

Effects of Geographic Region and Cultivar on Fatty Acid Profile and Thermal Stability of *Zanthoxylum bungeanum* Seed Oil

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Abstract: Fatty acid profile and thermal stability of 7 varieties *zanthoxylum bungeanum* (GZF, GDJ, CJJ, SHY, SMN, SJY, GTS) seed oils (ZBO) were studied. Fatty acid profile, thermal stability were determined using gas chromatography equipped with flame ionization detector (GC-FID) and thermogravimetry analysis (TGA), respectively. Chemical properties, total phenolics and antioxidant activities of ZBO were determined as well. Palmitoleic acid and oleic acid (OA) were the dominant fatty acids, the ratio of ω -6/ ω -3 polyunsaturated fatty acids (PUFA) of ZBO ranged from 0.66 ± 0.01 to 1.17 ± 0.01 , seven varieties ZBO showed a higher thermal stability, with the 50% mass loss temperature ranged from $397.35 \pm 4.02^\circ\text{C}$ to $412.50 \pm 2.35^\circ\text{C}$, GZF seed oil showed a balance fatty acid profile, the ratio of ω -6/ ω -3 PUFA was 0.90 ± 0.01 , GDJ seed oil showed a higher thermal stability, which the 50% mass loss temperature was $412.50 \pm 2.35^\circ\text{C}$. These results suggested that fatty acid profile and thermal stability of ZBO were affected by cultivars and geographic region, and it may serve as a functional dietary oil.

Key words: *Zanthoxylum bungeanum* seed oil, supercritical CO₂ extraction, fatty acid profile, thermal stability

1 Introduction

Zanthoxylum bungeanum (ZB) is also called Chinese pepper or Huajiao in Chinese, it is a perennial tree and belongs to the Rutaceae family¹. ZB is an economical crop which is widely cultivated from tropical to subtropical areas and consists of 250 species around the world, it can also be used as a spice in cuisine and herbal medicine^{2,3}. China is the largest producer of ZB in the world, and its yield over 450000 tons, which is the total output value of 18 billion US dollars in 2018⁴. ZB is composed of *Zanthoxylum bungeanum* pericarp (ZBP) and *Zanthoxylum bungeanum* seed (ZBS), and ZBP is usually used as a seasoning. ZBS contains about 22.39% oil, and it serves as an oil resource. ZBO is a dominant product of ZB further processing. The dominant fatty acids of ZBO were palmitoleic acid and α -linolenic acid (ALA)^{5,6}. ALA cannot be synthesized in mammals, is the precursor to synthesize eicosapentaenoic acid (EPA; C20:5 ω -3), docosapentaenoic acid (DPA; C22:5 ω -3) and docosahexaenoic acid (DHA; C22:6 ω -3) in the human body⁷. Previous study reported that regular intake of vegetable oils rich in ALA can reduce the risk of cardiovascular disease, cancer and inflammatory^{8,9}. Moreover, DHA has been named the Brain Gold, which can

improve infants' intellectual development, enhance memory and eyesight, EPA can affect atherosclerosis, hypertension and allergic reactions¹⁰. The proper ratio of palmitoleic acid in daily supplement plays a critical role in preventing various diseases, such as obesity, cardiovascular disease, diabetes and cancer¹¹. In addition, the parameter of ω -6/ ω -3 PUFA also affects the health benefit. Previous study reported high ratio of ω -6/ ω -3 PUFA may increase the risk of some diseases such as cancer, inflammatory, cardiovascular¹². On the contrary, the ratio was lower than 1:1 may provide a positive effect against tumor growth rates and final tumor volume¹³. Cultivar is the dominant factor to determine fatty acid profile, antioxidant capacities¹⁴ and thermal stability¹⁵ of vegetable oil, moreover, vegetable oil quality was affected by geographical location¹⁶ and climatic condition¹⁷.

Thermal stability is a crucial property for vegetable oil, and thermal processing is essential before intaking it. Thermal stability of oil is related to the profile of unsaturated fatty acids (UFA) and phytochemical components^{18,19}. In the present study, seven varieties of ZBS from the western province of P.R. China were selected, and ZBO were extracted using supercritical CO₂ equipment, physio-

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chemistry property, fatty acid profile, thermal stability, as well as total phenolics of these ZBO were determined. The nutritional value based on fatty acids, thermal stability and oxidative stability of ZBO from different geographic regions and cultivars were discussed.

2 Materials and Methods

2.1 Materials

Seven ZBS were supplied by Guizhou Xuande Biotechnology Co., Ltd. (Guiyang, Guizhou Province, China). ZB was harvested between July to September from a plantation located in Chongqing municipality, Guizhou, Sichuan and Gansu province. Pericarp and seed of ZB were separated through drying at 45°C, then the seed was collected by winnowing. The Guizhou region was represented by two ZBS samples: GZF (Zhenfeng County) and GDJ (Dejiang County). The Chongqing region was collected one ZBS sample: CJJ (Jiangjin District). The Sichuan region was represented by three ZBS samples: SHY (Hanyuan County), SMN (Mianning County) and SJY (Jinyang County). The Gansu region was represented by another one ZBS sample: GTS (TianShui County). Among them, GTS belongs to *Zanthoxylum bungeanum* Maxim (ZBM), and the GZF, GDJ, CJJ, SHY, SJY and SMN belong to *Zanthoxylum schinifolium* Sieb. Et Zucc (ZSZ). The crude oil was extracted from ZBS using supercritical CO₂ equipment (Shengyang Soufuer SFE Equipment Co., Ltd., China) at the processing conditions (16 MPa, 45°C, 180 min, 20 L/h of CO₂ flow rate). Then, the crude oil was refined with a sequence of processing, the degumming processing was performed by acidification and water scrubbing, then, free fatty acids were neutralized using sodium hydroxide solution, the deacidification oils were decolored using activated carbon and clay, at last, oils were deodorized using a lab-scale simulated deodorizer. ZBO were stored in a dark condition under 4°C until further analysis.

2.2 Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI company (Tokyo, Japan). Gallic acid, Folin-Ciocalteu's reagent and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other analytical grade chemicals were obtained from Chengdu Jinshan Chemical Reagent Co., Ltd. (Chengdu, Sichuan province, China).

2.3 Determination of chemical properties

Acid value (AV), peroxide value (POV) and saponification value (SV) were determined according to standard methods of American Oil Chemist's Society, AV was determined using potassium hydroxide solution titration, POV was de-

termined using sodium thiosulfate solution titration and SV was determined using hydrochloric acid solution titration²⁰⁾.

The conjugated dienes (K232) and conjugated trienes (K270) were measured according to Wali *et al.*²¹⁾ with a little modification, 0.25 g ZBO were diluted to 25 mL in hexane, the absorbances of solution were measured at 232 nm and 270 nm with UV spectrophotometer (Shanghai Yuanjie Instrument Co., Ltd., China).

2.4 Determination of color parameter

The ZBO color parameters (L*, a*, b*) were determined using a calibrated Chromameter (Hangzhou Optography Technology Co., Ltd., China), results were performed in triplicate for each of the ZBO.

2.5 Determination of total phenolics (TP)

Total phenolics of ZBO were determined using the Folin-Ciocalteu colorimetric method, as reported by previous study with minor modification^{9, 22)}. Briefly, 5 g ZBO was mixed with 7 mL methanol:water (80:20), mixtures were shaken (30 min) in an orbital shaker table (TS-100C, TENSUC, Shanghai, China) prior to centrifuge at 6791 g for 10 min at 4°C (CenLee16R, Hunan CenLee Scientific Instrument Co., Ltd., China), supernatants were collected, and mixtures were extracted for two times, mixtures were evaporated to dryness under vacuum at 45°C and reconstituted to 10 mL with distilled water, extracts were stored at -20°C until further analysis. ZBO phenolic extracts were presented in triplicate.

One hundred microliter phenolic extracts and gallic acid standard solution were mixed with 500 µL Folin-Ciocalteu reagent, 1.5 mL 2% Na₂CO₃ and 6 mL distilled water were added, mixtures were placed in a dark condition for 40 min, then absorbance of mixtures were determined at 760 nm using a UV spectrophotometer (Shanghai Yuanjie Instrument Co., Ltd., Shanghai, China). TP were calculated from the gallic acid (0-200 µg/mL) standard curve, and results were expressed as mg gallic acid equivalents (GAE)/100 g ZBO.

2.6 Determination of fatty acid profile

Fatty acid profile of ZBO were analyzed using GC-FID (7890A Infinity, Agilent Technologies, Santa Clara, CA, USA) according to the method reported by Yao *et al.*²³⁾ with minor modification. Briefly, 60 mg ZBO was mixed with 4 mL isooctane in a 10 mL centrifuge tube, and vortexed for 30 s, 200 µL 2 mol/L KOH-CH₃OH was added, the mixture was vortexed 30 s again, mixtures humidity was absorbed using 1 g Na₂SO₄, 1 µL fatty acid methyl esters supernatant was injected into GC, and it was separated using a DB-WAX column (30 m × 0.25 mm × 0.25 µm). GC program as followed, the initial temperature of the oven was 50°C for 1 min, then increasing by 25°C/min to 200°C for 8 min in this

temperature and finally increasing by 3°C/min to 230°C. The split ratio was 20:1. Fatty acids were quantified as relative percentages of the peak areas. Results were performed in triplicate for each of the ZBO.

2.7 Lipid quality indices

Calculated oxidizability value (Cox), hypocholesterolemic and hypercholesterolemic fatty acids (H/H), thrombogenicity index (TI) and atherogenic index (AI) of ZBO were calculated according to formula 1, formula 2, formula 3 and formula 4, respectively^{24, 25}.

$$\text{Cox} = \frac{[C18:1\omega9\% + 10.3 * C18:2\omega6\% + 21.6 * C18:3\omega3\%]}{100} \quad (1)$$

$$\text{H/H} = \frac{C18:1\omega9\% + C18:2\omega6\% + C18:3\omega3\%}{C14:0\% + C16:0\%} \quad (2)$$

$$\text{TI} = \frac{0.5(\Sigma\text{MUFA})\% + 3\Sigma\omega-3\% + 0.5\Sigma\omega-6\% + \left(\frac{\Sigma\omega-3}{\Sigma\omega-6}\right)\%}{1} \quad (3)$$

$$\text{AI} = \frac{[C12:0\% + (4 * C14:0\%) + C16:0\%]}{[\text{MUFA} + \text{PUFA}]\%} \quad (4)$$

Where: C12:0 is lauric acid, C14:0 is myristic acid, C16:0 is palmitic acid, C18:0 is stearic acid, C18:1 is OA, C18:2 is linoleic acid (LA), C18:3 is ALA, ΣMUFA is the sum of monounsaturated fatty acids (MUFA), Σω-3 is the sum of the ω-3 PUFA and Σω-6 is the sum of the ω-6 PUFA.

2.8 Free radical scavenging assay

Antioxidant activity of ZBO was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay²⁶. Briefly, 200 µL ZBO (Trolox) were mixed with 2 mL ethyl acetate, and 2 mL 0.1 mmol/L DPPH ethyl acetate solution was added to the mixture, then mixtures were placed in a dark condition for 2 h, absorbance was determined at 517 nm using UV spectrophotometer (L5S, Shanghai Yidian Analytical Instrument Co., Ltd., Shanghai, China). DPPH values were expressed as µg Trolox equivalent (TE)/mL ZBO. Results were performed in triplicate for each of the ZBO.

2.9 Thermogravimetric analysis

Thermal stability of ZBO were analyzed using thermogravimetric analyzer (TG209F1L, Brahm, Germany Naichi Instrument Manufacturing Co., Ltd., Germany)¹⁸. Briefly, 12 mg ZBO were stood on an alumina pan and heated from room temperature to 550°C under N₂ atmosphere at a flow rate of 50 mL/min. Results were performed in triplicate for each of the ZBO.

2.10 Statistical analysis

All data were presented as means ± standard deviations (SD), n=3. The significance of the data was analyzed by one-way analysis of variance (ANOVA) using SPSS 25.0 software (SPSS Inc. Chicago, IL, USA). The statistical difference was identified at a level of $p < 0.05$.

3 Results and Discussion

3.1 Physicochemical properties of ZBO

The results of different geographic regions and cultivars on physicochemical properties of ZBO, including AV, POV, SV, K232, K270 and color parameters were analyzed. Lipid oxidation is the main reason to reduce the nutritional value and shelf time by the formation of oxidation products, and some important physicochemical indexes of oils were used to detect the oxidation state during storage and process.

Chemical properties of ZBO were shown in Table 1, AV of ZBO ranged from 2.50 ± 0.16 mg KOH/g to 2.83 ± 0.11 mg KOH/g. AV of ZBO was lower than guava seed oil (0.4 g KOH/100 g) reported by Kapoor *et al.*²⁷. The AV indicated the degree of hydrolytic degradation of fatty acids, which represented the free acidity²⁸. In addition, POV, a value of hydroperoxides, which have adverse effects on the quality of the oil²⁸. POV of ZBO ranged from 4.63 ± 0.77 meq O₂/kg to 5.26 ± 0.28 meq O₂/kg. The lower POV of ZBO than that of *Helianthus annuus* (sunflower) seed oil (6.07 meq O₂/kg)²⁹, SV can reflect the average weight and chain length of fatty

Table 1 Physicochemical properties of ZBO.

Indicators	Varities	GZF	GDJ	CJJ	SHY	SMN	SIY	GTS
AV (mg KOH/g)		2.50 ± 0.16 ^b	2.83 ± 0.11 ^a	2.60 ± 0.13 ^{ab}	2.52 ± 0.12 ^b	2.50 ± 0.12 ^b	2.65 ± 0.22 ^{ab}	2.58 ± 0.25 ^{ab}
SV (mg KOH/g)		155.96 ± 2.14 ^{cd}	192.88 ± 8.20 ^a	180.17 ± 6.55 ^b	180.38 ± 10.57 ^b	190.78 ± 4.91 ^{ab}	167.20 ± 6.06 ^c	154.69 ± 4.58 ^d
POV (meq O ₂ /kg)		4.82 ± 0.29 ^a	5.06 ± 0.28 ^a	5.09 ± 0.73 ^a	4.63 ± 0.30 ^a	4.63 ± 0.77 ^a	5.26 ± 0.28 ^a	4.64 ± 0.30 ^a
L*		30.29 ± 1.24 ^d	33.20 ± 0.40 ^{cd}	30.91 ± 1.95 ^{cd}	33.70 ± 1.39 ^c	38.22 ± 0.15 ^b	36.92 ± 1.07 ^{bc}	43.88 ± 1.09 ^a
a*		8.16 ± 0.18 ^{ab}	8.33 ± 0.18 ^a	6.27 ± 0.94 ^{cd}	6.94 ± 0.15 ^{bc}	6.69 ± 0.49 ^{cd}	5.61 ± 0.34 ^d	2.83 ± 0.12 ^c
b*		11.35 ± 0.97 ^c	16.04 ± 0.15 ^b	10.31 ± 1.03 ^c	16.48 ± 0.81 ^b	20.44 ± 0.98 ^a	16.96 ± 0.25 ^b	22.54 ± 1.11 ^a
K270		0.17 ± 0.01 ^c	0.16 ± 0.02 ^d	0.16 ± 0.01 ^d	0.14 ± 0.02 ^d	0.25 ± 0.00 ^b	0.29 ± 0.01 ^a	0.23 ± 0.01 ^b
K232		0.24 ± 0.01 ^b	0.25 ± 0.00 ^b	0.25 ± 0.00 ^b	0.24 ± 0.01 ^b	0.25 ± 0.01 ^b	0.26 ± 0.01 ^a	0.25 ± 0.00 ^b
TP (mg GAE/100 g)		27.53 ± 1.26 ^b	28.05 ± 0.25 ^b	6.85 ± 0.14 ^d	24.00 ± 0.55 ^c	33.79 ± 1.02 ^a	3.64 ± 0.15 ^c	3.99 ± 0.19 ^c
DPPH (µg TE/mL)		205.84 ± 2.58 ^b	236.77 ± 0.70 ^a	120.66 ± 3.12 ^e	166.77 ± 1.25 ^c	135.19 ± 1.82 ^f	156.05 ± 1.02 ^c	161.21 ± 1.13 ^d

Data expressed as means ± standard deviation, values with different letters in the same row indicate significant differences ($p < 0.05$).

acids of oil, and it presents a negative effect on the carbon chain length of fatty acids¹⁵⁾, SV of ZBO ranged from 154.69 ± 4.58 mg KOH/g to 192.88 ± 8.20 mg KOH/g, GTS seed oil was lower than other ZBO, indicating that GTS seed oil contains a higher ratio of chain length of fatty acids than others. K232 and K270 values, which indirectly reflect the level of oxidation products, such as hydroperoxides, carbonyl compounds, and corresponding to the absorbance at 232 nm and 270 nm by spectrophotometer about primary and secondary products of oil oxidation²¹⁾. K270 and K232 values were presented in Table 1, K232 values ranged from 0.24 ± 0.01 to 0.26 ± 0.01 , SJY seed oil presented higher K232 value ($p < 0.05$) than other ZBO. K270 values ranged from 0.14 ± 0.02 to 0.29 ± 0.01 , SJY seed oil presented higher K270 value as well, these results suggested that SJY seed oil was prone to oxidation during the extracting process.

Color is a precious parameter to effect consumer's desire to purchase the product, calibrated Chromameter is becoming a common technique to analyze color parameters. L^* , a^* and b^* values, characterize the difference of color, which indicate the lightness, redness and yellowness, respectively, were shown in Table 1. As performed, there were significant difference in L^* , a^* and b^* values of GTS seed oil to compare with other ZBO ($p < 0.05$), and GTS seed oil presented better than other ZBO from the respect of color quality due to a higher value of L^* and b^* value. However, the a^* value of GTS seed oil presented to be lower than the rest of ZBO. These results might be caused by Maillard browning under the thermal treatment and the presence of pigments were extracted from the seed^{30, 31)}.

3.2 Total phenolics

Polyphenol is an important active ingredient, not only contributes to health function for human health, but also inhibits the oxidation and deterioration for oil³²⁾. TP of ZBO ranged from 3.64 ± 0.15 mg GAE/100 g to 33.79 ± 1.02 mg GAE/100 g, SMN seed oil presented the highest TP (33.79 ± 1.02 mg GAE/100 g), while SJY seed oil showed the lowest TP (3.64 ± 0.15 mg GAE/100 g), as shown in Table 1. The present results were lower than nectarine (*Prunus persica* var. *nucipersica*) kernel oil (334.5 mg GAE/100 g) reported by Sodeifian *et al.*²²⁾, this may be due to the difference of species.

3.3 Antioxidant activity based on DPPH assay

The radical scavenging capacity of oil deeply shows relation to endogenous active ingredients, which could reflect the oxidation stability to some extent. DPPH values of ZBO ranged from 120.66 ± 3.12 μ g TE/mL oil to 236.77 ± 0.70 μ g TE/mL oil, GDJ seed oil showed the highest radical scavenging capacity with the value of 236.77 ± 0.70 μ g TE/mL oil, as shown in Table 1. The range of DPPH values was inconsistent with TP, this agrees with the results reported by Sodeifian *et al.*²²⁾, who suggested that the antioxidant activity might not be completely related to the TP. This may be due to flavonoids and tocopherols endow radical scavenging capacity of ZBO⁶⁾. Many articles have been reported that the free radical scavenging capacity of oils had a positive correlation with the presence of bioactive compounds, such as tocopherols, polyphenols, phytosterols and so on^{33–35)}.

3.4 Fatty acid profile

Fatty acids were the dominant ingredients in oil, which can not only regulate blood lipid level, but also improve

Table 2 Fatty acid profile and nutritional quality parameters of ZBO.

Varieties		GZF	GDJ	CJJ	SHY	SMN	SJY	GTS
Fatty acid (%)	Palmitic (C16:0)	10.33 ± 0.02^c	10.27 ± 0.04^c	11.05 ± 0.01^d	11.54 ± 0.05^c	12.12 ± 0.01^b	11.06 ± 0.06^d	12.41 ± 0.11^a
	Palmitoleic (C16:1 ω 7)	56.78 ± 0.28^a	52.55 ± 0.13^b	42.91 ± 0.16^c	43.25 ± 0.18^c	37.77 ± 0.18^d	42.84 ± 0.34^c	1.56 ± 0.25^c
	Stearic (C18:0)	0.49 ± 0.01^f	0.70 ± 0.00^c	0.92 ± 0.02^d	1.00 ± 0.02^c	1.04 ± 0.01^b	0.92 ± 0.01^d	1.18 ± 0.01^a
	Oleic (C18:1 ω 9)	17.74 ± 0.10^d	18.34 ± 0.08^c	16.73 ± 0.02^c	17.61 ± 0.09^d	20.85 ± 0.07^b	16.76 ± 0.01^c	32.96 ± 0.11^a
	Linoleic (C18:2 ω 6)	6.94 ± 0.04^c	8.18 ± 0.03^d	11.29 ± 0.04^c	11.24 ± 0.07^c	13.01 ± 0.05^b	11.32 ± 0.17^c	27.80 ± 0.19^a
	Linolenic (C18:3 ω 3)	7.72 ± 0.12^c	9.96 ± 0.05^d	17.09 ± 0.08^b	15.23 ± 0.06^c	15.21 ± 0.14^c	17.09 ± 0.23^b	23.71 ± 0.30^a
	Gondoic (C20:1 ω 9)	ND	ND	ND	0.13 ± 0.22^b	ND	ND	0.37 ± 0.02^a
Quality parameters	SFA	10.82 ± 0.03^f	10.97 ± 0.04^c	11.97 ± 0.02^d	12.53 ± 0.07^c	13.16 ± 0.01^b	11.98 ± 0.06^d	13.59 ± 0.11^a
	MUFA	74.52 ± 0.18^a	70.89 ± 0.06^b	59.64 ± 0.14^d	60.99 ± 0.05^c	58.63 ± 0.11^c	59.61 ± 0.34^d	34.89 ± 0.38^f
	PUFA	14.66 ± 0.16^c	18.14 ± 0.08^d	28.38 ± 0.12^b	26.48 ± 0.03^c	28.21 ± 0.10^b	28.41 ± 0.40^b	51.51 ± 0.49^a
	ω -6/ ω -3PUFA	0.90 ± 0.01^b	0.82 ± 0.00^d	0.66 ± 0.00^f	0.74 ± 0.01^c	0.86 ± 0.01^c	0.66 ± 0.01^f	1.17 ± 0.01^a
	Cox	2.56 ± 0.03^f	3.18 ± 0.01^c	5.02 ± 0.02^b	4.62 ± 0.01^d	4.83 ± 0.03^c	5.02 ± 0.07^b	8.32 ± 0.08^a
	H/H	3.13 ± 0.02^c	3.55 ± 0.03^d	4.08 ± 0.01^b	3.82 ± 0.02^c	4.05 ± 0.02^b	4.08 ± 0.06^b	6.80 ± 0.09^a
	TI	0.17 ± 0.00^a	0.16 ± 0.01^b	0.14 ± 0.00^d	0.15 ± 0.00^c	0.16 ± 0.00^b	0.14 ± 0.01^d	0.13 ± 0.00^c
	AI	0.116 ± 0.000^c	0.115 ± 0.001^c	0.126 ± 0.001^d	0.132 ± 0.001^c	0.140 ± 0.001^b	0.126 ± 0.001^d	0.144 ± 0.001^a

Data expressed as means $\pm$ standard deviation, values with different letters in the same row indicate significant differences ($p < 0.05$). ND, not detected.

insulin sensitivity and glucose tolerance, anti-cancer and antioxidant activity³⁶). The relative content of fatty acids of ZBO were shown in **Table 2**. The main fatty acids of ZBO were palmitic acid (C16:0), palmitoleic acid (C16:1), OA (C18:1), LA (C18:2) and ALA (C18:3). GTS seed oil contained the highest level of OA (32.96%), followed by LA, ALA, palmitic acid, palmitoleic acid, but palmitoleic acid (C16:1) was the dominant fatty acids in other ZBO, which ranged from $37.77 \pm 0.18\%$ to $56.78 \pm 0.28\%$, moreover, ZBO presented a higher level of UFA than saturated fatty acid (SFA). The content of palmitoleic acid was significantly higher ($p < 0.05$) in ZBO extracted from Guizhou province than that of extracting from Sichuan province and Chongqing municipality, the difference in the content of fatty acid might be due to altitude variations.

The seed oil of ZBM and ZSZ exhibited a quite differences in UFA, especially, OA, LA, ALA and palmitic acid in the seed oil of ZBM were higher than that of ZSZ, but palmitoleic acid was the lowest levels in the seed oil of ZBM. These results were consistent with calendula seed oil reported by Krol *et al.*¹⁷) that the difference of fatty acids might be due to genetic background, climatic condition and harvest date.

Among these fatty acids, especially the profile of LA and ALA, which are essential fatty acids in our diet for prevention or improvement of some certain diseases, ALA and LA are ω -3 and ω -6 PUFA, a precursor to synthesize EPA and DHA through metabolism with the reaction of a series of the enzymes, it plays an important role for the human body to maintain the level of EPA and DHA^{37–39}). ZBO also contained abundant MUFA, and it presented a positive effect on people's benefit, such as improve postprandial blood glucose, lipid level and diabetes⁴⁰). Especially palmitoleic acid, an uncommon ω -7 MUFA, it was the predominant fatty acid in ZSZ, which have shown the capacity to reduce the risk of various diseases, including obesity, cardiovascular diseases and cancer^{11, 41}). Additionally, palmitoleic acid has a significant advantage in suppressing the expression of proinflammatory genes⁴²). Meanwhile, the presence of OA in oils provides health-promoting effects that include improvement of blood pressure, cardiovascular diseases, cancer and obesity⁴³). In addition, the fatty acid composition also affects lipid oxidation and shelf time. GDJ and GZF seed oil contained a lower level of SFA than other ZBO. Nehdi *et al.*¹⁵) have reported that the presence of high amounts of SFA endows oils a higher oxidation stability and prolong the shelf time. But the dietary supplement of oil with a high ratio of palmitic acid will increase the risk of atherosclerosis, coronary heart disease and tumors⁴⁴).

3.5 Lipid quality indices

Fatty acids profile is a dominant factor to affect the health benefit of lipid, previous studies reported that regular consumption of a lower ratio of ω -6/ ω -3 PUFA can

decrease the risk of certain disease, including breast cancer, depression and obesity^{45–47}), moreover, regular consumption of PUFA that the ratio of ω -6/ ω -3 closes to 1:1 associated with the reduced risk of chronic diseases⁴⁸). In the present study, ω -6/ ω -3 PUFA of ZBO ranged from 0.66 ± 0.01 to 1.17 ± 0.01 , and the proportion of GZF seed oil was close to 1. The ω -6/ ω -3 PUFA of ZBO were lower than soybean oil (9.40), rice bran oil (73.90), canola oil (3.45)⁴⁹). These results suggested ZBO may provide a superior health benefit to human than soybean oil, rice bran oil and canola oil.

AI, TI and H/H were identified by the ratio of SFA and UFA, respectively. AI and TI predict the potential health benefit of oil to improve atherosclerosis and platelet aggregation, H/H is related to cholesterol metabolism, and a higher ratio of H/H is beneficial to human health^{12, 25}). AI, TI and H/H were also adopted to evaluate the health benefit of ZBO, TI, AI and H/H of ZBO shown in **Table 2**, TI and AI ranged from 0.13 ± 0.00 to 0.17 ± 0.00 and 0.115 ± 0.001 to 0.144 ± 0.001 , which was lower than those of pulp oil and nut oil⁵⁰). Additionally, H/H ranged from 3.13 ± 0.02 to 6.80 ± 0.09 and GTS seed oil was higher than that of other ZBO ($p < 0.05$). According to Bezerra *et al.*⁵¹) that TI and AI were lower than 1 and exhibited the potential for the treatment of coronary diseases. On the contrary, the value of H/H for oil plays a positive relationship in metabolizing cholesterol and the formation of high-density lipoproteins. Thus, all of these results indicate that ZBO can serve as healthy supplement oil. Cox was only identified by the presence of UFA in oil, which can predict the oxidizability based on the fatty acid composition, and plays a negative relationship with the oxidizability. ZBO ranged from 2.56 ± 0.03 to 8.32 ± 0.08 , and GTS seed oil presented higher Cox than other ZBO, indicating GTS seed oil was prone to oxidate than that of the rest of ZBO. Similar result has been reported by Zhang *et al.*²⁴) which may be due to higher ratio of PUFA presence of oil.

3.6 Thermogravimetric analysis

Thermogravimetric analysis was usually adopted to evaluate the physicochemical properties related to the thermal stability of oil⁵²). The thermogravimetry curve was formatted from the kinetics of mass loss against temperature, initial decomposition temperature, 20% mass loss temperature, 50% mass loss temperature and final decomposition temperature were observed, moreover, the maximum rate of mass loss and its temperature were observed from the DTG curve, which presented the 1st derivative of mass loss curve, as shown in **Fig. 1** and **Table 3**. The initial decomposition temperature of ZBO ranged from $61.11 \pm 8.86^\circ\text{C}$ to $274.80 \pm 6.50^\circ\text{C}$, the initial decomposition temperature of seed oil of CJJ, SJY, SMN and GTS were $61.74 \pm 3.44^\circ\text{C}$, $61.11 \pm 8.86^\circ\text{C}$, $66.79 \pm 7.52^\circ\text{C}$, $78.35 \pm 4.59^\circ\text{C}$, respectively, this may be due to water desorption and other impurities

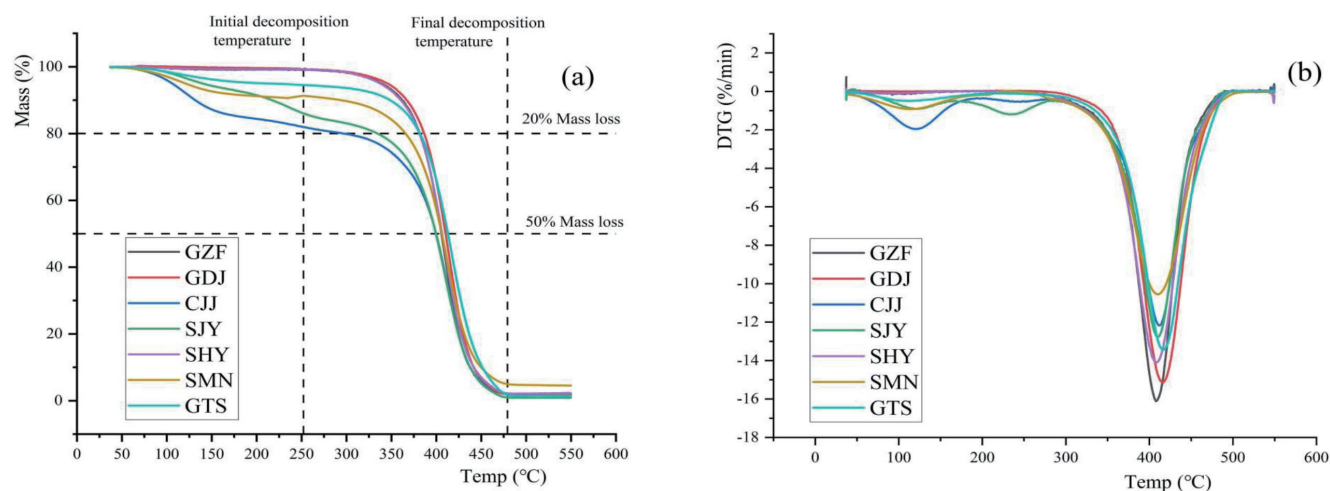


Fig. 1 Thermogravimetric curves of ZBO. (a) Thermogravimetric curve; (b) Differential thermal analysis curve.

Table 3 Mass loss temperature and maximum mass loss of ZBO.

Parameters	GZF	GDJ	CJJ	SHY	SJY	SMN	GTS
IDT * (°C)	252.77 ± 1.99 ^b	263.03 ± 9.76 ^b	61.74 ± 3.44 ^d	274.80 ± 6.50 ^a	61.11 ± 8.86 ^d	66.79 ± 7.52 ^{cd}	78.35 ± 4.59 ^c
MLT (20%, °C)	382.40 ± 0.73 ^a	384.35 ± 5.92 ^a	301.60 ± 1.86 ^d	381.10 ± 0.77 ^a	364.73 ± 4.04 ^b	335.32 ± 5.60 ^c	377.17 ± 9.29 ^a
MLT (50%, °C)	404.64 ± 2.56 ^{bc}	412.50 ± 2.35 ^a	397.35 ± 4.02 ^c	403.93 ± 5.92 ^{bc}	401.75 ± 3.25 ^{bc}	406.45 ± 4.19 ^{ab}	408.64 ± 4.37 ^{ab}
MMLT (°C)	407.73 ± 0.21 ^c	416.07 ± 2.19 ^a	409.27 ± 2.39 ^c	408.97 ± 0.55 ^c	408.83 ± 1.69 ^c	409.63 ± 0.35 ^c	413.40 ± 3.08 ^b
FMT (°C)	480.36 ± 1.68 ^b	479.59 ± 1.31 ^b	477.00 ± 2.19 ^b	478.68 ± 1.31 ^b	483.12 ± 4.01 ^{ab}	481.64 ± 2.33 ^{ab}	489.15 ± 10.45 ^a
MRML (%)	54.84 ± 2.38 ^b	57.14 ± 1.96 ^b	63.67 ± 2.35 ^a	54.38 ± 0.20 ^b	56.28 ± 3.11 ^b	62.05 ± 0.96 ^a	55.10 ± 3.20 ^b

Data expressed as means ± standard deviation, values with different letters in the same row indicate significant differences ($p < 0.05$). * IDT, initial decomposition temperature; MLT, mass loss temperature; MMLT, maximum mass loss temperature; FMT, final mass loss temperature; MRML, maximum rate of mass loss.

presented in the oil⁵³). The 20% mass loss temperature of ZBO ranged from $301.60 \pm 1.86^\circ\text{C}$ to $384.35 \pm 5.92^\circ\text{C}$, the 50% mass loss temperature of ZBO ranged from $397.35 \pm 4.02^\circ\text{C}$ to $412.50 \pm 2.35^\circ\text{C}$, the maximum mass loss temperature between $407.73 \pm 0.21^\circ\text{C}$ and $416.07 \pm 2.19^\circ\text{C}$, and the corresponding mass loss ranged from $54.38 \pm 0.20\%$ to $63.67 \pm 2.35\%$ of total content. The final decomposition temperature of ZBO ranged from $477.00 \pm 2.19^\circ\text{C}$ to $489.15 \pm 10.45^\circ\text{C}$. GDJ seed oil presented the highest 20% and 50% mass loss temperature, and the maximum mass loss temperature of GDJ seed oil was the highest as well, the corresponding mass loss was $57.14 \pm 1.96\%$ of total content. Contrarily, CJJ seed oil presented the lowest 20% and 50% mass loss temperature, other ZBO presented medium 20% and 50% mass loss temperature. It may be suggested that GDJ seed oil showed a higher thermal stability, with respect to 20% mass loss temperature of CJJ seed oil was $301.60 \pm 1.86^\circ\text{C}$, it may be suggested CJJ seed oil contained more protein components and organic matter, Bhutada *et al.*⁵⁴) reported that protein components and organic matter were decomposed from 130°C to 300°C . Twenty percent mass loss temperature of other ZBO between $335.32 \pm 5.60^\circ\text{C}$ and $384.35 \pm 5.92^\circ\text{C}$, it may be in-

dicated the decomposition of OA was occurred in this temperature range, Senrayan *et al.*⁵⁵) reported that OA was decomposed at the temperature from 300°C to 400°C , Ribeiro *et al.*⁵⁶) reported that SFA and MUFA were decomposed at the temperature from 380°C to 450°C , it may be indicated that 50% mass loss of ZBO caused by the decomposition of SFA and MUFA. Chemical composition results showed GDJ seed oil contained the highest TP, and presented the strongest scavenging activity to free radical, these properties may contribute to the thermal stability of GDJ seed oil.

4 Conclusions

In the present study, ZBO presented abundant UFA, palmitoleic acid was the dominant fatty acid in ZSZ, and ZBM presented a higher ratio of OA, LA and ALA. The ratio of ω -6/ ω -3 PUFA of GZF seed oil was 0.90 ± 0.01 , which may possess potential health benefits to human, and the thermal stability of GDJ seed oil was excellent. These results suggested that fatty acid profile and thermal stability of ZBO were affected by cultivars and geographic region.

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Author Contributions

Jingqi Ran: Writing, original draft preparation, data curation, software, validation, visualization. Likang Qin: Reviewing, editing, funding acquisition, methodology and supervision. Yong Zhu: Conceptualization, editing, investigation and writing-review. Tingyuan Ren: Reviewing, resources and editing.

Conflict of Interest

The authors have declared no conflict of interest.

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