

Comparison of fatty acid composition, phytochemical profile and antioxidant activity in four flax (*Linum usitatissimum* L.) varieties



Caisheng Qiu^{a,1}, Hong Wang^{b,1}, Yuan Guo^a, Songhua Long^a, Yufu Wang^a,
Arshad Mehmood Abbasi^c, Xinbo Guo^b, Devra I. Jarvis^{d,e,*}

^a Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, 410205, China

^b School of Food Science and Engineering, South China University of Technology, Guangzhou, 510640, China

^c Department of Environment Sciences, COMSATS University Islamabad, Abbottabad Campus, 22060, Pakistan

^d Platform for Agrobiodiversity Research C/o Alliance of Bioversity International and CIAT, Via Dei Tre Denari 472/a, 00057, Maccarese, Rome, Italy

^e Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA

ARTICLE INFO

Keywords:

Flaxseed
Phytochemicals
Lignan
Fatty acid
Antioxidant activity

ABSTRACT

The present study evaluates variations among flaxseed varieties in terms of fatty acid composition, phytochemical profile, and antioxidant activity determined by oxygen radical absorbance capacity (ORAC), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferrous ion reducing antioxidant power (FRAP) assays. Significant variations in fatty acid composition, phenolic acids and lignan were observed in flaxseed varieties from different countries. Among them, unsaturated fatty acids accounted over 4/5 of total fatty acid content. The highest ratio of linolenic acid of total fatty acid was observed in USPEA, whereas the lowest one was found in Yexiao. USPEA showed the highest content of total phenolics, as well as flaxseed lignan. In general, total phenolics appeared to be the main contributors of antioxidant capacity of flaxseed, which presented significant positive correlation. Our study revealed that both cultivar and origin of seed significantly affected fatty acid composition, phenolic acids, lignans and subsequent antioxidant activity of flaxseed. These results provided new aspects of breeding resources of flaxseed cultivars by presenting their quality specification and possible commercial value.

1. Introduction

Flax (*Linum usitatissimum* L.) originated from Mesopotamia and has been cultivated since 5,000 B.C. (Carraro et al., 2012). The original purpose of flax was for fabrication of cloths and paper, later it was also used for oil, and more recently it has been increasingly used for medicinal purposes and nutritional products (Singh et al., 2011). Flaxseed oil is one of the richest plant sources of unsaturated fatty acids, especially alpha-linolenic acid (C18:3, ALA) (Bhatty, 1995). In addition to ALA, flaxseed provides substantial amounts of phenolic compounds including lignans and phenolic acids (Eliasson et al., 2003). The beneficial effect of flaxseed and its bioactive components have been extensively investigated over the past years, including reducing risk of cardiovascular diseases, hormone-dependent cancers, and osteoporosis (Ezzat et al., 2018; Parikh et al., 2018; Toure and Xu, 2010).

Flaxseed is one of the most widely cultivated crops in the world and its area of production continues to increase. In 2018, world production of flaxseed was 3.18 million tones, with the highest production in Kazakhstan (29% of the global total), followed by Canada, Russia, and China (FAOSTAT, 2020). Fiber flax and oil flax are two main types cultivated worldwide. Studies have showed that cultivar and environmental conditions of planting area were important factors that influenced biochemical compositions of flaxseed, including fatty acids, mineral elements and phenolic compounds, affected different nutritional values of seed (Wang et al., 2017; Xing et al., 2014; Zhang et al., 2016). Zhang documented the strong influence of interaction of genotypes and ecological environments on fatty acid composition in flaxseed from China (Zhang et al., 2016). Genome-wide association analysis (GWAS) of 224 flaxseed fatty acid content suggested that 16 SNP loci were significantly associated with seed fatty acid oil content (Xie et al., 2019). Moreover,

* Corresponding author. Platform for Agrobiodiversity Research c/o Alliance of Bioversity International and CIAT, Via dei Tre Denari 472/a, 00057, Maccarese, Rome, Italy.

E-mail addresses: qcaisheng@163.com (C. Qiu), whongaq@163.com (H. Wang), guoyuan@caas.cn (Y. Guo), longsonghua@caas.cn (S. Long), wangyufu@caas.cn (Y. Wang), arshad799@yahoo.com (A.M. Abbasi), guoxinbo@scut.edu.cn (X. Guo), d.jarvis@cgiar.org (D.I. Jarvis).

¹ C.Q. and H.W. equally contributed to this work.

<https://doi.org/10.1016/j.ocsci.2020.08.001>

Received 2 June 2020; Received in revised form 26 July 2020; Accepted 4 August 2020

Available online 11 August 2020

2096-2428/© 2020 The Authors. Published by Elsevier B.V. on behalf of Oil Crops Research Institute, Chinese Academy of Agriculture Sciences. This is an open access

article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

differential transcriptional activity of desaturase genes including *SAD1*, *FAD2* and *FAD3* could contribute to various linolenic acid accumulation (Rajwade et al., 2014). A similar effect of cultivar genotype and environment interaction on oil characteristics was also observed in flaxseed grown in Pakistan (Yaqoob et al., 2016). Our previous studies indicated that fiber flaxseeds could also provide considerable phytochemicals as oil flaxseed varieties among six flaxseed varieties from China (Wang et al., 2017). These studies focused on flaxseed varieties from the same country; little information on comparing different varieties of flaxseed from different countries worldwide has been reported. Wild flaxseed has also not received much attention in its potential contribution to breeding programs after characterization of nutritional content.

2. Materials and methods

2.1. Materials and chemicals

Accept for Acetonitrile, which was purchased from the ANPEL Scientific Instrument Co., Ltd (Shanghai, China), all other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Four varieties of flaxseed including Zhongya 3, Ariane, Yexiao and USPEA were supplied by the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences (Changsha, China). They were plated at Experimental Station of the Institute of Bast Fiber Crops in Dali, Yunnan Province, China under the regular agronomic conditions from 2014 to 2016. Mean annual temperature and total rainfall were 15.67°C and 5,286 mm, respectively. Field management followed normal production practices. Among these varieties, Zhongya 3 and Ariane are fibrous flaxseeds, originating from China and France, respectively. While USPEA and Yexiao belong to oil flaxseed varieties, which individually come from America and China, respectively.

2.2. Analysis of oil and fatty acid composition

Oil content was measured using the Soxhlet extractor method (AOCS, 1997). Fatty acid composition was determined by gas chromatograph (Agilent 6890N with flame ionization detection) after derivatization to fatty acid methyl esters (Ackman, 2002). Each fatty acid was expressed as a percentage of total fatty acids.

2.3. Phenolics extraction and determination

Phenolics extraction of each sample was performed as described previously (Wang et al., 2015). Free phenolics were extracted with chilling 80% (V/V) acetone, concentrated and brought up to 10 mL in deionized water. Residue from free phenolic extraction was digested with sodium hydroxide, followed by extracting with ethyl acetated, evaporated the solvent, and then reconstituted in 10 mL deionized water. Phenolic content was determined using the Folin-Ciocalteu method with a slight modification as described (Chen et al., 2015; Singleton et al., 1999).

2.4. Phytochemical profile analysis

The main phytochemical compounds, including phenolic acids and lignans, were identified and quantified by HPLC-PAD analysis using a Waters series system equipped with C18 column (4.6 mm × 250 mm, 5 µm) as previously described (Wang et al., 2017). Absorbance monitored for phenolic acids was 323 nm, for lignans was 280 nm. Quantification of phytochemicals was performed by comparison with authentic standards. Results were expressed as µg per gram sample.

2.5. Antioxidant activity

The *in vitro* antioxidant activity of flaxseed varieties was determined by three complementary methods, 2,2-Di(4-tert-octylphenyl)-1-

picrylhydrazyl (DPPH) free radical scavenging activity (Brand-Williams et al., 1995), ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996), and oxygen radical absorbance capacity (ORAC) (Huang et al., 2002; Wang et al., 2016) assay, as previously reported. Trolox was used as standard and results were calculated by comparing with standard curve and expressed as µmol trolox equivalent (TE) per gram sample.

2.6. Statistical analysis

Data were expressed as mean ± standard deviation (SD) for at least three replicates. Analysis of variance (ANOVA) and Duncan's multiple comparison were performed using SPSS for Windows V. 21.0 (SPSS Inc., Chicago, IL). A *P* value less than 0.05 was regarded as statistically significant. Dose-effect analysis was performed using Calcsyn software V. 2.0 (Biosoft, Cambridge, U.K.).

3. Results

3.1. Biometrical and morphological characteristics

Four varieties of flaxseed, originating from different countries, including one wild flaxseed type were selected. Their biometrical and morphological characteristics were presented in Table 1. Generally, the fiber characteristics, such as greater plant height, less numbers of seed per capsule, and higher fiber yield were observed in Zhongya 3 and Ariane. Flaxseeds were predominantly glossy brown, except the yellowish Yexiao, a wild flaxseed with the lowest seed yield, 491.07 kg/ha. Seed yield of other varieties ranged from 1,203.27 to 1,912.57 kg/ha, and the peak value was found in oil flaxseed USPEA.

3.2. Total oil content and fatty acid composition





Flaxseed has been known as a good source of oil with significant proportion of highly unsaturated fatty acid content. In general, results showed notable variations among these varieties of flaxseed in total oil content and fatty acid composition (Table 2). Oil flaxseed USPEA had the highest oil content (37.37%). Oil contents obtained from fibrous flaxseed varieties i.e. Ariane and Zhongya 3 were lower than oil flaxseed USPEA. However, the lowest one was found in Yexiao (30.07%), a wild oil flaxseed.

Five major fatty acids were identified and quantified (Table 2), with the most abundant linolenic acid (almost half of the total fatty acids), followed by oleic acid (19.56% to 24.33%) and linoleic acid (12.80% to 15.01%), all of these are unsaturated fatty acid (UFA). Significant differences in values of UFA among flaxseed varieties were observed, ranging from 84.00% to 89.50% for Yexiao and Ariane, respectively. It was noteworthy that UFA were higher in fibrous flaxseed, leading to their lower ratio of saturated fatty acid (SFA) to UFA. The highest SFA/UFA ratio was observed in wild flaxseed Yexiao (0.19), followed by oil flaxseed USPEA (0.15).

3.3. Total phenolic content and phytochemical profiles

Contents of free and bound phenolics, expressed as milligrams of gallic acid equivalent per 100 grams of sample (mg GAE/100 g) were showed in Table 3. The highest content of total phenolics was observed in USPEA (389.65), followed by Zhongya 3 and Yexiao, while Ariane was the lowest one (69.20). Results of free phenolics were similar to those of total phenolic content. USPEA showed the highest content, 320.45 mg GAE/100 g, accounting for 82.2% of total phenolics. Ariane had the lowest free phenolics, 130.87 mg GAE/100 g, making the lowest contribution to total phenolic content (55.6%). Regarding the bound phenolics, however, the highest value was found in Zhongya 3, 120.62 mg GAE/100 g, and the lowest one was USPEA, 69.20 mg GAE/100 g.

Table 1
Biometrical and morphological characteristics of flaxseed varieties used in this study.

Characteristics	Zhongya 3	Ariane	USPEA	Yexiao
Snaps				
Native background	China	France	America	China
Type	Fiber	Fiber	Oil	Oil
Growth period (day)	180.6 ± 1.53 ^{ab}	178.3 ± 2.08 ^a	183.0 ± 1.00 ^b	190.0 ± 1.73 ^c
Plant height (cm)	103.0 ± 1.40 ^c	100.2 ± 1.51 ^c	69.50 ± 1.48 ^b	50.57 ± 2.47 ^a
Thousand seeds weight (g)	4.160 ± 0.04 ^b	4.320 ± 0.09 ^b	5.360 ± 0.09 ^c	1.940 ± 0.12 ^a
Seed yield (kg/ha)	1226.2 ± 93.69 ^b	1384.1 ± 46.2 ^c	1974.3 ± 115.1 ^d	433.7 ± 22.27 ^a
Fiber yield (%)	0.230 ± 0.01 ^c	0.220 ± 0.02 ^c	0.160 ± 0.01 ^b	0.110 ± 0.01 ^a

Notes: Values in the same line with different letters indicate significant differences at $P < 0.05$.

As for phenolic acids, ferulic acid was the predominated one, followed by caffeic acid and *p*-coumaric acid. Bound *p*-coumaric acid and ferulic acid markedly outweighed the free ones, although bound caffeic acid accounted for more than 40% of total caffeic acid in all varieties. Comparatively, Zhongya 3 showed the highest content of caffeic acid (13.06 µg/g), whereas lowest content of *p*-coumaric acid and ferulic acid (10.59 and 46.98 µg/g, respectively) were calculated in USPEA (Table 3).

As for lignans, elevated level of SDG was documented in the bound fraction of flaxseed ranging from 61.98 to 106.16 µg/g, while free SDG was only obtained in USPEA and Yexiao varieties. The highest concentration of total SDG content was found in oil flaxseed varieties, including USPEA and Yexiao (144.66 and 129.51 µg/g, respectively), compared to fibrous flaxseed. SECO, a glycone of SDG, prevailed in the free form of flaxseed extracts (Table 3). The highest content of total SECO was observed in USPEA (243.69 µg/g), whereas other flaxseed varieties showed comparable SECO content, ranging from 76.54 to 81.62 µg/g.

3.4. Antioxidant activities

Antioxidant activity in the free and bound fraction of flaxseed varieties was determined by DPPH, FRAP, and ORAC assays (Table 4).

Table 2
Major fatty acid composition (%) in four flaxseed varieties.

Fatty acids	Zhongya 3	Ariane	USPEA	Yexiao
Oil content	33.78 ± 0.07 ^b	35.21 ± 0.23 ^c	37.37 ± 0.17 ^d	30.07 ± 0.07 ^a
Palmitic acid (C16:0)	5.14 ± 0.01 ^a	5.47 ± 0.01 ^b	6.22 ± 0.01 ^c	6.43 ± 0.00 ^d
Stearic acid (C18:0)	7.31 ± 0.01 ^c	5.01 ± 0.01 ^a	6.77 ± 0.01 ^b	9.57 ± 0.01 ^d
Oleic acid (C18:1)	24.33 ± 0.02 ^d	20.84 ± 0.01 ^b	19.56 ± 0.01 ^a	23.77 ± 0.01 ^c
Linoleic acid (C18:2)	12.98 ± 0.01 ^b	15.01 ± 0.02 ^d	14.29 ± 0.01 ^c	12.80 ± 0.02 ^a
Linolenic acid (C18:3)	50.24 ± 0.03 ^b	53.67 ± 0.04 ^d	53.15 ± 0.02 ^c	47.44 ± 0.01 ^a
SFA	12.5 ± 0.02 ^b	10.5 ± 0.02 ^a	13.0 ± 0.02 ^c	16.0 ± 0.01 ^d
UFA	87.6 ± 0.02 ^c	89.5 ± 0.02 ^d	87.0 ± 0.02 ^b	84.0 ± 0.01 ^a
SFA/UFA	0.14	0.12	0.15	0.19

Notes: Values of fatty acid (% total fatty acids) in the same line with different letters indicate significant differences at $P < 0.05$. SFA means saturated fatty acid and UFA means unsaturated fatty acid.

The highest DPPH free radical scavenging capacity was obtained in bound and free fractions of Zhongya 3 with the value of 3.91 and 4.49 µmol TE/g respectively, whereas the lowest DPPH values for bound and free phytochemicals were found in USPEA and Ariane at 0.23 and 2.01 µmol TE/g, respectively.

Table 3
Total phenolics and phytochemical profile in flaxseed varieties.

Phytochemicals		Zhongya 3	Ariane	USPEA	Yexiao
Total phenolics (mg GAE/100 g)	Bound	120.62 ± 1.59 ^d	104.28 ± 1.38 ^c	69.20 ± 1.56 ^a	93.120 ± 4.19 ^b
	Free	237.20 ± 4.21 ^b	130.87 ± 3.33 ^a	320.45 ± 2.54 ^c	239.37 ± 3.66 ^b
	Total	357.82 ± 2.67 ^c	235.15 ± 4.63 ^a	389.65 ± 3.68 ^d	332.48 ± 1.81 ^b
Phenolic acid (µg/g)					
Caffeic acid	Bound	6.28 ± 0.37 ^c	5.22 ± 0.25 ^{ab}	4.83 ± 0.10 ^a	5.50 ± 0.15 ^b
	Free	6.78 ± 0.38 ^c	6.08 ± 0.25 ^{ab}	6.64 ± 0.45 ^{bc}	5.88 ± 0.18 ^a
	Total	13.06 ± 0.34 ^b	11.29 ± 0.03 ^a	11.47 ± 0.51 ^a	11.38 ± 0.22 ^a
<i>p</i> -coumaric acid	bound	5.52 ± 0.36 ^b	5.90 ± 0.05 ^b	8.12 ± 0.18 ^c	4.37 ± 0.06 ^a
	Free	2.98 ± 0.25 ^c	1.76 ± 0.06 ^a	2.48 ± 0.19 ^b	4.14 ± 0.33 ^d
	Total	8.51 ± 0.61 ^b	7.66 ± 0.11 ^a	10.59 ± 0.31 ^c	8.50 ± 0.34 ^b
Ferulic acid	Bound	31.28 ± 0.49 ^d	10.52 ± 0.41 ^a	25.49 ± 0.60 ^c	12.06 ± 0.16 ^b
	Free	6.74 ± 0.68 ^b	4.44 ± 0.08 ^a	21.49 ± 1.29 ^c	4.88 ± 0.45 ^a
	Total	38.03 ± 1.01 ^b	14.96 ± 0.33 ^a	46.98 ± 1.81 ^c	16.94 ± 0.60 ^a
Lignan (µg/g)					
SDG	Bound	71.61 ± 7.22 ^{ab}	61.98 ± 19.69 ^a	90.40 ± 3.54 ^{bc}	106.16 ± 1.34 ^c
	Free	nd	nd	54.25 ± 2.72 ^b	23.34 ± 6.11 ^a
	Total	71.61 ± 7.22 ^a	61.98 ± 19.69 ^a	144.66 ± 3.83 ^b	129.51 ± 6.68 ^b
SECO	Bound	22.77 ± 0.42 ^{ab}	24.64 ± 0.41 ^c	21.90 ± 0.71 ^a	23.18 ± 0.18 ^b
	Free	58.85 ± 4.74 ^a	52.60 ± 4.10 ^a	221.80 ± 14.90 ^b	53.36 ± 3.43 ^a
	Total	81.62 ± 4.91 ^a	77.24 ± 4.38 ^a	243.69 ± 14.82 ^b	76.54 ± 3.25 ^a

Notes: Data in the same line with different letters indicate significant differences at $P < 0.05$. "nd" means not detected. SDG means secoisolarisiresinol diglucoside. SECO means secoisolariciresinol.

Table 4
Antioxidant activities ($\mu\text{mol TE/g}$) in four flaxseed varieties.

Antioxidant activity		Zhongya 3	Ariane	USPEA	Yexiao
DPPH	Bound	3.91 \pm 0.81 ^d	1.98 \pm 0.05 ^c	0.23 \pm 0.03 ^a	1.02 \pm 0.14 ^b
	Free	4.49 \pm 0.90 ^c	2.01 \pm 0.31 ^a	3.03 \pm 0.54 ^{ab}	3.20 \pm 0.24 ^b
Total		8.40 \pm 1.60 ^b	3.99 \pm 0.33 ^a	3.25 \pm 0.54 ^a	4.23 \pm 0.38 ^a
FRAP	Bound	22.34 \pm 2.31 ^c	26.22 \pm 2.77 ^d	3.49 \pm 0.25 ^a	16.09 \pm 1.25 ^b
	Free	39.31 \pm 3.16 ^b	32.31 \pm 3.09 ^a	28.73 \pm 2.46 ^a	34.35 \pm 2.86 ^{ab}
	Total	61.65 \pm 5.47 ^c	58.53 \pm 0.45 ^c	32.22 \pm 2.70 ^a	50.44 \pm 4.11 ^b
ORAC	Bound	23.61 \pm 2.55 ^a	22.18 \pm 0.82 ^a	21.08 \pm 0.70 ^a	21.50 \pm 1.26 ^a
	Free	39.19 \pm 2.68 ^b	16.24 \pm 5.41 ^a	67.08 \pm 3.03 ^c	36.46 \pm 2.60 ^b
	Total	62.80 \pm 5.12 ^b	38.43 \pm 5.98 ^a	88.16 \pm 2.36 ^c	57.96 \pm 3.74 ^b

Notes: DPPH means 2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity. FRAP means ferric reducing antioxidant power. ORAC means oxygen radical absorbance capacity. Values of antioxidant activities expressed as $\mu\text{mol TE/g}$ in the same line with different letters indicate significant differences at $P < 0.05$.

Free fractions of flaxseed varieties exhibited high FRAP value compared to bound fractions. In the free fractions, FRAP values ranged from 28.73 to 39.31 $\mu\text{mol TE/g}$ in USPEA and Zhongya 3 varieties, respectively. Whereas, in the case of bound fractions, Ariane had the highest FRAP value of 26.22 $\mu\text{mol TE/g}$, while lowest level was calculated for USPEA (3.49 $\mu\text{mol TE/g}$). On the whole, Ariane and USPEA depicted the highest and lowest FRAP values in bound fractions, respectively. Zhongya 3 showed the maximum ability of inhibit ferric ion potential in free fraction. Finally, higher total FRAP values were found in both Zhongya 3 and Ariane, which was almost double than the lowest one found in USPEA (32.22 $\mu\text{mol TE/g}$).

Oxygen radical absorbance capacity (ORAC) was also found higher in free fractions of flaxseed varieties, as it ranged from 21.08 to 23.61 $\mu\text{mol TE/g}$ in bound fractions and from 16.24 to 67.08 $\mu\text{mol TE/g}$ in free fractions. The highest free ORAC value was calculated for free fraction of USPEA cultivar (67.08 $\mu\text{mol TE/g}$), which also had maximum total ORAC contents (88.16 $\mu\text{mol TE/g}$). The lowest free ORAC value was obtained in Ariane, which could be attributed to lower concentration of phytochemicals (Table 3).

Relationship between content of phenolic compounds and antioxidant activity of flaxseed extract was obtained using Person's correlation. High correlation coefficients were found between DPPH, FRAP and ORAC values and contents of total phenolics and caffeic acid ($P < 0.05$, Table 5). Besides, contents of ferulic acid and SECO were positively correlated with ORAC values ($R^2 = 0.634$, $P < 0.05$; $R^2 = 0.878$, $P < 0.01$).

Table 5
Pearson correlation between phytochemicals and antioxidant activities.

Variables	DPPH	FRAP	ORAC
Total phenolics	0.681*	0.682*	0.946**
Caffeic acid	0.744**	0.821**	0.721**
Coumaric acid	0.252	0.235	0.477
Ferulic acid	0.355	0.053	0.634*
SDG	-0.060	-0.085	0.456
SECO	0.184	0.193	0.878**

Notes: DPPH means 2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity. FRAP means ferric reducing antioxidant power. ORAC means oxygen radical absorbance capacity. * and ** mean significant differences at $P < 0.05$ and $P < 0.01$ level, respectively.

4. Discussion

This study compared fatty acid, phytochemical profile and antioxidant activity of flaxseed varieties originating from different countries including a wild relative. This research suggested considerable variation among flaxseed varieties of different geographical origin growing under the same climatic condition.

Oil content of USPEA was 37.37%, which comparable to other oil flaxseed varieties reported from China (Zhang et al., 2016), and higher than that of fibrous flaxseeds including Ariane and Zhongya 3 (Table 2). Yexiao is a wild oil flaxseed without improvement which might explain its lowest oil content, less than 19.5% of that of USPEA. As to fatty acid composition, our results were in agreement with previous studies reporting the distribution of five major fatty acid contents in flaxseed from China (Zhang et al., 2016; Zou et al., 2017) and Pakistan (Yaqoob et al., 2016). These researches suggested that oil content and fatty acid composition were primarily depended on variety, but also ecological environment, as reported in almond cultivated in Serbia (Yaqoob et al., 2016). Besides, significant differences did not observed between fibrous and oil flaxseed varieties, except for a slightly higher ratio of SFA/UFA in oil flaxseed varieties.

Results of free and bound phenolic contents indicated considerable variation among different flaxseed varieties. Bound phenolics usually linked with cell wall material that may released by intestinal microflora during colonic digestion to play a role in health benefits (Adom and Liu, 2002). In general, contribution of free phenolics to total phenolic content was higher than bound phenolics in all four varieties, accounting for 55.7%–82.2%. These findings were in accordance with previous results from different flaxseed varieties with the value of 55.3% in Shuangya 12 and 76.5% in Longya 10 (Wang et al., 2017). Lower content of phenolics in flaxseed varieties from India was reported, with the values from 61.76 to 85.24 $\mu\text{g GAE/g}$ sample, which might be explained by underestimation of bound phenolics and different methods of extraction except for genotype and agronomic conditions (Kaur et al., 2017).

Three phenolic acids and two flaxseed lignans were identified in flaxseeds from different countries (Table 3). Similar to total phenolic content, clear differences were noted in phytochemical profile. Contents of *p*-coumaric acid, ferulic acid and SDG were higher in bound fractions than those in free fractions. Evidences have shown that phenolic acids mostly existed in bound fraction of grains, especially ferulic acid and *p*-coumaric acid (Adom and Liu, 2002; Multari et al., 2018; Okarter and Liu, 2010).

Flaxseed is one of the richest dietary sources of lignan (Milder et al., 2005), a class of diphenolic compound, which widely existed in plant kingdom. In the present study, two kind of lignan compounds: secoisolarisiresinol diglucoside (SDG) and secoisolarisiresinol (SECO) were identified in extracts of different flaxseeds (Table 3). Measured values of SDG, however, were relatively lower than previous reports (Johnsson et al., 2000; Lorenc-Kukula et al., 2005), potentially explained by variations in growing conditions and extraction method. Nevertheless, comparable SDG content was reported in flaxseed varieties using similar extraction method (Wang et al., 2017). The attentive observation of flaxseed lignan suggested that USPEA was rich in SDG and SECO, indicating its potential as a good breeding parent for accumulation of flaxseed lignan. It was also interesting that SDG content in wild Yexiao was comparatively higher than Zhongya 3 and Ariane, also suggesting that this wild type could be a good candidate for phytochemical resource.

Antioxidant activity is a multiple reaction according to different free radicals or oxidant sources. Thus, no single assay can reflect all radical sources or antioxidants in a complex system. In general, potent variability of antioxidant activities based on different assay methods was observed in flaxseed varieties (Table 4). DPPH is a well-known organic nitrogen radical, which could be used to measure either electron transfer or hydrogen atom transfer of antioxidant compounds (Xie and Schaich, 2014). FRAP method determines reduction of ferric 2,4,6-tripryridyl-s-triazine (TPTZ) to a colored product based on single electron transfer

mechanism (Prior et al., 2005). ORAC assay is based on hydrogen atom transfer mechanism to detect antioxidant protection of fluorescence damaged by peroxy radical (Prior et al., 2003). Most studies showed that antioxidant capacity was associated with phenolic composition of plant extracts (Kwee and Niemeyer, 2011; Wang et al., 2016). Total DPPH and FRAP contents were 3.25–8.40 and 32.22–61.65 $\mu\text{mol TE/g}$ respectively, which were higher than the reported values in methanol-water extracts of flaxseed cultivated in North and Northwest China (1.47–1.77 $\mu\text{mol TE/g}$ for DPPH and 3.20–3.92 $\mu\text{mol TE/g}$ for FRAP, respectively) (Deng et al., 2018). Total ORAC values ranged from 38.43 to 88.16 $\mu\text{mol TE/g}$, which is little lower than previous reports in selected Chinese flaxseed varieties (67.47 to 107.16 $\mu\text{mol TE/g}$) by Wang (Wang et al., 2017). Higher antioxidant activities were found in Zhongya 3 and USPEA, which were positively associated with phenolics and other phytochemicals (Tables 3 and 4). The dissimilarities showed in different antioxidant methods might be explained by variation of type and proportion of antioxidant compounds detected by different antioxidant activity assays. These results indicated that flaxseed varieties originating from different counties have the potential to be good sources of different antioxidant compounds according to different free radicals or oxidants.

Therefore, comparison and characterization of flaxseed varieties from different countries will be helpful to provide new breeding resources of flaxseed by presenting their quality specifications and possible commercial values.

Author contributions

Conceptualization, X.G. and C.Q.; methodology, H.W.; software, H.W.; validation, C.Q. and H.W.; formal analysis, C.Q. and H.W.; investigation, C.Q.; resources, Y.W.; data curation, C.Q. and D.I.J.; writing—original draft preparation, C.Q. and D.I.J.; writing—review and editing, A.M.A. and X.G.; visualization, A.M.A.; supervision, X.G.; project administration, Y.W.; funding acquisition, Y.W.

Declaration of competing interest

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

Acknowledgements

This study was supported by the China Agriculture Research System Fund (No. CARS-16-E13), the National Natural Science Foundation of China (31401469), the Agricultural Science and Technology Innovation Program in Chinese Academy of Agricultural Sciences (No. ASTIP-IBFC06), and the Central Public-interest Scientific Institution Basal Research Fund (No.1610242019006).

References

Ackman, R.G., 2002. The gas chromatograph in practical analyses of common and uncommon fatty acids for the 21st century. *Anal. Chim. Acta* 465 (1–2), 175–192. [https://doi.org/10.1016/S0003-2670\(02\)00098-3](https://doi.org/10.1016/S0003-2670(02)00098-3).

Adom, K.K., Liu, R.H., 2002. Antioxidant activity of grains. *J. Agric. Food Chem.* 50 (21), 6182–6187. <https://doi.org/10.1021/jf0205099>.

AOCS, 1997. *Official and Tentative Methods of the American Oil Chemists' Society*, 7 ed. American Oil Chemists' Society, Champaign, IL.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239 (1), 70–76. <https://doi.org/10.1006/abio.1996.0292>.

Bhatty, R.S., 1995. Nutritional composition of whole flaxseed and flaxseed meal. In: Cunnane, S.C., Thomp-son, L.H. (Eds.), *Flaxseed in Human Nutrition*. AOCS Press, Champaign, IL, pp. 22–45.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 28 (1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).

Carraro, J.C.C., Dantas, M.L.D., Espeschit, A.C.R., Martino, H.S.D., Ribeiro, S.M.R., 2012. Flaxseed and human health: reviewing benefits and adverse effects. *Food Rev. Int.* 28 (2), 203–230. <https://doi.org/10.1080/87559129.2011.595025>.

Chen, Y., Chen, G., Fu, X., Liu, R.-h., 2015. Phytochemical profiles and antioxidant activity of different varieties of *Adinandra* tea (*Adinandra Jack*). *J. Agric. Food Chem.* 63 (1), 169–176. <https://doi.org/10.1021/jf503700v>.

Deng, Q., Yu, X., Ma, F., Xu, J., Huang, F., Huang, Q., Sheng, F., 2018. Comparative analysis of the in-vitro antioxidant activity and bioactive compounds of flaxseed in China according to variety and geographical origin. *Int. J. Food Prop.* 20, S2708–S2722. <https://doi.org/10.1080/10942912.2017.1402029>.

Eliasson, C., Kamal-Eldin, A., Andersson, R., Aman, P., 2003. High-performance liquid chromatographic analysis of secoisolaricresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction. *J. Chromatogr., A* 1012 (2), 151–159. [https://doi.org/10.1016/S0021-9673\(03\)01136-1](https://doi.org/10.1016/S0021-9673(03)01136-1).

Ezzat, S.M., Shouman, S.A., Elkhoely, A., Attia, Y.M., Elsexy, M.S., El Senousy, A.S., Choucry, M.A., El Gayed, S.H., El Sayed, A.A., Sattar, E.A., El Tanbouly, N., 2018. Anticancer potentiality of lignan rich fraction of six flaxseed cultivars. *Sci. Rep.* 8, 544. <https://doi.org/10.1038/s41598-017-18944-0>.

FAOSTAT, 2020. Production of linseed in 2018, 2020-03-04 update. <http://www.fao.org/faostat/en/#data/QC>.

Huang, D.J., Ou, B.X., Hampsch-Woodill, M., Flanagan, J.A., Prior, R.L., 2002. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* 50 (16), 4437–4444. <https://doi.org/10.1021/jf0201529>.

Johnsson, P., Kamal-Eldin, A., Lundgren, L.N., Aman, P., 2000. HPLC method for analysis of secoisolaricresinol diglucoside in flaxseeds. *J. Agric. Food Chem.* 48 (11), 5216–5219. <https://doi.org/10.1021/JF0005871>.

Kaur, R., Kaur, M., Gill, B.S., 2017. Phenolic acid composition of flaxseed cultivars by ultra-performance liquid chromatography (UPLC) and their antioxidant activities: effect of sand roasting and microwave heating. *J. Food Process. Preserv.* 41 (5), e13181. <https://doi.org/10.1111/jfpp.13181>.

Kwee, E.M., Niemeyer, E.D., 2011. Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. *Food Chem.* 128 (4), 1044–1050. <https://doi.org/10.1016/j.foodchem.2011.04.011>.

Lorenc-Kukula, K., Amarowicz, R., Oszmianski, J., Doermann, P., Starzycki, M., Skala, J., Zuk, M., Kulma, A., Szopa, J., 2005. Pleiotropic effect of phenolic compounds content increases in transgenic flax plant. *J. Agric. Food Chem.* 53 (9), 3685–3692. <https://doi.org/10.1021/jf047987z>.

Milder, I.E.J., Arts, I.C.W., van de Putte, B., Venema, D.P., Hollman, P.C.H., 2005. Lignan contents of Dutch plant foods: a database including laricresinol, pinoresinol, secoisolaricresinol and matairesinol. *Br. J. Nutr.* 93 (3), 393–402. <https://doi.org/10.1079/bjn20051371>.

Multari, S., Pihlava, J.-M., Ollenu-Chuasam, P., Hietaniemi, V., Yang, B., Suomela, J.-P., 2018. Identification and quantification of avenanthramides and free and bound phenolic acids in eight cultivars of husked oat (*Avena sativa* L) from Finland. *J. Agric. Food Chem.* 66 (11), 2900–2908. <https://doi.org/10.1021/acs.jafc.7b05726>.

Okarter, N., Liu, R.H., 2010. Health benefits of whole grain phytochemicals. *Crit. Rev. Food Sci.* 50 (3), 193–208. <https://doi.org/10.1080/10408390802248734>.

Parikh, M., Netticadan, T., Pierce, G.N., 2018. Flaxseed: its bioactive components and their cardiovascular benefits. *Am. J. Physiol. - Heart C.* 314 (2), H146–H159. <https://doi.org/10.1152/ajpheart.00400.2017>.

Prior, R.L., Hoang, H., Gu, L.W., Wu, X.L., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D.J., Ou, B.X., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(F))) of plasma and other biological and food samples. *J. Agric. Food Chem.* 51 (11), 3273–3279. <https://doi.org/10.1021/jf0262256>.

Prior, R.L., Wu, X.L., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 53 (10), 4290–4302. <https://doi.org/10.1021/jf0502698>.

Rajwade, A.V., Kadoo, N.Y., Borikar, S.P., Harsulkar, A.M., Ghorpade, P.B., Gupta, V.S., 2014. Differential transcriptional activity of SAD, FAD2 and FAD3 desaturase genes in developing seeds of linseed contributes to varietal variation in alpha-linolenic acid content. *Phytochemistry* 98, 41–53. <https://doi.org/10.1016/j.phytochem.2013.12.002>.

Singh, K.K., Mridula, D., Rehal, J., Barnwal, P., 2011. Flaxseed: a potential source of food, feed and fiber. *Crit. Rev. Food Sci.* 51 (3), 210–222. <https://doi.org/10.1080/10408390903537241>.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Packer, L. (Ed.), *Oxidants and Antioxidants*, Pt A, pp. 152–178.

Toure, A., Xu, X.M., 2010. Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. *Compr. Rev. Food Sci. F.* 9 (3), 261–269. <https://doi.org/10.1111/j.1541-4337.2009.00105.x>.

Wang, H., Chen, G., Guo, X.B., Abbasi, A.M., Liu, R.H., 2016. Influence of the stage of ripeness on the phytochemical profiles, antioxidant and antiproliferative activities in different parts of *Citrus reticulata* Blanco cv. Chachiensis. *LWT-Food Sci. Technol.* 69, 67–75. <https://doi.org/10.1016/j.lwt.2016.01.021>.

Wang, H., Qiu, C., Abbasi, A.M., Chen, G., You, L., Li, T., Fu, X., Wang, Y., Guo, X., Liu, R.H., 2015. Effect of germination on vitamin C, phenolic compounds and antioxidant activity in flaxseed (*Linum usitatissimum* L.). *Int. J. Food Sci. Technol.* 50, 2545–2553. <https://doi.org/10.1111/ijfs.12922>.

Wang, H., Wang, J.H., Qiu, C.S., Ye, Y.T., Guo, X.B., Chen, G., Li, T., Wang, Y.F., Fu, X., Liu, R.H., 2017. Comparison of phytochemical profiles and health benefits in fiber and oil flaxseeds (*Linum usitatissimum* L.). *Food Chem.* 214, 227–233. <https://doi.org/10.1016/j.foodchem.2016.07.075>.

Xie, D.W., Dai, Z.G., Yang, Z.M., Tang, Q., Deng, C.H., Xu, Y., Wang, J., Chen, J., Zhao, D.B., Zhang, S.L., Zhang, S.Q., Su, J.G., 2019. Combined genome-wide association analysis and transcriptome sequencing to identify candidate genes for flax

- seed fatty acid metabolism. *Plant Sci.* 286, 98–107. <https://doi.org/10.1016/j.plantsci.2019.06.004>.
- Xie, J., Schaich, K.M., 2014. Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. *J. Agric. Food Chem.* 62 (19), 4251–4260. <https://doi.org/10.1021/jf500180u>.
- Xing, L., Zhao, F.-M., Cao, Y.-F., Wang, M., Mei, S., Li, S.-P., Cai, Z.-Y., 2014. Principal component analysis of mineral elements and fatty acids composition in flaxseed from ten different regions. *Spectrosc. Spectr. Anal.* 34 (9), 2538–2543. [https://doi.org/10.3964/j.issn.1000-0593\(2014\)09-2538-06](https://doi.org/10.3964/j.issn.1000-0593(2014)09-2538-06).
- Yaqoob, N., Bhatti, I.A., Anwar, F., Mushtaq, M., Artz, W.E., 2016. Variation in physico-chemical/analytical characteristics of oil among different flaxseed (*Linum Usitatissimum* L.) cultivars. *Ital. J. Food Sci.* 28 (1), 83–89. <https://doi.org/10.14674/1120-1770/ijfs.v461>.
- Zhang, J.P., Xie, Y.P., Dang, Z., Wang, L.M., Li, W.J., Zhao, W., Zhao, L., Dang, Z.H., 2016. Oil content and fatty acid components of oilseed flax under different environments in China. *Agron. J.* 108 (1), 365–372. <https://doi.org/10.2134/agronj2015.0224>.
- Zou, X.-G., Chen, X.-L., Hu, J.-N., Wang, Y.-F., Gong, D.-M., Zhu, X.-M., Deng, Z.-Y., 2017. Comparisons of proximate compositions, fatty acids profile and micronutrients between fiber and oil flaxseeds (*Linum usitatissimum* L.). *J. Food Compos. Anal.* 62, 168–176. <https://doi.org/10.1016/j.jfca.2017.06.001>.