



Investigation of Nut Qualities of Pecan Cultivars Grown in China

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Abstract: Pecan had been introduced into China for over one hundred years and had raised great attentions as a woody oil crop these years, but the detailed nut qualities of pecan cultivars grown in China had not been evaluated before. To access the adaptabilities of different pecan cultivars after introduce and for future utilization of these cultivars, the pecan nut quality had been evaluated in this article. Both physical and chemical nut quality traits were evaluated on Chinese-grown pecan cultivars, including nut weight, nut shape, shell thickness, kernel, lipid, fatty acid content, tocopherol content, antioxidant capacities of lipids, phenolics contents and their antioxidant capacities. Great variability existed among different cultivars in most of the nut quality traits. After introduced, physical traits changed in different ways, which suggested that cultivars have different adaptabilities. Cultivars 'Desirable', 'Western' or 'Kanza' are high in oleic acid, while cultivar 'Pawnee' is high in γ -tocopherol. Cultivar 'Mahan' had significant high values of total phenolic content (TPC), condensed tannins (CT), total flavonoid content (TFC), ellagic acid content and antioxidant capacity of defatted kernel compared to other cultivars, which made it a suitable material for further study of phenolics in pecan. This article provided detailed analyses of the nut qualities of Chinese-grown pecan cultivars for the first time, which can be useful for future studies.

Keywords: *Carya illinoensis* (Wangenh.) K. Koch, Nut Quality, Physical Trait, Lipid, Phenolic, Antioxidant Capacity

1. Introduction

Pecan [*Carya illinoensis* (Wangenh.) K. Koch] is a phreatophyte arbor original from North America. It had been introduced into China around 1900s and had raised great attention as a woody oil crop in China these years [1-2]. The cultivation area increased about 20,000 acre per year and the increasing speed is still accelerating. After decades of practice, some of these introduced cultivars were more welcomed and widely cultivated due to their good adaptability and good traits such as large nut shape and high yield [3]. Our previous research showed that genetic variation occurred among pecan cultivars after introduced into China [4]. But the detailed nut qualities of these popular cultivars grown in China had not been evaluated or compared yet. The evaluation of nut qualities is critical for accessing

the adaptability of cultivars or better utilization of these cultivars.

Physical traits directly determined the price and popularity of pecan cultivars. People prefer to purchase pecan nuts with big long shape and thin shell, so these physical traits have direct influence on the price of the nuts, while traits such as kernel percentage and shell thickness are especially valuable for processing industry. Besides physical traits, chemical traits such as nutritional components and their health benefits were also important for nut qualities. Therefore, comprehensive evaluation of nut traits is more conducive to fully understand of different cultivars. The dominant nutritional component in pecan nut is lipid. Mature pecan nuts are composed of 65-75% lipid, in which over 95% are triglycerides [5]. Lipid quality is mainly decided by the type and proportion of fatty acids attached to the glycerol. Fatty acids in pecan kernel were mainly unsaturated, including

oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), while low in saturated fatty acid, including palmitic acid (C16:0) and stearic acid (C18:0) [6]. Pecan lipid also contains tocopherols [5, 7, 8]. Tocopherol is one of the most powerful antioxidant in living organisms, it can protect cells from harms of radicals [9], and it is also the main component which prevents oil from peroxidation [10]. There are several types of tocopherols existed in pecan kernel, in which γ -tocopherol is the dominant, while α - and δ -tocopherols are in small amounts [5, 6, 11]. Another type of important nutritional components in pecan kernel is phenolics [3, 6, 7]. Phenolics in pecan kernel were mainly phenolic acids, flavones, proanthocyanidins and hydrolysable tannins [3, 12, 13]. Although their contents were low, the phenolics in pecan process great activities such as antioxidant [7, 14, 15], antiviral [16], antidiabetic activity [17] and insect resistant [18]. So, phenolic contents and their antioxidant capacities were also important aspects which need to be evaluated.

It is well known that environment factors can affect nut qualities, while different cultivars have different adaptabilities in the same area. The comprehensive evaluation of nut quality and adaptability is of great significance for future utilization of these cultivars. In this article, we conducted a detailed phenotypic analysis of nut-quality traits. Both the physical traits and chemical aspects, including nut weight, nut shape, shell thickness, kernel, lipid, fatty acid content, tocopherol content, antioxidant capacities of lipids, phenolics contents and their antioxidant capacities were evaluated on 14 Chinese-grown pecan cultivars. These data will be useful for future utilization of these cultivars.

2. Materials and Methods

2.1. Pecan Samples

Nuts of fourteen pecan cultivars were collected from the pecan germplasm orchard of Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Jiangsu Province, China in 2015. All experimental trees were cultivated with the same regular practice. When mature, nuts were handpicked from trees at four directions of east, south, west and north. Two nuts were picked from each direction makes eight nuts from each tree. Each cultivar had three biological replicates, each replicate contained three trees. Nuts were naturally dried in shed place as commercial practice until the moisture was down to 4-5%. There are two types of cultivars, cultivar 'Jinhua', 'Lvzhou 1' and 'Shaoxing' were domestic seedling selected cultivars, while the rest 11 cultivars were introduced from USA.

2.2. Physical Traits

Seven physical traits, including nut weight (g), longitudinal diameter (mm), transverse diameter (mm), nut index, kernel weight (g), shell thickness (mm) and kernel percentage (%) were measured or calculated to evaluate the physical differences among cultivars. Nut weight and kernel weight

were measured with analytical scale (Mettler Toledo ME55, German). Diameters and shell thickness were measured with millimetric calipers. Nut index is the ratio of longitudinal diameter to transverse diameter. Kernel percentage is the ratio of kernel weight to nut weight. Pecan nuts were cracked when measuring the shell thickness. The kernels were carefully collected, dried in oven, powdered using a high speed pulverizer (Xifu FW100, China) for homogeneity and stored at -20°C before use. The results of nut weight and kernel percentage were also compared with data reported by the United States Department of Agriculture [19]. All data are means \pm standard deviation (SD) of three replicates, each replicate contained 24 nuts.

2.3. Chemical Traits

2.3.1. Lipid and Fatty Acids

Lipids were extracted using the Soxhlet extraction method. Pecan kernel powder was refluxed in Soxhlet's apparatus with n-hexane (1:10, w/v) for 6 h. Solvent was then removed and the lipid was stored at -20°C for further use. Fatty acid methyl esters were prepared following the standard procedure of GB/T 17376-2008. Briefly, pecan oil (30 mg) was refluxed in a water bath under nitrogen protection together with 4 mL sodium hydroxide methanol solution (NaOH/MeOH, 0.5%) for about 10 min, and kept refluxing for another 3 min after adding 5 mL boron trifluoride catalyst reagent. Then 3 mL isooctane was added, and the reaction was stopped immediately. Saturated solution of NaCl (20 mL) was added with violent shaken for extraction and separation. The supernatant layer was collected and filtrated with 0.45 μ m polytetrafluoroethylene (PTFE) filters before gas chromatography (GC) analysis. GC was carried on with an Agilent 6890N gas chromatograph (Supelco SP-2340 column, 100 m \times 0.25 mm, 0.20 μ m, USA). The initial temperature was set at 100°C, last for 2 min, ramp at 5°C per minute to 200°C and hold for 1 min, ramp at 10°C per minute to 280°C and hold for 10 min. The temperature of injector and flame ionization detector (FID) were set at 250°C and 200°C. The helium, air, and hydrogen flows were set at 1.6, 300, and 35 mL \cdot min⁻¹, respectively. Methyl heptadecanoate (Nu-Chek-Prep, USA) was used as internal standard and Supelco FAME mixture of standards (Nu-Chek-Prep, USA) was used for identification. All data are means \pm SD of three replicates.

2.3.2. Tocopherol

Tocopherols were analyzed according to the report of Villarreal-Lozoya [6] with some modification. Pecan oil (0.5 g) and methanol (2 mL) were mixed and vortexed for 1 min. The mixture was centrifuged at 6000 rpm for 5 min, the upper methanol layer was collected and filtered using 0.45 μ m PTFE filters, removed into brown glass bottles (1.5 mL), and stored at -20°C until analysis. HPLC analysis was carried on with Agilent 1100 HPLC (Agilent Technologies, USA) using a C18 column (Elipse XDB, 250 mm \times 4.6 mm, 5 μ m, USA) conducted at 35°C. An isocratic flow of 1.0 mL \cdot min⁻¹ of methanol/water (97:3, v/v) was used as mobile phase. The

wavelength was set at 298 nm. Standard compounds (Sigma-Aldrich, USA) were used for identification and quantification. The results were expressed as mg tocopherols per kg oil ($\text{mg}\cdot\text{kg}^{-1}$). All data are means \pm SD of three replicates.

2.3.3. Antioxidant Capacity of Lipid

The antioxidant capacity of pecan lipid was accessed using DPPH method according to previous reports [6, 20] with some modification. Methanol extract (10 μL) was mixed with 0.1 mM solution of DPPH radical (4 mL) and kept in dark for 30 min for fully reaction. The absorbance was measured at 515 nm with a UV spectrophotometer (Shimadzu UV-2100, Japan). Methanol was used as the blank and trolox was used as the standard reference. The absorbance of blank was subtracted from each sample and the results were expressed as μmol trolox equivalents (TE) per gram of pecan oil ($\mu\text{mol TE}\cdot\text{g}^{-1}$). All data are means \pm SD of three replicates.

2.3.4. Total Phenolic Content, Condensed Tannins, Total Flavonoid Content and Phenolic Acids

Phenolic compounds were extracted using the defatted kernels (1 g) with 20 mL of 80% acetone. The mixture was ultrasonic extracted twice for 2 h each time, and the supernatants were combined. Acetone was removed by nitrogen blowing (Anpel nitrogen evaporator, China) at 50°C. Samples were further lyophilized (Songyuan Huaxing LGJ-12, China), re-dissolved in methanol and stored at -20°C until further analysis. The total phenolic content (TPC) was measured according to the procedure reported by de la Rosa [21]. Briefly, methanol extract (10 μL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (10%, v/v), 2 mL of sodium carbonate (7.5%, w/v) and reacted at 50°C for 15 min in dark. After cooling to room temperature, the absorbance was measured at 760 nm using UV spectrophotometer. Ellagic acid was used as the standard reference because most phenolics in pecan kernel are ellagic acid derivatives [3, 7]. Methanol was used as the blank for TPC, condensed tannins (CT) and total flavonoid content (TFC). The results were expressed as milligrams of ellagic acid equivalent (EAE) per gram of defatted kernel ($\text{mg EAE}\cdot\text{g}^{-1}$). CT and TFC were measured according to our previous report [3]. The results were expressed as milligrams of (+)-catechin equivalents (CE) per gram of defatted kernel ($\text{mg CE}\cdot\text{g}^{-1}$). Phenolic acids were quantified by HPLC using a C18 column (Gemini, 250 mm \times 4.6 mm, 5 μm , USA) conducted at 35°C. The mobile phase consist acetonitrile (A) and 2% of acetic acid (v/v, B) and conducted as 5-37% of A for 0-40 min at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. Phenolic acids were detected at 280 nm. All data are means \pm SD of three replicates.

2.3.5. Antioxidant Capacity of Defatted Kernel

The antioxidant capacity of defatted kernel was accessed using both DPPH and ABTS method. The DPPH method was the same as in 2.3.3. The ABTS was measured according to previous reports [22-23]. Methanol extract (40 μL) was mixed with 2 mL ABTS⁺ solution (7.0 mM) and kept in dark for 6 min for reaction. Then the absorbance was

measured at 734 nm with UV spectrophotometer. Methanol was used as the blank and trolox was used as the standard reference. The absorbance of blank was subtracted from each sample and the results were expressed as μmol trolox equivalents (TE) per gram of defatted kernel ($\mu\text{mol TE}\cdot\text{g}^{-1}$). All data are means \pm SD of three replicates.

2.4. Statistical Analysis

One-way analysis of variation (ANOVA) and Pearson correlations were performed with the SPSS software (18.0). Hierarchical clustering and heat-map analysis were performed with the Heml software (1.0).

3. Results and Discussions

3.1. Comparison of Physical Traits

3.1.1. Nut Weight

Great variability existed among different cultivars in nut weight from 6.55 to 11.45 g (Table 1). Cultivar 'Desirable' had the highest nut weight after introduced in China, the same as in USA. Cultivar 'Kanza' had the lowest nut weight after introduced, while cultivar 'Caddo' was the lowest in USA among the 14 cultivars analyzed in this article. After introduced, nut weights were increased in 'Chickasaw', 'Desirable', 'Western', 'Mahan', 'Caddo' and 'Wichita', while decreased in 'Pawnee', 'Choctaw', 'Stuart', 'Kanza' and 'Cheyenne'. The number of cultivars which gain weight was a little bit more, but the losses of weights were more intensive.

3.1.2. Longitudinal Diameter, Transverse Diameter, Nut Index and Shell Thickness

There was also great variability in longitudinal diameter, nut index and shell thickness (Table 1). For both longitudinal diameter and nut index, cultivar 'Mahan' was the biggest and 'Chickasaw' was the smallest. As in shell thickness, 'Stuart' had the thickest shell of 1.25 mm, while 'Wichita' had a very thin shell of only 0.70 mm. On the other hand, the coefficient of variation (CV) of transverse diameter was the smallest among all physical traits, which means that variations of nut shape were more likely to occur on the longitudinal direction. Large and long nuts with thin shell can be sold at higher prices. So cultivars like 'Mahan', 'Desirable' which have big long nut and thin shell seem to have good potential to be cultivated in bigger areas, while cultivars like 'Chickasaw', 'Kanza' which have small nut and thick shell will be more suitable to be used as seeds for rootstocks.

3.1.3. Comparison of Kernel Weight and Kernel Percentage

It is not surprised that 'Desirable' had the heaviest kernel weight since it had the heaviest nut weight, the same as in 'Kanza' of the lightest kernel weight and nut weight (Table 1). The highest CV among physical traits was observed in kernel weight of 21.58%. After introduced, 'Wichita' had the highest kernel percentage and 'Chickasaw' had the lowest kernel percentage. The kernel percentage of 'Desirable' increased for 8.77% after introduced. The kernel percentages of 'Wichita', 'Pawnee' and 'Stuart' were also increased, but the kernel

percentages of the other 7 cultivars were decreased. It should be noticed that more cultivars were decreased in kernel percentage than increased and the decreases were more intensive than increases.

Table 1. Physical traits of pecan cultivars.

Varieties	Nut Weight			Longitudinal Diameter (mm)	Transverse Diameter (mm)
	After Introduced (g)	In USA (g) *	Variation (%)		
Caddo	7.0 ± 0.3 de	6.7	5.1	46.2 ± 1.9 c	22.2 ± 0.8 de
Cheyenne	7.5 ± 0.6 de	8.0	-6.8	46.1 ± 2.9 c	21.6 ± 1.0 e
Chickasaw	7.6 ± 0.7 d	6.7	12.7	34.3 ± 1.9 f	23.1 ± 1.0 cd
Choctaw	8.2 ± 1.2 cd	9.9	-16.9	41.4 ± 3.8 d	23.7 ± 1.0 cd
Desirable	11.4 ± 0.8 a	10.3	11.5	49.0 ± 1.5 b	26.3 ± 0.3 a
Jinhua	9.9 ± 1.2 b	-	-	42.8 ± 1.8 d	24.0 ± 1.0 c
Kanza	6.6 ± 0.7 e	7.4	-10.9	35.6 ± 1.2 f	22.8 ± 0.6 d
Lvzhou 1	8.3 ± 0.5 cd	-	-	42.6 ± 1.4 d	22.6 ± 0.6 de
Mahan	10.4 ± 1.6 b	9.6	8.2	56.3 ± 3.5 a	25.2 ± 0.9 b
Pawnee	8.0 ± 1.2 cd	9.8	-18.6	38.8 ± 2.7 e	23.1 ± 1.2 cd
Shaoxing	7.7 ± 0.6 cd	-	-	36.6 ± 1.6 ef	24.1 ± 0.6 c
Stuart	7.6 ± 1.1 d	9.0	-15.9	38.4 ± 1.8 e	24.2 ± 0.9 bc
Western	8.6 ± 1.2 c	8.0	8.3	44.4 ± 2.8 cd	23.7 ± 1.8 cd
Wichita	7.6 ± 0.5 d	7.6	0.5	44.0 ± 2.3 cd	22.4 ± 0.8 de
Max	11.4			56.3	26.3
Min	6.6			34.3	21.6
Average	8.3			42.6	23.5
CV (%)	16.5			13.7	5.2

Table 1. Continued.

Varieties	Nut Index	Kernel Weight (g)	Shell Thickness (mm)	Kernel Percentage		
				After Introduced (%)	In USA (%) ²	Variation (%)
Caddo	2.1 ± 0.1 b	3.7 ± 0.2 d	0.8 ± 0.1 c	52.5 ± 3.0 b	55.5	-5.5
Cheyenne	2.1 ± 0.2 b	3.9 ± 0.3 d	0.7 ± 0.1 c	51.2 ± 3.2 bc	56.3	-9.2
Chickasaw	1.5 ± 0.1 e	3.4 ± 0.4 d	1.1 ± 0.2 b	45.5 ± 1.8 c	51.8	-12.1
Choctaw	1.8 ± 0.2 cd	4.7 ± 1.0 c	0.9 ± 0.2 bc	53.5 ± 7.6 b	55.5	-3.6
Desirable	1.9 ± 0.1 c	6.6 ± 0.5 a	0.7 ± 0.1 c	56.7 ± 1.7 ab	52.1	8.8
Jinhua	1.8 ± 0.1 c	5.6 ± 0.7 b	1.0 ± 0.1 b	54.3 ± 2.1 b	-	
Kanza	1.6 ± 0.1 de	3.2 ± 0.5 d	1.2 ± 0. ab	47.4 ± 3.6 c	54.1	-12.3
Lvzhou 1	1.9 ± 0.1 c	4.5 ± 0.3 cd	0.9 ± 0.2 bc	53.8 ± 0.9 b	-	
Mahan	2.2 ± 0.2 a	5.5 ± 1.0 bc	0.8 ± 0.1 c	53.2 ± 4.9 b	53.6	-0.8
Pawnee	1.7 ± 0.1 d	4.7 ± 0.7 c	0.8 ± 0.0 c	58.7 ± 5.4 ab	58.0	1.1
Shaoxing	1.5 ± 0.1 e	3.5 ± 0.4 d	1.1 ± 0.1 ab	46.1 ± 3.5 c	-	
Stuart	1.6 ± 0.1 de	3.9 ± 0.1 d	1.2 ± 0.1 a	46.8 ± 0.8 c	46.6	0.5
Western	1.9 ± 0.2 c	4.9 ± 1.9 bc	0.8 ± 0.0 c	56.5 ± 6.9 ab	58.0	-2.7
Wichita	2.0 ± 0.1 dc	4.6 ± 0.3 cd	0.7 ± 0.1 c	61.6 ± 1.160 a	59.1	4.2
Max	2.2	6.6	1.2	61.6		
Min	1.5	3.2	0.7	45.5		
Average	1.8	4.5	0.9	52.7		
CV (%)	13.0	21.6	19.0	9.2		

+Max, The maximum value; Min, The minimum value; AV, Average value; CV, Coefficient of variation. Different letters in the same column meant significantly different according to the Turkey's test at $p < 0.05$. ²data were obtained from the reports of the United States Department of Agriculture.

These results reminded us that except high nut yield which is normally easy to notice, growers should also pay more attentions to physical traits which are normally easy to ignore to avoid fall down of nut quality. It is reported that fruit qualities can be influenced by environment factors, cultivation practices and adoption abilities [7]. From the above results, we can observe that nut qualities of pecan cultivars had changed in different ways after introduced, which indicated their different adaptabilities after introduced.

3.2. Comparison of Chemical Traits

3.2.1. Lipid and Fatty Acid

Lipids of all cultivars exceeded 70% of kernel weight with a very low CV of 3.92%, which means all cultivars had

relatively high lipid content (Table 2). Cultivar 'Desirable' had the highest C18:1 content, while 'Stuart' had the lowest. The CV of C18:1 content was only 4.89%. The C18:1 contents in 'Western' and 'Kanza' were also over 70%. The content of C18:2 was just opposite to the content of C18:1. Such as 'Desirable' contained the highest content of C18:1 and the lowest content of C18:2, while 'Stuart' had the lowest content of C18:1 and the highest content of C18:2. The content of C18:3 in pecan kernel was relatively low and great variations existed among cultivars. Great variations can also be seen in saturated fatty acids of palmitic acid (C16:0) and stearic acid (C18:0).

Table 2. Quality traits of pecan oil.

Varieties	Lipid (%)	Fatty acid (%)				
		C16:0	C18:0	C18:1	C18:2	C18:3
Caddo	72.4 ± 4.0 d	6.2 ± 0.1 b	1.6 ± 0.0 d	69.6 ± 3.4 b	21.5 ± 0.6 d	1.1 ± 0.0 b
Cheyenne	74.8 ± 4.1 cd	6.6 ± 0.2 b	2.0 ± 0.0 bc	68.6 ± 2.6 b	21.8 ± 0.6 d	1.0 ± 0.0 bc
Chickasaw	71.0 ± 2.8 e	8.1 ± 0.1 a	1.8 ± 0.0 c	63.1 ± 2.9 de	25.8 ± 0.4 b	1.2 ± 0.0 ab
Choctaw	72.1 ± 2.9 d	6.6 ± 0.1 b	2.0 ± 0.0 bc	64.8 ± 1.7 d	25.6 ± 0.6 b	1.1 ± 0.0 b
Desirable	79.7 ± 1.5 a	6.1 ± 0.2 bc	1.9 ± 0.0 c	74.2 ± 3.4 a	17.2 ± 0.6 f	0.7 ± 0.0 c
Jinhua	75.1 ± 4.4 c	6.9 ± 0.3 b	1.8 ± 0.0 c	67.3 ± 2.1 c	23.2 ± 0.6 c	0.7 ± 0.0 c
Kanza	71.4 ± 5.3 de	5.6 ± 0.0 c	2.9 ± 0.0 a	70.9 ± 4.0 ab	19.6 ± 0.6 e	1.0 ± 0.0 b
Lvzhou 1	75.8 ± 5.6 b	3.7 ± 0.1 e	2.6 ± 0.0 ab	68.9 ± 1.3 b	23.6 ± 0.4 cd	1.2 ± 0.0 ab
Mahan	70.1 ± 4.6	5.7 ± 0.5 c	2.9 ± 0.0 a	69.8 ± 3.1 b	20.8 ± 0.7 de	0.8 ± 0.0 c
Pawnee	77.2 ± 7.3 bc	6.1 ± 0.1 bc	2.3 ± 0.0 b	69.4 ± 3.1 b	21.4 ± 0.4 d	0.8 ± 0.0 c
Shaoxing	71.4 ± 6.1 de	4.2 ± 0.3 d	2.2 ± 0.0 b	68.2 ± 3.1 bc	24.2 ± 0.4 bc	1.2 ± 0.0 a
Stuart	72.7 ± 6.3 d	6.3 ± 0.1 b	2.0 ± 0.0 bc	62.1 ± 3.1 e	28.5 ± 0.9 a	1.2 ± 0.0 a
Western	75.3 ± 5.2 c	5.3 ± 0.2 c	1.4 ± 0.0 d	72.8 ± 4.4 a	19.8 ± 0.6 e	0.8 ± 0.0 c
Wichita	77.8 ± 5.8 bc	6.0 ± 0.3 bc	2. ± 0.0 bc	69.1 ± 5.2 b	21.8 ± 0.6 d	1.0 ± 0.0 bc
Max	79.7	8.1	2.9	74.2	28.5	1.2
Min	70.1	3.7	1.4	62.1	17.2	0.7
Average	74.1	6.0	2.1	68.5	22.5	1.0
CV (%)	3.9	18.2	21.0	4.9	13.0	20.0

+C16:0, Palmitic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; C18:3, α -Linolenic acid; Max, The maximum value; Min, The minimum value; AV, Average value; CV, Coefficient of variation. Each value is a mean \pm SD of replicate analysis results. Different letters in the same column meant significantly different according to the Turkey's test at $p < 0.05$.

3.2.2. Tocopherol

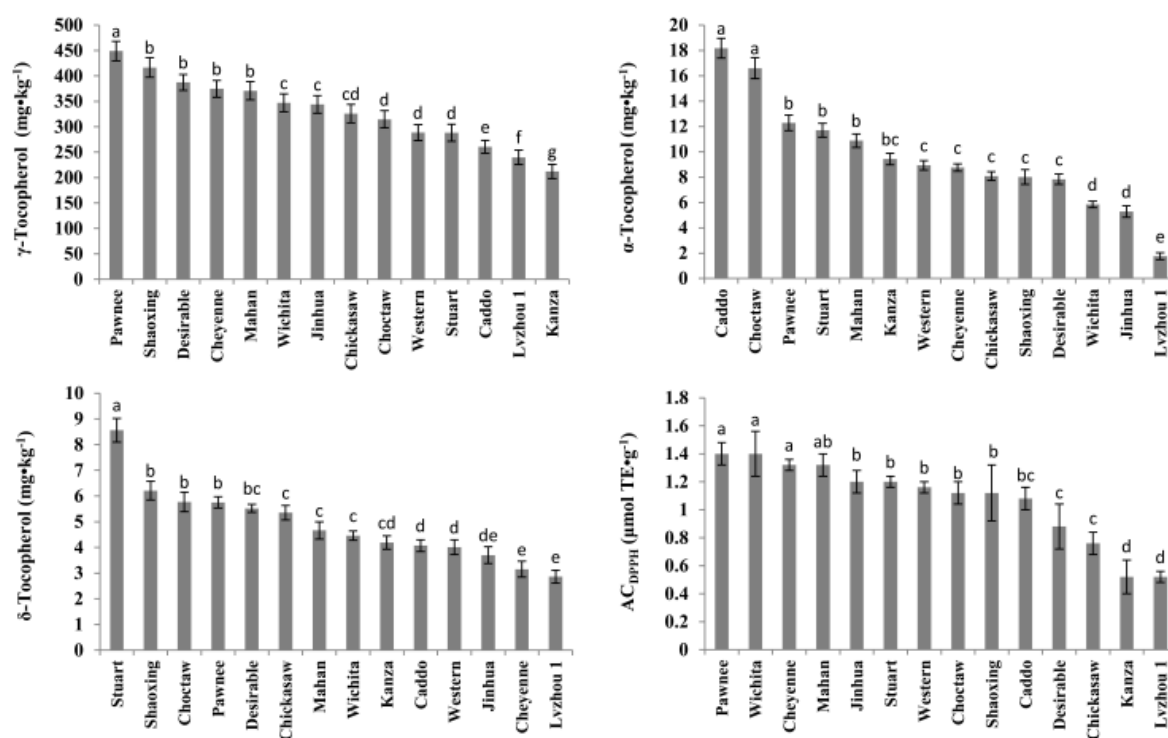


Figure 1. Comparison of γ -tocopherols, α -tocopherols, δ -tocopherols content and antioxidant capacities of pecan lipids. Data are mean \pm SD of replicate analysis results. Different letters meant significantly different according to the Turkey's test at $p < 0.05$.

The γ -tocopherol is the dominant tocopherol in pecan kernel, its content of all cultivars ranged from 211.99 to 448.92 $\text{mg}\cdot\text{kg}^{-1}$ (Figure 1). 'Pawnee' had the highest γ -tocopherol content, followed by 'Shaoxing' and 'Desirable'. The γ -tocopherol content in 'Kanza' was the lowest. An research on cultivar 'Desirable', 'Stuart', 'Schley' and seedlings showed that the γ -tocopherol contents were 201–293 $\text{mg}\cdot\text{kg}^{-1}$,

which is similar to our results [8]. Cultivars had significant effects on the content of γ -tocopherol. The contents of α - and δ -tocopherol were much lower than γ -tocopherol, but the effects of cultivars were also highly significant. The content of tocopherol in different cultivars can be very different, which indicated the possibility of get cultivars with high tocopherol content by selection.

3.2.3. Antioxidant Capacity of Lipid

The highest antioxidant capacity (AC) of pecan lipid was $1.40 \mu\text{mol TE}\cdot\text{g}^{-1}$ of 'Pawnee', while the lowest was $0.52 \mu\text{mol TE}\cdot\text{g}^{-1}$ of 'Lvzhou 1' (Figure 1). This result is comparable to previous research. Miraliakbari & Shahidi had analyzed the antioxidant capacities of several tree nuts and found out the AC_{ABTS} and AC_{DPPH} of pecan oil were 329 and $107 \mu\text{g TE}\cdot\text{g}^{-1}$ respectively, which equaled to 1.2 and $0.3 \mu\text{mol TE}\cdot\text{g}^{-1}$ [24]. The antioxidant capacity of lipid was significantly ($p < 0.05$) correlated with the γ -tocopherol content (data not showed). The antioxidant capacity of pecan lipid was much lower compared to the rest part of pecan kernel, which informed that there are more constituents with great antioxidant capacities existed in the rest part of pecan kernel.

3.2.4. Phenolics

The remaining part of pecan kernel after lipid extraction was used to test the TPC, CT and TFC, the results were listed in Table 3. What stands out is that the cultivar 'Mahan' had the highest values in all three traits. The TPC of all cultivars ranged between 11.37 and $21.41 \text{ mg EAE}\cdot\text{g}^{-1}$, the CT values were from 14.54 to $35.72 \text{ mg CE}\cdot\text{g}^{-1}$, while the TFC values were from 2.40 to $6.40 \text{ mg CE}\cdot\text{g}^{-1}$. Our results were comparable with previous reports [3, 7, 15, 21, 25-28]. The CT values were higher than that of TPC of all cultivars. Similar results can be found in previous reports [6, 12, 29], which may on account of the fact that there are highly

polymeric phenolics existed in pecan kernels [30]. Lowest TPC and CT values were found in 'Kanza', whereas, the lowest TFC was found in 'Lvzhou 1'. Two representative phenolic acids were chosen according to previous reports [3], the comparison of their contents were showed in Table 3. The ellagic acid contents varied from $0.46 \text{ mg}\cdot\text{g}^{-1}$ of 'Jinhua' to $1.72 \text{ mg}\cdot\text{g}^{-1}$ of 'Mahan'. The contents of gallic acid were much less than that of ellagic acid. Great variations can be observed in both phenolic acids among different cultivars, which indicated that cultivars have significant effects on the levels of phenolic acids.

3.2.5. Antioxidant Capacity of Defatted Kernels

The analyses of the antioxidant capacity were also carried on using the defatted kernel. Both DPPH and ABTS methods were used (Figure 2). The antioxidant capacities were 56.22 - $124.48 \mu\text{mol TE}\cdot\text{g}^{-1}$ of DPPH method and 47.21 - $134.13 \mu\text{mol TE}\cdot\text{g}^{-1}$ of ABTS method. 'Mahan' had the highest antioxidant capacity in both methods, while 'Jinhua' and 'Stuart' had relatively lower antioxidant capacities. Our results are comparable to previous reports. Villarreal-Lozoya reported an AC_{DPPH} of 81 - $135 \text{ mg TE per gram}$ defatted kernel among 6 pecan cultivars collected in USA [6], while de la Rosa reported an AC_{DPPH} of 102.6 - $108.7 \mu\text{mol TE per gram}$ fresh kernel in Mexico-grown pecans [21]. Our AC_{ABTS} values are also similar to the reports of de la Rosa [21].

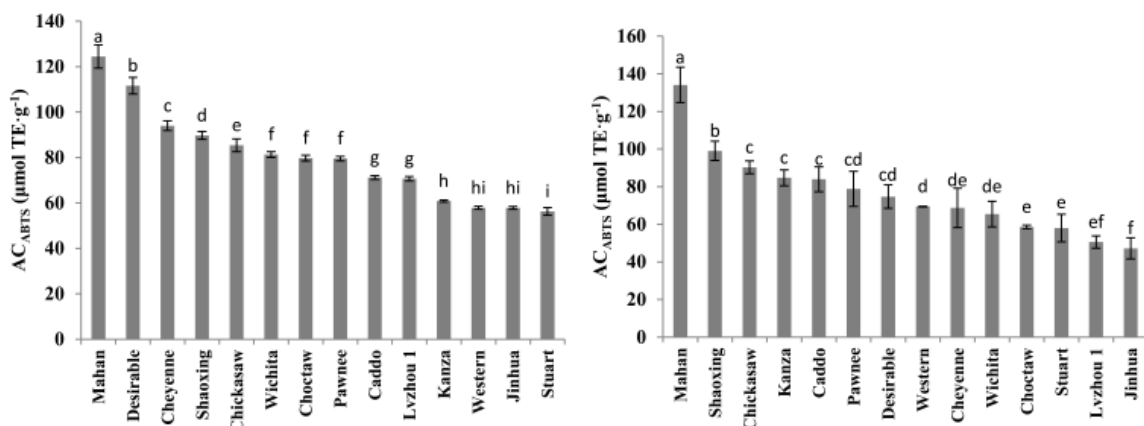


Figure 2. Comparison of antioxidant capacity of phenolics. Data are mean \pm SD of replicate analysis results. Different letters meant significantly different according to the Turkey's test at $p < 0.05$.

3.2.6. Heat-map Analysis and Hierarchical Clustering

Through heat-map analysis, we can get comprehensive views of nut-qualities of different cultivars which are convenient for choosing cultivars of certain purpose (Figure 3). Such as 'Mahan' which not only had high values of TPC, CT and TFC, but also had high values of antioxidant capacities can be a good material for researches related to antioxidant constituents. Hierarchical clustering divided pecan cultivars into three subclusters: the upper subcluster mostly contained pecan cultivars with small to medium nut size, while the lower subcluster mostly contained pecan cultivars with large nut size, and the third branch only contained 'Mahan' (Figure 3). We had accessed the genetic diversity of Chinese-grown pecan

using ISSR and SSR previously [4], while analyses of the nut quality traits were a good assistance of molecular markers. Furthermore, seedling cultivar 'Jinhua' was clustered with 'Desirable', while 'Shaoxing' was clustered with 'Chickasaw' and 'Stuart'. The evaluation of nut quality traits of these seedling cultivars is helpful to understand their genetic backgrounds.

The 24 nut-quality traits can be grouped into three subclusters by Pearson correlation analyses and hierarchical clustering (Figure 4). Phenolic traits were grouped together, such as TPC, CT, TFC, gallic acid content, ellagic acid content, AC_{DPPH} and AC_{ABTS} , together with nut weight, longitudinal diameter and nut index, which suggested that

these quality traits were mutually related. Many of the lipid traits were also clustered together, including lipid, C18:1, γ -tocopherol and AC_{DPPH} of oil. Furthermore, many of the

lipid traits with small quantity values were showed to be more related to each other, like the other four fatty acids and α -, δ -tocopherol.

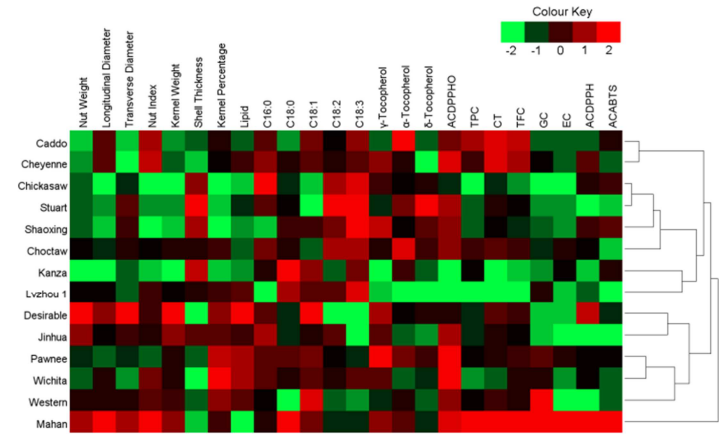


Figure 3. Hierarchical clustering of pecan cultivars and heat-map analysis of nut-quality traits. The units in the color scale are standard deviations. C16:0, Palmitic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; C18:3, α -Linolenic acid; ACDPPHO, AC_{DPPH} of pecan oil; TPC, Total phenolic content; CT, Condensed tannins; TFC, Total flavonoid content; GC, Gallic acid; EC, Ellagic acid; ACDPPH, AC_{DPPH} of defatted kernel; ACABTS, AC_{ABTS} of defatted kernel.

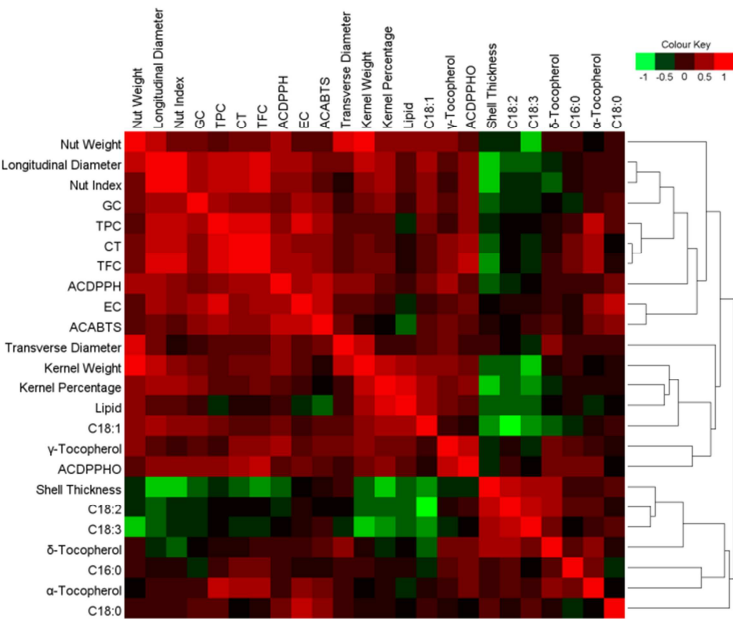


Figure 4. Hierarchical clustering and Pearson correlations among nut-quality traits. The units in the color scale are positive and negative correlations. C16:0, Palmitic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; C18:3, α -Linolenic acid; ACDPPHO, AC_{DPPH} of pecan oil; TPC, Total phenolic content; CT, Condensed tannins; TFC, Total flavonoid content; GC, Gallic acid; EC, Ellagic acid; ACDPPH, AC_{DPPH} of defatted kernel; ACABTS, AC_{ABTS} of defatted kernel.

4. Conclusion

This article provided detailed analyses of the nut qualities of 14 Chinese-grown pecan cultivars for the first time, which can be useful for future studies. For example, cultivars 'Mahan' and 'Desirable' which have big long nut seem to have good potential for fresh consumption. Cultivars 'Desirable', 'Western' or 'Kanza' are high in oleic acid, while cultivar 'Pawnee' is high in γ -tocopherol. As for cultivar 'Mahan', it had the highest content of TPC, CT, TFC, ellagic acid and antioxidant capacity of defatted kernel, which makes it a

good material for both high phenolic cultivar breeding and researches related to phenolics.

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