack and fall to the ground which threatens quality and safety¹⁷⁾. It is vital to properly time the fruit harvest to obtain high nut quality and yield¹⁸⁾. Compared with camellia (*Camellia oleifera* Abel.), olive (*Olea europaea* L.), walnut (*Juglans regia* L.), and other woody oil species, the research on the harvesting and maturation of *T. grandis* is minimal. There are no reports about the fruit traits, accumulation of oil content and nutrients at the different harvest periods.

Determination of the optimal fruit-ripening stage for the production of *T. grandis* nut represents a critical choice based on the best combination of quantity and quality. Usually, the optimum harvest time of nuts varies by years, depending on ecological conditions. Accordingly, determination of harvest time should be made by phenological observation. The quite flushing of husks, efficient rotation of fruit in the husk, taking its color of hard shell, and the dropping of nuts (about 3/4 rate) when shaken indicate the optimum harvest time for nuts^{18, 19)}. As *T. grandis* ripening proceeds, nut characteristics such as weight, kernel rate, oil content, profiles of various phytochemicals, including fatty acids, protein, starch, soluble sugar, tocopherol and total reducing capacity, are changing. These changes influence the chemical composition and qualities of *T. grandis* nut and its oil²⁰⁾. This study evaluates the influence of harvest timing on *T. grandis* nutrient quality and identifies the optimal time for harvest, thereby providing a reference and a guide for actual production.

2 Materials and Methods

2.1 Sampling site

The study site was located in Fuyang (30° 06' N, 119° 95' E), Zhejiang Province (Southeast China). The region is characterized by mountains and hills (105 m in altitude). It is a mild rainy region with a sub-tropical humid monsoon climate. Yearly precipitation is 1477.9 mm (average of 30 years). Monthly mean temperature is 4.3°C for January and 28.8°C for July (Fig. S1). The agro-climatic data were supplied by the local weather bureau in Fuyang district of Hangzhou.

2.2 Sampling and processing

The *T. grandis* trees were managed and had no nutrient deficiencies or pest damage. 8-year-old trees were selected and labeled in groves. To determine the best harvest time in terms of oil yield and other fruit quality parameters, *T.*

grandis fruit samples were harvested by hand five times in 2016: Aug. 25 (mature green stage), Aug. 31, Sept. 6, Sept. 12 and Sept. 18 (ripe fruits with epicarp longitudinal cracks), when the fruit was 30% mature until fully maturity. Only healthy fruits without any infection or physical damage were used. All the fruits were collected from the same 10 trees with a representative subsample (about 100 g) collected from each tree. Immediately after harvesting, fruits were sealed in plastic bags and transported to the laboratory where they were separated from the hulls, then cleaned nuts-in-shell and dried to 7% kernel moisture content²¹⁾ in fan-forced oven at 50°C, and stored at -18°C before analysis.

2.3 Methods

2.3.1 Oil extraction and quality analysis

T. grandis nuts were pressed by a vertical hydraulic oil press (6YY-190, Luoyang, China). The following quality evaluation parameters were tested: the kernel percent, kernel moisture content, kernel oil yield, fatty acid profile, free fatty acids (FFA), peroxide value, reducing capacity, tocopherols, soluble sugar content, starch content and the kinds and contents of free amino acids.

Kernel percent was calculated from the total nut and kernel weights after drying. Kernel moisture was determined by differencing weight before and after drying. Kernel oil content was determined using the Soxhlet method whereby seed samples were finely ground in household food grinder and extracted with petroleum ether (boiling range 30~60°C) in a B811-Soxhlet apparatus (Buchi, Switzerland) for 8 h at a constant temperature of 70°C. Protein, starch and total soluble sugar content were determined according to the procedure described by Wang²²⁾. FFA content and peroxide value were carried out following the analytical methods described in ISO (International Organization for Standardization) 660 and 3960, respectively.

2.3.2 Fatty acid profile

The fatty acid profile was analyzed by gas chromatography. Methyl esters were prepared in compliance with the standard method to determine the fatty acid profile, and oil was analyzed by GC-2010 plus gas chromatography (Shimadzu, Japan) equipped with a flame-ionization detector (FID) detector. The gas-chromatographic condition used were based on the previous study²³⁾. The gas chromatograph is equipped with an autosampler, a split/splitless injector, and a FID. A fused silica capillary column FAMEWAX (50 m length × 0.25 mm i.d. and 0.25 µm film thickness, Restek, America) was used. The carrier gas was nitrogen with 2 mL/min flow rate, and the temperature of injection block and detector was 220°C. The GC oven heating was initiated at 150°C. That temperature was maintained for 1 min and then increased to 190°C (at a rate of 5°C/min) and those conditions were maintained for 20

min. The injection volume was 1 μ L, the split ratio was 1:10. Commercial mixtures of fatty acid methyl esters (Sigma Aldrich, Milan, Italy) were used as reference data for the relative retention times. The results were expressed as relative area percent of the total.

2.3.3 Determination of tocopherols

Oil samples weighing 1 g (to an accuracy of 0.01 g) were dissolved with *n*-hexane in a 10-mL brown volumetric flask and filtrated through a 0.22- μ m PTFE filter for HPLC analysis. Tocopherols were measured using an Agilent 1290 high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA, USA) equipped with a silica column (250 $\times$ 4.6 mm i.d., 5 μ m, Waters Co., Milford, MA, USA) and a fluorescence detector (Agilent Technologies Inc., Santa Clara, CA, USA). Mobile phase was *n*-hexane and isopropanol with the volume ratio of 98.5:1.5. The flow rate was 1.2 mL/min, and the sample size was 10 μ L. The column temperature was 30°C. The fluorescence detection was operated with the excitation wavelength at 295 nm and the emission wavelength at 333 nm. The α -, β -, γ -, and δ -tocopherols were identified and quantified by comparing with the standards (MilliporeSigma, Billerica, MA). The results were expressed as milligrams of tocopherol per 100 gram of oil.

2.3.4 Reducing capacity assay

Folin-Ciocalteu reagent has a meaningful disadvantage since the reacting not only with the phenolic compounds but also with vitamin C, some amino acids, peptides, reduction sugars, organic acids, Maillard reaction products and some more compounds, therefore several researchers advise to interpret obtained results as reducing capacity instead of total phenolics content^{24, 25)}. The Folin-Ciocalteu method was used to measure sample reducing capacity in the research as reported by Mahmood Ghasemnezhad *et al.*²⁶⁾ with some variations. The extracting solution was prepared by solid-liquid extraction using ground kernels (5 g) and methanol-water (30:70, v/v, 50 mL) at 70°C for three times. 1 mL of extract was added to 5 mL of Folin-Ciocalteu's phenol reagent (5% w/v) and to 4 mL of sodium carbonate (7.5% w/v). The mixture was kept in the dark for 30 min after 1 min of vortex oscillation. Finally, the absorptions of the solution at 765 nm were measured. Reducing capacity were expressed as mg gallic acid/g of kernel.

2.3.5 Determination of free amino acids

The composition and contents of free amino acid in *T. grandis* nut were determined using L-8800 Amino Acid Analyzer (Hitachi, Japan) as reported by Mo R.H. *et al.*²⁷⁾.

All analyses were performed at least in triplicate except where there was insufficient equipment capacity, in which case appropriate repeat analyses were performed.

2.4 Statistical analysis

All data were processed and analyzed by Microsoft Office Excel 2010 and SPSS 19.0 statistical software. Results were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $p < 0.05$ level.

3 Results and Discussion

3.1 The kernel moisture, kernel yield and oil content under different harvesting times

The quality and economic indicators of *T. grandis* fruits at different harvest timepoints were determined (Table 1). The results showed that the kernel moisture content, kernel yield and oil content of *T. grandis* were significantly different ($p < 0.01$), which indicated that the fruit maturity had a substantial impact on the yield of *T. grandis* nut and its oil. The kernel moisture content decreased from 41.98 $\pm$ 0.07% on Aug. 25 to 35.73 $\pm$ 0.09% on Sept. 12, and the moisture content further decreased to 30.41 $\pm$ 0.04% on Sept. 18 which was more than 5% lower than the moisture value determined six days prior. The kernel yield increased by 1.71% from Aug. 25 to Sept. 6, after which time kernel yield did not significantly change ($p > 0.05$). The non-significant differences among harvest dates suggested that the kernels had attained their maximum size before the earliest harvest date (Aug. 25, 2019).

On the contrary, kernel oil content had a substantial increase in the early stages of maturity (before Sept. 6) followed by a significant increase in the latter part of maturation (from 42.32% on Sept. 6 to 48.4% on Sept. 18). These data inconsistent were inconsistent with the results obtained by other researchers about grape berry development^{28, 29)}. Oil content is one of the important indexes to

Table 1 Effect of harvest time on *T. grandis* nut kernel moisture, kernel yield and kernel oil content.

Indicators	Harvest date				
	Aug. 25th	Aug. 31st	Sept. 6th	Sept. 12th	Sept. 18th
Kernel moisture content /%	41.98 $\pm$ 0.04a	39.41 $\pm$ 0.30b	37.47 $\pm$ 0.03c	35.73 $\pm$ 0.06d	30.41 $\pm$ 0.04e
Kernel yield /%	68.84 $\pm$ 0.31a	69.80 $\pm$ 0.59b	70.55 $\pm$ 0.40c	70.57 $\pm$ 0.44c	70.72 $\pm$ 0.40c
Kernel oil content /%	41.75 $\pm$ 0.57a	42.19 $\pm$ 0.22b	42.32 $\pm$ 0.14b	43.47 $\pm$ 1.07c	48.40 $\pm$ 0.38d

Note: Kernel yield and kernel oil content expressed as percent of dry weigh. Results are mean $\pm$ standard error. Means with different letters are significantly different ($p < 0.05$).

evaluate oil plants' quality, and the amount of oil content directly affects economic value of the oil plants. Usually high oil content is associated with high edible quality and good storage stability. Several factors such as cultivar, species, growing conditions, maturity, and extraction method affect plant oil yield²⁸⁾. Previous studies have shown that the crispy taste of *T. grandis* nuts is positively correlated with its oil content^{3, 30)}. Oil accumulation in fruits increased consistently with postponement of harvest time. This increase may be attributed to continued activity of the triglyceride-forming biosynthesis pathway until the fruit reaches full maturation.

3.2 Fatty acid profile of nut oil on different harvest date

The fatty acid profile of oil is its most useful chemical property. The primary fatty acid compositions and contents of oil from the *T. grandis* nut at the different harvest times is shown in Table 2. The results indicate that *T. grandis* nut oil was rich in unsaturated fatty acid (UFA) (nearly 90% of the total fatty acids), especially linoleic acid and oleic acid. High oleic-linoleic oils are of interest because of their superior stability and nutritional importance. Linoleic acid is recognized as one of the most significant polyunsaturated fatty acids (PUFA) in human food because it has preventive effects on heart diseases^{31, 32)}. *T. grandis* nut oil also contained higher proportion of *cis*-5,11,14-eicosatrienoic acid (above 8%).

In the predominating fatty acid, the oleic acid content increased ($p < 0.05$) with a delay of harvest time from $30.76 \pm 0.41\%$ on Aug. 25 to $34.85 \pm 0.45\%$ on Sept. 18. Both linoleic acid and *cis*-5,11,14-eicosatrienoic acid contents decreased between Aug. 25 and Sept. 18, 2019, but there

was no significant difference ($p > 0.05$), and there was a negative correlation between the content of oleic acid and linoleic acid. Research has shown that oleic and linoleic acid content are related to maturity or the degree of filling³³⁾, and the content of linoleic acid decreases and the oleic acid increases during seed development³⁴⁾. Between Aug. 25 and Sept. 18, 2019, palmitic acid and *cis*-11,14-eicosadienoic acid decreased slightly, while stearic acid increased slightly, but these changes were not significant ($p > 0.05$).

The amounts of fatty acids in the *T. grandis* nut oil were in the order of PUFA > monounsaturated fatty acid (MUFA) > saturated fatty acid (SFA) for all five harvesting times, this difference was a result of higher levels of linoleic acid and the lower amount of oleic acid in the *T. grandis* nut oil sample. Total UFAs increased with postponement of harvest time, MUFA were not significant at the beginning of the ripening process of the *T. grandis* fruit (Aug. 25th Aug. 31st) and significant increase at the end (last three points of harvesting, Sept. 6th to Sept. 18th). This is consistent with the results of the author's research group on camellia oil³⁵⁾.

The PUFA constituted over 50% of the oil, while the MUFA content was relatively lower, ranging from $31.34 \pm 0.41\%$ to $35.44 \pm 0.04\%$. The SFA content was low (about 11%) and did not show an apparent change ($p > 0.05$) during ripening. The dietary fats abundant in PUFA can prevent chronic health conditions such as atherosclerosis, coronary heart disease, and high blood pressure. Furthermore, *cis*-5,11,14-eicosatrienoic acid is a special fatty acid also known as sciadonic acid, which often is found in the leaves and seeds of Gymnosperms, its name comes from its

Table 2 Fatty acid composition and relative content of *T. grandis* nut oil at different harvest times.

Fatty acid relative content/%	Harvest date				
	Aug. 25th	Aug. 31st	Sept. 6th	Sept. 12th	Sept. 18th
Palmitic acid (C16:0)	$8.66 \pm 0.01a$	$8.58 \pm 0.03ab$	$8.64 \pm 0.03a$	$8.49 \pm 0.02c$	$8.26 \pm 0.01d$
Stearic acid (C18:0)	$2.31 \pm 0.03a$	$2.49 \pm 0.32b$	$2.71 \pm 0.15c$	$2.95 \pm 0.23d$	$2.92 \pm 0.03d$
Oleic acid (C18:1)	$30.76 \pm 0.41a$	$30.86 \pm 0.08a$	$32.48 \pm 0.25b$	$33.87 \pm 0.31c$	$34.85 \pm 0.45d$
Linoleic acid (C18:2)	$43.61 \pm 0.23a$	$43.79 \pm 0.10b$	$42.82 \pm 0.31c$	$41.58 \pm 0.48d$	$41.13 \pm 0.48e$
Linolenic acid (18:3)	$0.46 \pm 0.07a$	$0.45 \pm 0.03b$	$0.45 \pm 0.03c$	$0.44 \pm 0.03d$	$0.45 \pm 0.22e$
Eicosanoic acid (C20:0)	$0.12 \pm 0.00a$	$0.17 \pm 0.04a$	$0.13 \pm 0.00a$	$0.14 \pm 0.00a$	$0.13 \pm 0.03a$
<i>cis</i> -11-eicosenoic acid (C20:1)	$0.57 \pm 0.03a$	$0.55 \pm 0.07ab$	$0.55 \pm 0.07ab$	$0.57 \pm 0.00ab$	$0.59 \pm 0.06b$
<i>cis</i> -11,14-eicosadienoic acid (C20:2)	$2.90 \pm 0.05a$	$2.83 \pm 0.21a$	$2.70 \pm 0.12b$	$2.70 \pm 0.06bc$	$2.71 \pm 0.03bd$
<i>cis</i> -5,11,14-eicosatrienoic acid (C20:3)	$9.89 \pm 0.07a$	$9.46 \pm 0.04b$	$8.98 \pm 0.01c$	$8.66 \pm 0.03d$	$8.35 \pm 0.14e$
Unsaturated fatty acid (UFA)	$85.54 \pm 0.49a$	$85.78 \pm 0.16a$	$87.92 \pm 0.55b$	$90.03 \pm 0.08c$	$90.84 \pm 0.14d$
Monounsaturated fatty acid (MUFA)	$31.34 \pm 0.41a$	$31.43 \pm 0.08a$	$32.97 \pm 0.02b$	$34.44 \pm 0.03c$	$35.44 \pm 0.04d$
Polyunsaturated fatty acid (PUFA)	$54.20 \pm 0.34a$	$54.35 \pm 0.13a$	$54.95 \pm 0.54a$	$55.59 \pm 0.11ab$	$55.40 \pm 0.10ac$
Saturated fatty acid (SFA)	$11.09 \pm 0.01a$	$11.24 \pm 0.06b$	$11.48 \pm 0.05c$	$11.57 \pm 0.01cd$	$11.33 \pm 0.01be$

Note: Results are mean $\pm$ standard error. Means with different letters are significantly different ($p < 0.05$).

rich in a plant of *Sciadopitys verticillata* (golden pine) in Japan³⁶). Previous research showed that sciadonic acid presents a strong role in lipid regulation in rats. Moreover, sciadonic acid could inhibit the activity of fatty acid synthase and 6-phosphate glucose dehydrogenase in liver and plasma of rats^{37, 38}). Thus, based on the contents of PUFA and sciadonic acid, the oil from *T. grandis* nuts is a higher nutritional and functional edible oil, and has a great value for exploitation and utilization. The fatty acid composition of seed oils varies widely among different plant species, and often the occurrence of unusual fatty acids can be used for the differentiation of particular plant families³⁹). Therefore, sciadonic acid can also be used as an essential fatty acid to distinguish *T. grandis* oil from other plant oils.

3.3 FFA and peroxide value on different harvest time

FFA and peroxide value are important indexes to evaluate the quality of oil plants. The amounts of FFA and peroxide value in *T. grandis* nut oil at different fruit maturity times were analyzed (Fig. 1). The effect of harvesting time on the FFA and peroxidation value were significant ($p < 0.05$), with both factors declining with delayed harvesting time. This result was consistent with previous research on camellia seeds¹⁷). The FFA and peroxide value of *T. grandis* sharply decreased from 0.39 ± 0.06 mg/g and 2.92 ± 0.30 g/100 g at first harvest to 0.23 ± 0.02 mg/g and 0.70 ± 0.41 g/100 g at the last harvest, respectively. Combined with the fact that riper *T. grandis* fruits have higher oil content, we could draw the conclusion that more FFA in cell tissues are used to synthesize triglyceride as *T. grandis* fruit matures, which leads to the decrease of FFA. This result is consistent with prior study⁴⁰). Also, ripening fruits contained more antioxidants that entered the oil, thereby reducing the oxidation of the oil to some extent⁴¹).

Fig. 1 FFA(●) and peroxide value(■) of *T. grandis* nut at different harvest times.

3.4 Protein, soluble sugar and starch contents on different harvest times

Protein is one of the main nutritional components in *T. grandis* nuts, and starch and soluble sugar are the standard indexes to evaluate the quality of oil seed. With the *T. grandis* fruit maturing, the protein content in its nut increased ($p < 0.05$) from $23.3 \pm 0.08\%$ on Aug. 25 to $25.3 \pm 0.12\%$ on Sept. 18. As shown in Fig. 2, there were no significant differences in terms of starch and soluble sugar amounts for different harvest times ($p > 0.05$). The soluble sugar content was $4.24 \pm 0.03\%$ on Aug. 25, and then it increased to $6.06 \pm 0.32\%$ on Sept. 18 with the delay of harvest time. On the contrary, the starch content dropped from $5.95 \pm 0.21\%$ on Aug. 25 to $5.50 \pm 0.07\%$ on Sept. 18. It was interesting that the increased in soluble sugar content coincided with a decline in starch content during ripening; the two factors were negatively correlated ($r = -0.8773$, 95% CI = $-0.718 \sim -1.000$, $p = 0.05$), which suggesting that the increased soluble sugar mainly came from the hydrolysis of starch.

3.5 The composition and content of tocopherol on different harvest times

Tocopherol is an important natural antioxidant, which can effectively block free radical chain reactions, prevent and reduce lipid peroxidation damage of cell membranes to protect cell membranes, remove free radicals and prevent cancer *in vivo*⁴²). It is present in plants in four forms which possess different molecular structures and functions: α -, β -, γ - and δ -, of which α -tocopherol has the strongest physiological activity.

The tocopherol composition and contents of *T. grandis* nut collected at the different maturation dates are presented in Table 3. α -tocopherol and β -tocopherol were identified from *T. grandis* nut, the content of β -tocopherol (more than 100 mg/100 g) was about four times higher than

Fig. 2 Protein(●), soluble sugar(▲) and Starch(◇) content of *T. grandis* nut at different harvest times.

Table 3 Tocopherols composition and content in *T. grandis* nut at different harvest times.

Content/(mg · 100 g ⁻¹)	Harvest date				
	Aug. 25th	Aug. 31st	Sep. 6th	Sep. 12th	Sep. 18th
α-tocopherol	23.45 ± 0.05a	24.67 ± 0.03b	24.48 ± 0.02c	25.39 ± 0.01d	26.15 ± 0.05e
β-tocopherol	103.95 ± 0.05a	105.96 ± 0.05b	108.97 ± 0.10c	111.18 ± 0.15d	111.52 ± 0.10de
γ-tocopherol	ND	ND	ND	ND	ND
δ-tocopherol	ND	ND	ND	ND	ND
Total tocopherol	127.5 ± 0.04a	130.7 ± 0.36b	133.5 ± 0.30c	136.4 ± 0.05d	137.1 ± 0.02de

Note: ND, not detectable. Results are mean ± standard error. Means with different letters are significantly different ($p < 0.05$).

α-tocopherol (when concentrations are above 23 mg/100 g), but no γ-tocopherol and δ-tocopherol had been detected, which is consistent with previous study⁴¹. The total tocopherol contents gradually increased from 127.5 ± 0.04 mg/100 g on the first date to 137.1 ± 0.02 mg/100 g on the last date. The levels of α-tocopherol and β-tocopherol increased with ripening, and reached the highest values at Sept. 18, which increased 11.51% and 7.28% from the first fruit picking respectively. Besides the influence of the harvest time, also other factors should be taken into account. For instance, in acorns, genotype and the year have a significant impact on the concentration of tocopherols⁴³. Commonly, plant seeds are rich in homologues α- and γ- but little or no β- and δ-⁴⁴, but *T. grandis* nut oil is rich in β-tocopherol. There was rarely reported about the predominance of β-tocopherol on other plants, only in last decade some meaning reports in acorns⁴³, coffee beans⁴⁴, wheat germ⁴⁵, apple seeds⁴⁶, European cranberrybush seeds⁴⁷, kerkir seeds⁴⁸ had been published. Therefore, in *T. grandis* β-tocopherol is a main tocopherol homologue which can serve as a characteristic indicator of *T. grandis* nut oil. It can be used as an unconventional natural source of the rare tocopherol homologue.

3.6 The reducing capacity on different harvest times

The antioxidant activity of a substance is directly related to its reducing capacity. The stronger the reducing capacity is, the stronger the antioxidant activity. Therefore, the antioxidant activity of a substance can be illustrated by measuring its reducing capacity. **Figure 3** lists the reducing capacity in the *T. grandis* nuts harvested at the five harvest dates. The results showed that the reducing capacity initially increased reaching a peak value of 17.87 ± 0.03 mg/g on Sept. 6, then declined gradually during the later ripening stages, though values were still higher than the initial value. With the harvest time delay, the color of *T. grandis* aril changed from light red to crimson which might be related to oxidation of phenols; phenols were oxidized to quinones which could be polymerized to produce colored substances that caused tissue browning⁴⁹. The degree of browning is usually positively correlated with the content of phenols. This falling of reducing capacity at the late

Fig. 3 The reducing capacity in *T. grandis* nuts at different harvest times.

harvest date was mainly attributed to the decline in astringent substance, for example, phenolic substances occurring as part of the physiological maturation of the fruits¹⁸. In addition, the content of phenolic substances in fruits is closely related to the edible qualities of fruits such as texture, fragrance and astringent and bitter taste⁵⁰. The higher level of phenolics could bring strong astringency, which affected the taste and flavor of the *T. grandis* nuts. But at the same time, phenolic compounds can raise the stability, sensory and health properties of oil, the amount of which was an important factor affecting the quality of oil²⁰. It is known that the content of antioxidant compounds in plant materials such as phenolics varies greatly with, for example, processing, genotype, harvest time, growing environment and conditions.

3.7 Effects of harvest time on free amino acids

Amino acids are the primary composition unit of protein and the decomposition product of protein, which are also one of the components of fruit quality and participate in the synthesis of other quality characteristic components and flavor substances⁵¹. Since the content of free amino acids is closely related to food processing and food flavor, we studied the composition and content of free amino acid in the *T. grandis* nut. **Table 4** shows that there were 17 free amino acids in the *T. grandis* nut, the total content of

Table 4 The free amino acid composition and content in *T. grandis* nut with different harvest times.

Content /%	Harvest date				
	Aug. 25th	Aug. 31st	Sept. 6th	Sept. 12th	Sept. 18th
Aspartic acid	0.52 ± 0.22c	0.32 ± 0.01b	0.30 ± 0.08b	0.60 ± 0.02a	0.18 ± 0.03d
Threonine*	0.38 ± 0.11a	0.35 ± 0.01a	0.34 ± 0.10a	0.61 ± 0.07b	0.75 ± 0.01c
Serine	0.46 ± 0.05a	0.47 ± 0.02a	0.67 ± 0.07b	1.45 ± 0.27c	1.54 ± 0.06d
Glutamate	1.26 ± 0.31a	1.27 ± 0.23a	1.40 ± 0.28b	1.72 ± 0.18c	1.95 ± 0.39d
Glycine	0.75 ± 0.24d	0.60 ± 0.17a	0.61 ± 0.13a	0.69 ± 0.11c	0.66 ± 0.09b
Alanine	0.78 ± 0.28a	0.83 ± 0.24a	0.76 ± 0.02a	1.34 ± 0.41b	1.55 ± 0.44c
Valine*	1.08 ± 0.37a	1.10 ± 0.02a	1.16 ± 0.56b	1.14 ± 0.02b	1.28 ± 0.06c
Cystine	0.14 ± 0.01c	0.16 ± 0.05d	0.12 ± 0.27b	0.12 ± 0.05b	0.09 ± 0.31a
Methionine*	0.26 ± 0.11a	0.23 ± 0.01a	0.33 ± 0.06b	0.26 ± 0.03a	0.35 ± 0.00b
Isoleucine*	0.97 ± 0.23d	0.82 ± 0.12c	0.76 ± 0.01b	0.75 ± 0.32b	0.61 ± 0.02a
Leucine*	1.19 ± 0.06b	1.30 ± 0.31c	1.43 ± 0.22d	1.14 ± 0.25b	0.95 ± 0.07a
Tyrosine	1.31 ± 0.03a	1.37 ± 0.09a	2.10 ± 0.30b	2.80 ± 0.31c	3.40 ± 0.12d
Phenylalanine*	1.30 ± 0.19a	1.45 ± 0.21b	1.75 ± 0.35c	1.92 ± 0.09c	2.19 ± 0.32d
Lysine*	0.50 ± 0.32a	0.79 ± 0.13b	0.74 ± 0.15b	0.93 ± 0.24c	0.96 ± 0.08c
Histidine	0.35 ± 0.08c	0.23 ± 0.06b	0.21 ± 0.02b	0.25 ± 0.14b	0.12 ± 0.04a
Arginine	0.69 ± 0.10b	1.39 ± 0.11c	0.41 ± 0.01a	0.63 ± 0.13b	1.43 ± 0.09c
Proline	0.44 ± 0.06a	0.44 ± 0.07a	0.26 ± 0.07b	0.20 ± 0.10b	0.22 ± 0.05b
Total amino acid	12.38 ± 0.27a	13.12 ± 0.37b	13.35 ± 0.13b	16.55 ± 0.43c	18.23 ± 0.43d

Note: *, essential amino acids. Results are mean ± standard error. Means with different letters are significantly different ($p < 0.05$).

these free amino acids is more than 12%, among which the contents of tyrosine, phenylalanine, glutamate and valine were higher than 1%. Humans require seven types of amino acids that we cannot naturally synthesis: threonine, valine, methionine, leucine, isoleucine, phenylalanine and lysine. In our analysis, tyrosine content was highest in the *T. grandis* nut, while the content of histidine was the lowest. With the delay in harvesting time, the content of all essential amino acids except isoleucine and leucine showed an upward trend.

Amino acid compositions are nutritionally important and are influence the taste of plant fruits⁵²⁾. Amino acids which have flavor properties can be divided into sweet (e.g. glycine, alanine, serine, threonine, proline, histidine and glutamine), bitter (e.g. valine, leucine, isoleucine, methionine, tryptophan and arginine) and delicious (e.g. lysine, glutamine, aspartic acid and asparagine) and aromatic amino acids (e.g. phenylalanine, tyrosine and cysteine)⁵³⁾. As shown in Fig. 4, among the flavor amino acids in *T. grandis* nuts, the content of sweet amino acids and aromatic amino acids were relatively high (more than 30% and 20% of the total free amino acids, respectively). Both the sweet and aromatic amino acids showed rapid increases during the early stage from 4.42% and 2.75% on Aug. 25 to 6.79% and 5.68% on Sept. 18, respectively. By contrast,

Fig. 4 The free amino acid types in *T. grandis* nuts at different harvest times. Values are mean and standard error.

the content of bitter amino acids decreased with the delay of picking time and drop more slowly early (from Aug. 25 to Sept. 6), since then it has fallen rapidly to 1.56%. These results indicate that a late harvest times is beneficial to the flavor of *T. grandis* nuts.

These results were in agreement with previous research that there was an improvement in flavor quality of pecans after they reached full size, but that the flavor was subject

to impairment, mostly through the onset on oxidation, staleness and rancidity⁵⁴⁾.

4 Conclusion

The study showed that *T. grandis* nuts were a rich source of tocopherols, mainly homologue β -tocopherol, which was a unique finding, due to previous studies often reporting low abundance of this homologue in other plant materials. Therefore, *T. grandis* nut can be used as an unconventional natural resource of the rare tocopherol homologue.

Based on the results of different harvest time in this study, we concluded that the kernel yield of *T. grandis* had no clear change in later harvesting. With the exception of moisture, protein, and starch contents, the content levels of most analytes increased with prolonged ripening time, reaching their highest values on Sept. 18. The acid values and peroxide values declined with prolonged ripening time, and reached their lowest values at Sept. 18. This showed that *T. grandis* kernels were no longer gaining weight, but their nutrients were still accumulating, and at that point, their nutrition and quality indicators were high. Therefore, prolonged ripening is helpful to the accumulation of dry matter and nutrients in *T. grandis* nuts. This results are helpful for the choosing suitable harvest time for *T. grandis* fruit to maximize their beneficial effects for human health.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Supporting Information

This material is available free of charge via the Internet at <http://dx.doi.org/jos.70.10.5650/jos.ess20155>

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