Oil chemical traits of kernels of different almond cultivars from China

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ABSTRACT

Traditional cultivars account for a large part of worldwide almond cultivars. However, the oil chemical traits of traditional almond cultivars in China remain unclear. Here, the oil chemical traits of four native and two traditional almond cultivars grown in China were determined. The content of unsaturated and monounsaturated fatty acids ranged from 93.02% to 93.29% and 75.24% to 77.98%, respectively. The tocopherol content varied between 25.15 and 18.37 mg/100 g. β-sitosterol content varied between 93.06% and 94.40%. VB_1, VB_2, VB_3, and VB_6 contents ranged from 0.11 to 0.23, 0.26 to 0.73, 370.00 to 1272.00, and 0.05 to 0.07 mg/100 g, respectively. The levels of squalene and phospholipids ranged from 131.20 to 274.80 mg/kg and 1.18 to 1.47 mg/g, respectively. These results indicated that the composition and content of fatty acids, tocopherols, sterols, VB_1, VB_2, VB_3, VB_6, squalene, and phospholipids in almond kernels were significantly different among the investigated cultivars.

Keywords: almond; cultivar; fatty acids; tocopherols; sterols.

Practical Application: This study will show the oil chemical traits of kernels from six main almond cultivars in China.

1 Introduction

Almonds are one of the most popular edible nuts worldwide. In the global dry fruit market, their annual output is the highest compared to those of hazelnut, walnut, and cashew. Almonds are cholesterol- and gluten-free. They are high in monounsaturated fats, antioxidants, and are a great energy source, which is beneficial for heart health and weight management (Cassady et al., 2009; Abaspour et al., 2012; Maestri et al., 2015; Čolić et al., 2017). They are consumed not only as snack foods, but also used as ingredients in a variety of processed foods and in the pharmaceutical and cosmetic industries (Bolling et al., 2010; Socias i Company et al., 2017).

In China, the almond growing region mainly includes Shache County, Kashgar Prefecture, Xinjiang Uygur Autonomous Region. The region accounts for 95% of the total Chinese almond output. Many native almond cultivars are grown here, such as 'Zhipi', 'Wanfeng', 'Xiaoruanke', 'Shuangruan', and 'Yingzui'. Traditional cultivars, such as 'Nonpareil' and 'Mission', were introduced from California in 1996, and they have been extensively popularised in the Shache region.

To date, the studies on almond cultivars in the Shache region have mainly focused on kernel qualities and amino acid composition. Even though the oil chemical traits of native cultivars have been reported, the chemical content of the oil of traditional cultivars is still unknown. Zhou et al. (2016) studied the characteristics of cold pressed oil extracted from eight almond cultivars in Xinjiang and found that the nutritional value, oxidation stability, and flavour quality of the oil of 'SC-9', 'SC-Zhipi', and 'SC-Taxi' cultivars were better than those of the other five cultivars and

that they were suitable for the cold-press process. Yin et al. (2015) determined the physical (shell thickness, kernel percentage, and kernel mass) and chemical qualities (crude fat content, crude protein content, and amygdalin content) of 38 almond cultivars in Xinjiang. They showed that the comprehensive qualities of cultivars, such as 'Zhipi', 'Butte', 'Thompson', 'Sonora', 'Nonpareil', 'Ye'erqiang', and 'Badanwang', were the best among the investigated cultivars. Li et al. (2004) used gas chromatography (GC) to test the contents and composition of fatty acids in 28 almond varieties from Xinjiang. They found that the unsaturated acid content of almond accounted for 92.3% of the total fatty acid content, and the share of oleic acid ranged from 63.72% to 75.89%.

Throughout the world, traditional cultivars are the main and most popular cultivars in the almond industry, but the oil chemical traits of traditional almond cultivars in China have not been investigated to date. Therefore, the objective of this study was to determine the oil chemical traits of kernels in typical and traditional native almond cultivars from the Shache region, including the contents of fatty acids, tocopherols, sterols, B vitamins (VB_1, VB_2, VB_3, and VB_6), squalene, and phospholipids, in order to provide the basis for the production and development of Chinese almond industry.

2 Materials and methods

2.1 Study material

Raw almonds (six cultivars: 'Zhipi', 'Shuangruan', 'Xiaoruanke', 'Wanfeng', 'Nonpareil', and 'Mission') were collected from the No. 2 state forestry farm of Shache County in 2018. The almond trees in

the orchard were 16 years old with a spacing of 4 m × 6 m. Shache County has a warm temperate continental arid climate and four distinct seasons. It is dry and rainless, with strong evaporation and abundant sunshine and heat. Sand wind and floating dust are present in spring and less snow falls during winter. The average annual temperature is 11.3 °C, average annual precipitation is 47.6 mm, and annual frost-free period is 220 days.

For each almond cultivar, samples of 3.0 kg were taken collected the entire canopy of five selected trees. The almonds were hand-harvested at full maturity and dried on the ground with sun exposure until the kernel moisture content reached 5%. The almonds were cracked and shelled manually, and the kernels were ground using a universal cutting mill.

2.2 Oil analysis

Oil extraction. The kernels were dried in an oven at 80 °C until their moisture content was under 10%, and they were then stored in desiccators until further analysis. Oil extraction was carried out according to Sorkheh et al. (2016). The ground kernels were extracted by n-hexane in a Soxhlet apparatus. The extracted oil bottled in flasks was dried under vacuum conditions in an oven at 80 °C for 30 min and cooled in a dryer.

Fatty acid composition. Fatty acid composition was analysed by the normalisation method according to Zhou et al. (2016). A total of 20.0 mg of oil, 2.5 mL of n-hexane, and 100.0 μL of 0.5 mol/L sodium methoxide were added to a 10 mL centrifuge tube. The solution was mixed for 5 min and then centrifuged at 5000 rpm for 10 min. The supernatant was used for analysing the fatty acid profile using an Agilent 7890A gas chromatograph (Agilent Corporation, Santa Clara, CA, USA). The GC conditions were as follows: the chromatographic column was the Agilent HP-INNOWAX (30 m × 0.32 mm × 0.25 m); nitrogen was the carrier gas; flow rate was 1.5 mL/min; inlet temperature was 260 °C; split ratio was 80:1. After keeping the solution at 210 °C for 9 min, the temperature was increased to 250 °C at a rate of 20 °C/min, and the solution was kept at 250 °C for 10 min. The injection volume was 1.0 μL. The area normalisation method was used to quantitatively determine the percentage of fatty acids.

Tocopherol composition. Tocopherol content was determined by reversed-phase high performance liquid chromatography (RP-HPLC) according to Al Juhaimi et al. (2018). After oscillating 5.0 g of ground kernels and 0.5 g of amylase in a 60 °C water bath without sunshine for 30 min, they were mixed with 1.0 g of ascorbic acid, 0.1 g of BHT, 30.0 mL of anhydrous ethanol, and 10.0 mL of 5.0 g/ml potassium hydroxide solution. The mixture was saponified in a water bath at 80 °C and oscillated for 30 min. A total of 50.0 mL of petroleum ether-ethyl ether mixture (1:1 v/v) was added into the extracted saponification liquid. After cleaning the ether layer with 100.0 mL of water, it was concentrated with 3.0 g of anhydrous sodium sulphate and 15.0 mL of petroleum ether in a Senco R-501 rotary evaporator (Shanghai Shensheng Biotech Co. Ltd., Shanghai, China). Then, the concentration was stabilised by methanol to 10.0 mL. The tocopherol profile was analysed using an Agilent 1100 liquid chromatograph (Agilent Corporation, Santa Clara, CA, USA). The HPLC conditions were as follows: the chromatographic column was a C18 Kromasil column (5 μm × 4.6 mm × 250 m);

column temperature was 20 °C; mobile phase was A (water) and B (methanol). The gradient elution conditions were as follows: 0–13 min, 96%–96% B; 13–20 min, 96%–100% B; 20–24 min, 100%–100% B; 24–24.5 min, 100%–96% B; 24.5–30 min, 100%–96% B. The flow rate was 0.8 mL/min, UV detection wavelength was 294 nm, and injection volume was 10 μL. The retention time was used for qualitative analysis, and the internal standard method was used for quantitative analysis.

Sterol composition. Sterol was extracted by GC according to the method described in Amaral et al. (2003). A total of 250.0 mg of extracted oil was saponified with 1.0 mL of 1.0 mg/mL acetone and 5.0 mL of 0.5 mol/L ethanolic potassium hydroxide solution for 15 min. After heating, 5 mL of ethanol was added to the mixture while the mixture was hot. An aluminium oxide column was used to isolate the unsaponifiable fraction by solid-phase extraction. The unsaponifiable fraction was extracted with 5.0 mL of ethanol and 30.0 mL of diethyl ether, and then dissolved in diethyl ether by thin-layer chromatography. Methanol was sprayed to visualise the band. After scraping off the silicone layer with a blade and adding 0.5 mL of ethanol, the solution was concentrated. After adding 100.0 μL of silyl reagents into the reaction flask, the flask was sealed and placed in an oven at 105 °C for 15 min. The sterol profile was analysed using an Agilent 7890A gas chromatograph (Agilent Corporation, Santa Clara, CA, USA). The GC conditions were as follows: chromatographic column was an Agilent HP-5 column (0.25 μm × 0.320 mm × 30 m); hydrogen was the carrier gas; flow rate was 36 cm/s; split ratio was 1:20; injector and detector temperatures were both 320 °C. The column temperature was increased from 240 °C to 255 °C at a rate of 4 °C/min. The injection volume was 1.0 μL. The retention time was used for qualitative analysis, and the internal standard method was used for quantitative analysis.

B vitamin contents. B vitamins (VB_1, VB_2, VB_3, and VB_6) were evaluated by HPLC according to Köksal et al. (2006).

VB_1 content. Ground kernels (0.5 g) were weighed in a 100 mL centrifuge tube. After adding 50.0 mL of 0.5 mol/L hydrochloric acid solution, the mixture was ultrasonically extracted in an 80 °C water bath for 30 min. Then, the solution was centrifuged at 4000 rpm for 10 min. The supernatant was used for VB_1 content analysis. VB_1 content was analysed using an Agilent 1100 liquid chromatograph (Agilent Corporation, Santa Clara, CA, USA). The HPLC conditions were as follows: the chromatographic column was a C18 Kromasil column (5 μm × 4.6 mm × 250 m); column temperature was 30 °C; mobile phase was A (acetonitrile) and B (0.1% trifluoroacetic acid solution). The gradient elution conditions were as follows: 0–0.7 min, 100%–100% B; 0.7–5 min, 100%–97% B; 5–6 min, 97%–85% B; 6–11 min, 85%–80% B; 11–11.5 min, 80%–10% B; 11.5–15.5 min, 10%–10% B; 15.5–16 min, 10%–100% B; 16–20 min, 100%–100% B. The flow rate was 1.3 mL/min, UV detection wavelength was 280 nm, and injection volume was 10 μL. The retention time was used for qualitative analysis, and the internal standard method was used for quantitative analysis.

VB_2 content. A total of 10.0 g of ground kernels was weighed in a 100 mL centrifuge tube. After adding 60.0 mL of 0.1 mol/L hydrochloric acid solution, the mixture was kept in a high-handed sterilisation pan at 121 °C for 30 min. After adjusting

the pH value to 6.0 by 1 mol/L sodium hydroxide solution, the mixture was added 2.0 mL of mixed enzyme solution, and it was enzymed in an incubator at 37 °C. The volume of the enzymed liquid was adjusted to 100.0 mL and filtered. The supernatant was used for VB_2 content analysis. VB_2 was analysed using an Agilent 1100 liquid chromatograph (Agilent Corporation, Santa Clara, CA, USA). The HPLC conditions were as follows: the chromatographic column was a C18 Kromasil column (5 μm × 4.6 mm × 250 m); column temperature was 30 °C; mobile phase was 0.05 mol/L sodium acetate solution 65% (v/v) containing methanol 35% (v/v); flow rate was 1 mL/min; excitation wavelength was 462 nm; emission wavelength was 522 nm; injection volume was 20 μL. The retention time was used for qualitative analysis, and the internal standard method was used for quantitative analysis.

VB_3 content. After mixing 5.0 g of ground kernels and 25.0 mL of 45–50 °C water, 20.0 g of samples and 0.50 g of amylase were added to a 150 mL conical flask. The mixture was charged with nitrogen, covered with a plug, and then cultured in a 50–60 °C incubator for 30 min. A total of 5.0 mol/L of hydrochloric acid solution and 0.1 mol/L of hydrochloric acid solution were used to adjust the pH value of the sample solution to 4.5. Then, the sample was placed in a bath ultrasonic oscillator at 50 °C water and oscillated for 10 min. The volume of the solution was adjusted to 100 mL, and the solution was then filtered. The supernatant was used for VB_3 content analysis. VB_3 was analysed with a Waters 2695 liquid chromatograph (Waters Corporation, Milford, CT, USA). The HPLC conditions were as follows: the chromatographic column was a C18 Kromasil column (5 μm × 4.6 mm × 250 m); column temperature was 25 °C; mobile phase was as follows: 70.0 mL of methanol, 20.0 mL of isopropanol, and 1.0 g of sodium 1-heptanesulfonate heptane sulphonate dissolved in 910.0 mL of water. Perchloric acid was used to adjust the pH value to 2.1, and the sample liquid was then filtered through a 0.45 μm membrane. The flow rate was 1.0 mL/min, UV detection wavelength was 261 nm, and injection volume was 10 μL. The retention time was used for qualitative analysis, and the internal standard method was used for quantitative analysis.

VB_6 content. A total of 5.0 g of grounded kernels, 25.0 mL of 45–50 °C water, and 0.5 g of amylase were added to a 150 mL conical flask. The mixture was charged with nitrogen, covered with a plug, and then cultured in a 50–60 °C incubator for about 30 min. A total of 5.0 mol/L of hydrochloric acid solution was used to adjust the pH value of the sample solution to 1.7. The sample was incubated for 1 min. A total of 5.0 mol/L sodium hydroxide solution was used to adjust the pH value of the sample solution to 4.5. After ultrasonic oscillation for 10 min, the volume of the solution was adjusted to 50.0 mL, and the solution was filtered. The supernatant was used for VB_6 content analysis. VB_6 was analysed using an Agilent 1100 liquid chromatograph (Agilent Corporation, Santa Clara, CA, USA). The HPLC conditions were as follows: the chromatographic column was a C18 Kromasil column (5 μm × 4.6 mm × 250 m); column temperature was 30 °C; mobile phase was as follows: 50.0 mL of methanol, 2.0 g of sodium 1-octanesulfonate, and 2.5 mL of triethylamine were dissolved and adjusted to 1000 mL by water. Glacial acetic acid was used to adjust the pH value to 3.0. Then, the sample liquid was filtered through a 0.45 μm membrane. The flow rate was 1.0 mL/min, excitation wavelength was 293 nm, emission wavelength was 395 nm, and injection volume was 10 μL. The retention time was used for qualitative analysis, and the internal standard method was used for quantitative analysis.

Squalene content. Squalene was detected using the GC method as described in Maguire et al. (2004). Extracted oil 0.2 g and 50.0 mL of 1 mol/L potassium hydroxide-ethanol solution were saponified (reflux in a water bath pot at 80 °C for 50 min), after which 50.0 mL of water was added. The saponification liquid was extracted with 100.0 mL of n-hexane by separating the funnel. The extraction liquid was cleaned with 25 mL of 10% ethanol solution until the pH value of the lower effluent was 7.0. Then, it was filtered through a filter paper laying 5.0 g of anhydrous sodium sulphate, concentrated with 20.0 mL of n-hexane by rotation in a water bath at 40 °C. When there was about 2.0 mL of n-hexane left in the bottle, the evaporation bottle was removed and immediately put into a pressure blowing concentrator to dry. Then the volume of the solution was adjusted to 10.0 mL by n-hexane. The squalene content was analysed using an Agilent 7890A gas chromatograph (Agilent Corporation, Santa Clara, CA, USA). The conditions of the GS were as follows: the chromatographic column was an Agilent HP-5 column (0.25 μm × 0.320 mm × 30 m); nitrogen was the carrier gas; inlet temperature was 250 °C, split ratio was 1:10. First, the temperature was raised from 160 °C to 220 °C at a rate of 15 °C/min, and the solution was kept at 220 °C for 2 min. Second, the temperature was increased to 280 °C at a rate of 5 °C/min, and the solution was kept at 280 °C for 2 min. Finally, the temperature was increased to 300 °C at a rate of 40 °C/min and the solution was kept at 300 °C for 2 min. The flame ionization detector (FID) temperature of the detector was 300 °C, its hydrogen flow rate was 40 mL/min, air flow rate was 450 mL/min, and tail gas flow rate was 30 mL/min. The injection volume was 1.0 μL. The percentage of fatty acids was quantitatively determined by the area normalisation method.

Phospholipid content. Phospholipid content was determined by the molybdenum blue method according to Jiang et al. (2015).

Extracted oil 3.0 g and 0.5 g of zinc oxide were gradually heated until all the mixture carbonised in the crucible. The crucible was burned in a muffle furnace at 550–600 °C for 2 h until the mixture was completely ashed. The ash was dissolved with 10.0 mL of 1.19 g/mL salt acid solution, heated to slightly boiling temperature for 5 min, and filtered using 15.0 mL of hot water. Then, it was neutralised by 50% potassium hydroxide solution until it gained a muddy appearance. The zinc oxide precipitate was dissolved in a hydrochloric acid solution (1:1 v/v), and the volume was adjusted to 100.0 mL using water. A total of 10.0 mL of the measured liquid was absorbed with pipette and injected into a 50 mL colorimetric tube. After mixing 8.0 mL of 0.015% hydrazine sulphate solution and 2.0 mL of 2.5% sodium molybdate solution, the colourimetric tube was heated in a boiling water bath for 10 min. The tube was diluted with water to scale, shaken well, and allowed to rest for 10 min. Then, the solution was moved to a dry and clean cuvette, and its absorbance was determined by dividing the photometer at 650 nm.

2.3 Statistical analysis

Each test index was repeated three times. The data were analysed using SPSS version 16.0 for Windows (SPSS Inc., USA). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the significance of the differences among samples at p < 0.05. The results are reported as mean ± SD of triplicate experiments. Principal component analysis (PCA) was used to evaluate the relationship between the almond cultivars and oil chemical traits.

3 Results and discussion

3.1 Fatty acid composition

Fatty acid composition of the investigated almond cultivars is summarised in Table 1. Oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and palmitic acid ($C_{16:0}$) were the three main constitutive fatty acids in all samples. The ranges for these three fatty acids were 74.60% to 77.30%, 15.20% to 17.90%, and 5.59% to 5.90%, respectively. These results were consistent with those of a previous study showing that all almond cultivars contained five fatty acids, including oleic ($C_{18:1}$), linoleic ($C_{18:2}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), and palmitoleic acid ($C_{16:1}$) (Sathe et al., 2008). In the present study, each cultivar had different types of fatty acids. 'Shuangruan' contained the highest number of fatty acids (11), and it was also the only cultivar that contained linolenic acid ($C_{18:2}$). 'Zhipi' contained the lowest number of fatty acids (5).

The composition of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) in the investigated almond cultivars is summarised in Figure 1. The SFA content ranged from 6.64% to 7.02%. The main contributing SFA in all cultivars were stearic ($C_{18:0}$) and palmitic acid ($C_{16:0}$). 'Shuangruan' and 'Xiaoruanke' had three other SFAs, including myristic ($C_{14:0}$), heptadecanoic ($C_{17:0}$), and arachidic acid ($C_{20:0}$). The lowest and highest levels of SAF were found in 'Xiaoruanke' and 'Shuangruan', respectively.

The UFA content ranged from 93.02% to 93.29%. Oleic ($C_{18:1}$) and linoleic acid ($C_{18:2}$) were the main constituents of the UFA. Compared with that of walnut and hazelnut, the content of UFA in almonds was found to be higher (Ostrowska-Ligeza et al., 2010). The UFA have a variety of physiological functions, such as regulating blood glucose and blood fat and reducing cholesterol content. The content of monounsaturated fatty acids (MUFAs)

accounted for 75.24% to 77.98% of total fatty acid content. In all cultivars, oleic acid ($C_{18:1}$) was the dominant MUFA, accounting for over 74.6% of total fatty acid content. 'Xiaoruanke' had the highest oleic acid ($C_{18:1}$) content, accounting for 77.3% of total fatty acid content. In all cultivars, the polyunsaturated fatty acid (PUFA) content ranged from 15.23% to 17.90%. Only 'Shuangruan' cultivar contained linolenic acid ($C_{18:2}$) which accounted for 0.03% of total fatty acid content. Linolenic acid ($C_{18:2}$) is beneficial for brain development, and it can be converted into docosahexaenoic acid (DHA) by various enzymatic reactions in the human body. The main PUFA present in all six almond cultivars was linoleic acid ($C_{18:2}$).

The unsaturated/saturated fatty acids ratio of the investigated almond cultivars ranged from 13.26% to 14.05%, and this ratio was the highest in 'Xiaoruanke' cultivar. Compared to those in walnuts, MUFA content in almonds was 3.52 times higher, whereas PUFA content in almonds was 0.22 times higher (Maguire et al., 2004). Almonds have longer shelf life than walnuts.

3.2 Tocopherol content

Tocopherol content of the investigated almond cultivars is shown in Table 2. Tocopherol can prevent the production of peroxides, premature cell ageing, and coronary heart disease (López-Ortiz et al., 2008; Zhu et al., 2015). It is the most important antioxidant in the human body. From highest to lowest, the tocopherol content in the six cultivars was 'Wanfeng', 'Shuangruan', 'Nonpareil', 'Xiaoruanke', 'Zhipi', and 'Mission', and this content ranged from 25.15 to 18.37 mg/100 g. Tocopherol content differed among cultivars. There were four types of tocopherols in the samples. α-tocopherol was the predominant form of tocopherol, ranging between 85.16% and 90.91%, followed by γ-tocopherol, β-tocopherol, and δ-tocopherol. Previous studies have reported that γ-tocopherol is a more potent antioxidant than other tocopherols (Wang et al., 2019). 'Wanfeng' had the highest levels of total tocopherol, α-tocopherol, β-tocopherol, and γ-tocopherol, whereas 'Mission' had the lowest. The tocopherol content in almonds was 7.50–10.27 times higher than that of walnuts, 5.22–7.14 times higher than that of pistachios, and 33.71–46.15 times higher than that of cashews (Al Juhaimi et al., 2018). Almond cultivars from the Shache region were rich in tocopherol.

Table 1. Fatty acid contents in kernels of six almond cultivars (%).

Fatty acids	'Zhipi'	'Shuangruan'	'Xiaoruanke'	'Wanfeng'	'Nonpareil'	'Mission'
$C_{14:0}$	-	0.03±0b	0.036±0a	-	-	-
$C_{16:0}$	5.67±0.24b	5.89±0.09a	5.59±0.04b	5.90±0.02a	5.76±0.08ab	5.72±0.06ab
$C_{16:1}$	0.41±0.01c	0.60±0.03a	0.49±0.01b	0.58±0.01a	0.48±0.02b	0.47±0b
$C_{17:0}$	-	0.05±0a	0.04±0a	-	-	-
$C_{17:1}$	-	0.12±0b	0.13±0b	0.11±0d	0.12±0c	0.13±0a
$C_{18:0}$	1.18±0.03a	0.10±0.01c	0.90±0.01d	0.89±0.01d	1.16±0.01a	1.13±0.01b
$C_{18:1}$	76.90±0.26b	77.00±0.11b	77.30±0.04a	75.50±0.04c	74.60±0.05d	76.90±0.05b
$C_{18:2}$	15.90±0.04c	15.20±0.06f	15.30±0.01e	17.10±0.02b	17.90±0.05a	15.70±0.01d
$C_{18:3}$	-	0.03±0	-	-	-	-
$C_{20:0}$	-	0.06±0a	0.06±0a	-	-	-
$C_{20:1}$	-	0.07±0a	0.06±0a	-	0.05±0b	-

Values are mean ± S.D. Different letters in each row indicate significant differences at the 0.05 confidence levelaccording to ANOVA by Duncan's test. -, not analysed.
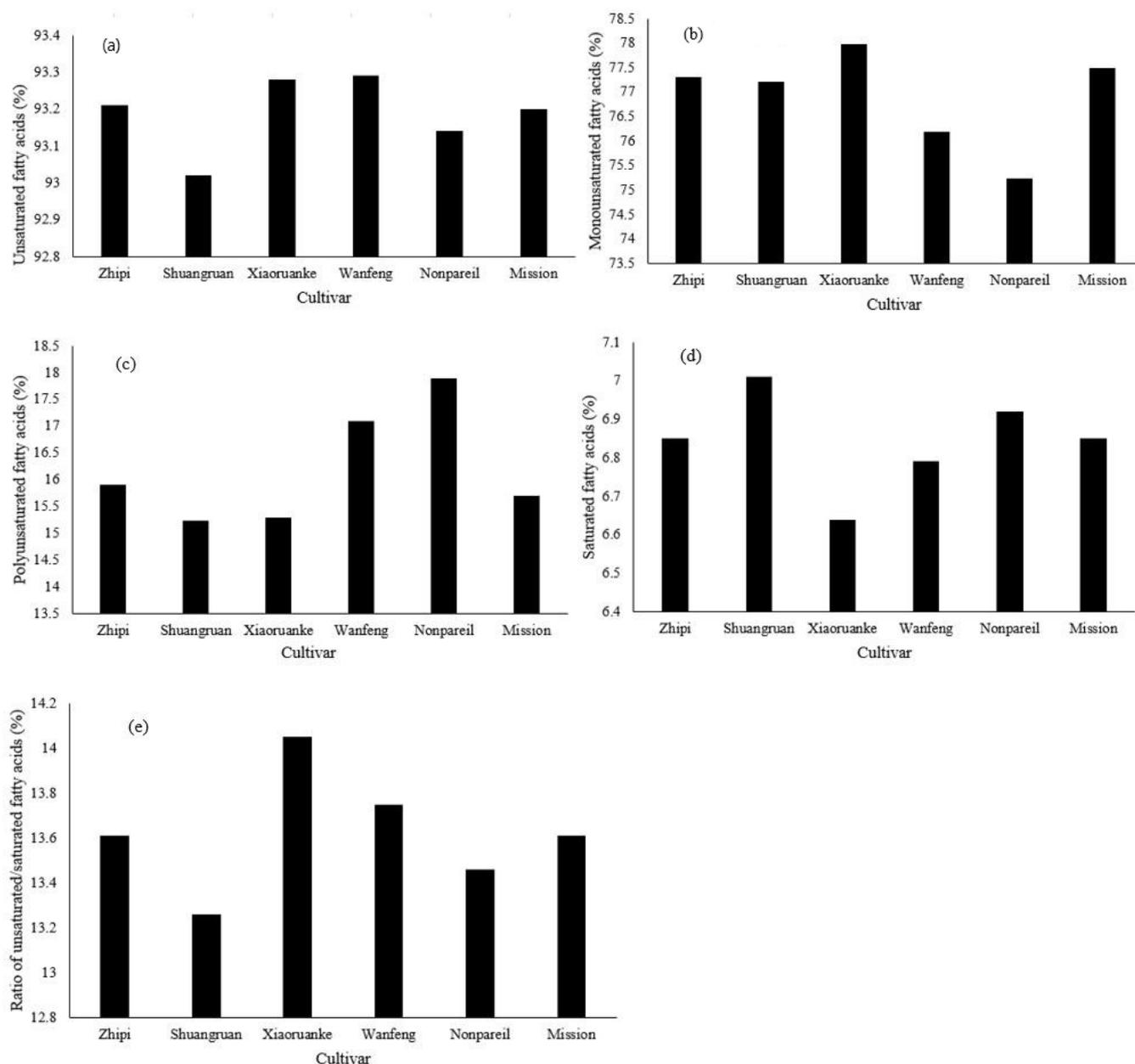
Figure 1. The contents of unsaturated fatty acids (a), monounsaturated fatty acids (b), polyunsaturated fatty acids (c), saturated fatty acids (d), and the ratio of unsaturated/saturated fatty acids (e) in kernels of six almond cultivars.

Table 2. Tocopherol and sterol contents in kernels of six almond cultivars (mg/100 g).

Compounds	'Zhipi'	'Shuangruan'	'Xiaoruanke'	'Wanfeng'	'Nonpareil'	'Mission'
α-tocopherol	19.40±0.16e	20.60±0.06c	20.30±0.07d	21.60±0.07a	21.00±0.14b	16.70±0.22f
β-tocopherol	0.39±0c	0.45±0b	0.37±0d	0.47±0a	0.27±04e	0.22±0f
γ-tocopherol	2.95±0.03b	2.87±0c	2.48±0d	3.00±0a	2.42±0.01e	1.38±0f
δ-tocopherol	0.04±0d	0.04±0d	0.10±0a	0.08±0b	0.06±0.01c	0.07±0.01b
Total tocopherols	22.78	23.96	23.25	25.15	23.75	18.37
Campesterol	8.76±0.03a	7.57±0.06d	7.38±0.02e	8.54±0.07b	7.77±0.06c	6.53±0.01f
Stigmasterol	4.45±0b	3.43±0.05d	3.88±0.02c	3.08±0.07e	5.20±0.03a	3.12±0.03e
β-sitosterol	188.00±0.25b	162.00±1.50e	178.00±1.50c	196.00±1.65a	174.00±1.00d	153.00±0.65f
Total sterols	201.21	173.00	189.26	207.62	186.97	162.65

Values are mean ± S.D. Different letters in each row indicate significant differences at the 0.05 confidence level according to ANOVA by Duncan's test.

3.3 Sterol content

Sterol content in the investigated almond cultivars is shown in Table 2. Sterol can significantly reduce the content of triacylglycerin and cholesterol in the plasma, decrease the content of plasma low-density lipoprotein, and inhibit the activity of cancer cells (Derewiaka & Obiedziński, 2012; Lehtonen et al., 2012; Vanmierlo et al., 2013). There were three types of sterols in the samples, and their percentage distributions varied among the cultivars. β-sitosterol is the dominant sterol, ranging between 93.06% and 94.40%. Campesterol and stigmasterol varied between 6.53–8.76 mg/100 g and 3.12–5.20 mg/100 g, respectively. From highest to lowest, total sterol contents in the six cultivars were: 'Wanfeng', 'Zhipi', 'Xiaoruanke', 'Nonpareil', 'Shuangruan', and 'Mission'. Sterol content in 'Wanfeng' was 1.28 times higher than that in 'Mission'. Sterol content in almonds was higher than that found in walnuts, macadamia nuts, and hazelnuts (Maguire et al., 2004).

3.4 Content of B vitamins

B vitamins are essential nutrients for the maintenance of life as they play an important role in metabolism (Hälvin et al., 2014; Hu et al., 2018). They cannot be synthesised in bodies and must be obtained from the outside.

VB_1, called thiamine, is a coenzyme of thiamine pyrophosphate (TPP) (Settembre et al., 2003; Fitzpatrick & Thore, 2014). In the present study, 'Xiaoruanke' had the highest VB_1 level of 0.23 mg/100 g, and 'Mission' had the lowest VB_1 level of 0.11 mg/100 g (Figure 2).

VB_2, called riboflavin, is present in the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in different organisms (Schwechheimer et al., 2016). It is a coenzyme of some oxidoreductases, taking part in many redox reactions. In the present study, the content of VB_2 among the cultivars ranged from 0.26 to 0.73 mg/100 g. The highest VB_2 content in the cultivar 'Mission' was 2.78 times higher than that in 'Wanfeng', which had the lowest VB_2 content.

VB_3, called nicotinic acid, is the precursor of nicotinamide adenine dinucleotide (NAD+, coenzyme I) and nicotinamide adenine dinucleotide phosphoric acid (NADP+, coenzyme II) in different organisms. It is involved in various enzymatic redox reactions. From highest to lowest, VB_3 content was as follows: Nonpareil'> 'Zhipi'> 'Shuangruan'> 'Xiaoruanke'> 'Mission'> 'Wanfeng'. The highest VB_3 content was observed in 'Nonpareil' (1272 mg/100 g), whereas the lowest was observed in 'Wanfeng' (639 mg/100 g).

VB_6, called pyridoxine, exists as phosphate in living organisms. It is the main coenzyme of various enzymes involved in amino acid and fatty acid metabolism (Clase et al., 2013; Fitzpatrick & Thore, 2014). The content of VB_6 in 'Xiaoruanke' was the highest among the six cultivars (0.07 mg/100 g), and it was 1.49 times higher than that of 'Zhipi', which had the lowest VB_6 content.
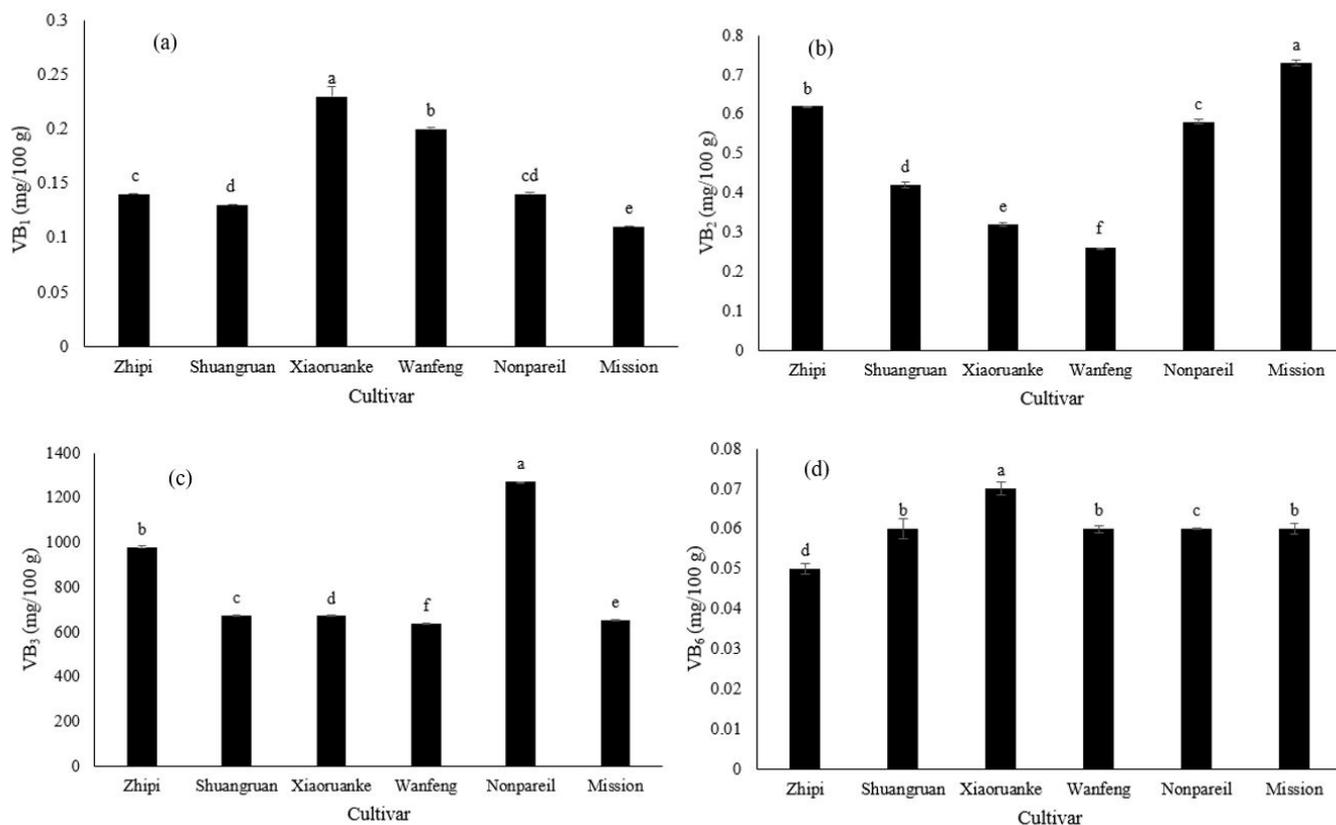
Figure 2. Vitamin B_1 (a), B_2 (b), B_3 (c), and B_6 (d) contents in kernels of six almond cultivars. Different lowercase letters above the columns indicate significant differences at the 0.05 confidence level according to ANOVA by Duncan's test.

3.5 Squalene and phospholipid contents

Nowadays, the bioactivity of squalene is a hot topic. Squalene can regulate cholesterol metabolism, oxidisation, and radiation. It has oxygen carrying capacity (He & Corke, 2003; Gunawan et al., 2008; Reddy & Couvreur, 2009; Lou-Bonafonte et al., 2012). In the present study, the levels of squalene differed between almond cultivars, ranging from 131.20 to 274.80 mg/kg (Figure 3). Squalene content was the highest in 'Shuangruan', and it was 2.09 times higher than that of 'Nonpareil', which had the lowest squalene content.

Phospholipids can promote fat metabolism, prevent fatty liver, reduce blood viscosity, and enhance haematopoietic function. They were detected in all cultivars, in levels ranging from 1.18 to 1.47 mg/g. Phospholipid content was the highest in 'Zhipi' and the lowest in 'Xiaoruanke'.

3.6 Principal component analysis

Figure 4 shows the loading plot on the plane defined by the first and second principal components as well as the corresponding projection of samples. The variance contribution rate of the
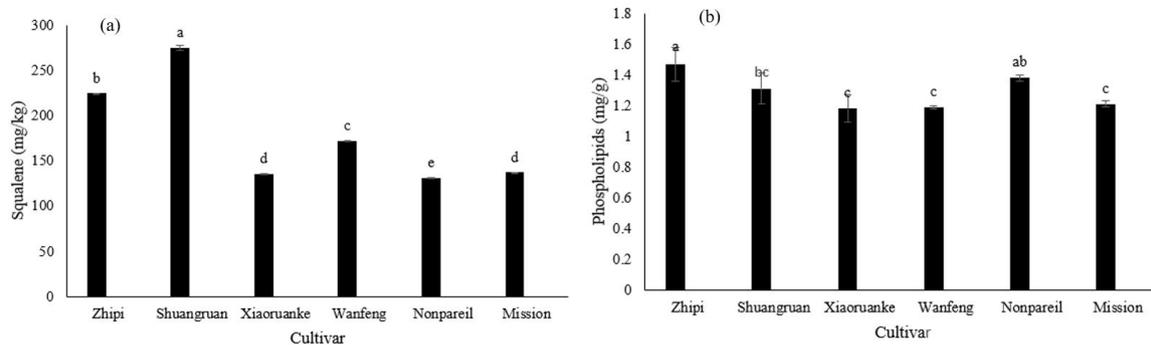
Figure 3. Squalene (a) and phospholipid (b) contents in kernels of six almond cultivars. Different lowercase letters above the columns indicate significant differences at the 0.05 confidence level according to ANOVA by Duncan's test.
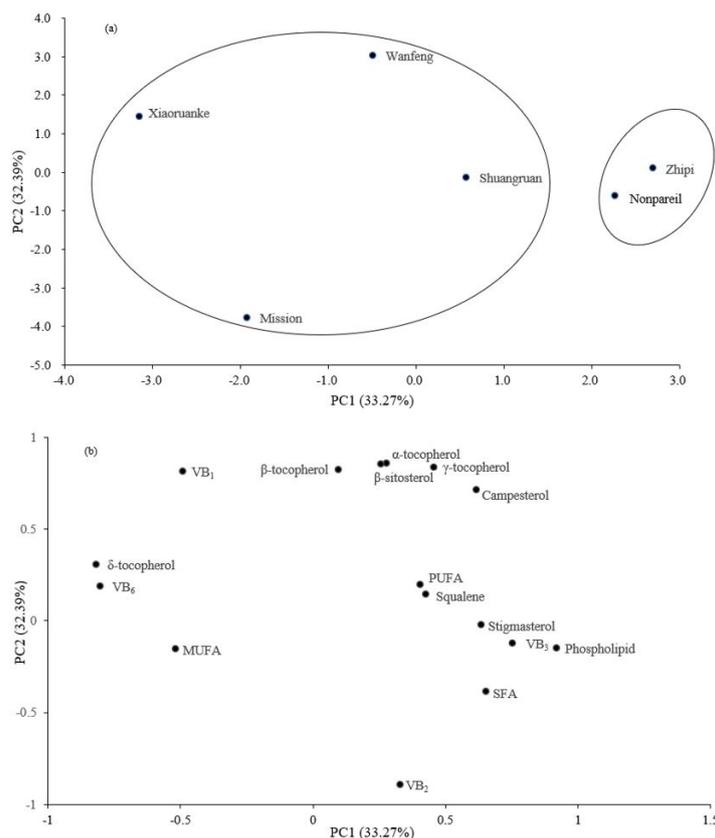
Figure 4. Representation of the six almond cultivars (a) and their measured oil chemical components, (b) defined by the first (PC1) and second principal component (PC2) obtained by Principal Component Analysis (PCA) ordination. "VB", "MUFA", "PUFA", and "SFA" represent "B vitamins", "monounsaturated fatty acids", "polyunsaturated fatty acids", and "saturated fatty acids", respectively.

first principal component (PC1) and the second principal component (PC2) was 33.27% and 32.39%, respectively. PC1 correlated highly with phospholipids, VB$_3$, SFA, stigmasterol, and campesterol. PC2 correlated highly with α-tocopherol, β-sitosterol, γ-tocopherol, β-tocopherol, VB$_1$, and campesterol. The six almond cultivars could be divided into two groups based on the relationships between scores and their oil chemical traits. The first group consisted of 'Xiaoruanke', 'Mission', 'Wanfeng', and 'Shuangruan', with a lower negative value of PC1. The second group was composed of 'Nonpariel' and 'Zhipi', with a higher positive value for PC1.

4 Conclusions

In the present study, we evaluated the oil chemical traits of kernels from six main almond cultivars in China, including four native and two traditional cultivars. The results indicated that there were some differences in the composition or content of fatty acids, tocopherols, sterols, B vitamins (B$_1$, B$_2$, B$_3$, B$_6$), squalene, and phospholipids among the six cultivars. UFAs were the most important contributors to fatty acids, containing significant amounts of MUFAs. Tocopherol levels were high, and α-tocopherol was the predominant form of tocopherol. β-sitosterol was the most abundant phytosterol. The six cultivars were rich in squalene and had a wide range of B vitamin and phospholipid variability.

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