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Florets of Sunflower (*Helianthus annuus* L.): Potential New Sources of Dietary Fiber and Phenolic Acids

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Supporting Information

ABSTRACT: Ray florets (Rf) and disc florets (Df) are agricultural byproducts of sunflower seeds. Their nutrition-related compounds were determined. The dietary fiber contents in Rf and Df were 42.90 mg/100 g and 58.97 mg/100 g. In both florets, palmitic, linoleic, and linolenic acids were identified as the three most abundant fatty acids, and the saturated ones constitute approximately two-thirds (w/w) of the total fatty acids. Lysine was the limiting amino acid in both florets by World Health Organization standards. Sixteen phenolic compounds, nine free and eight bound, mainly depsides, were identified in florets by RP-HPLC-DAD/ESI-TOF-MS. The free and bound phenolic compounds in Df were higher than in Rf. 1,5-di-*O*-caffeoylquinic acid was the predominant free phenolic compound in both florets. The present study revealed that the florets of sunflower are rich sources of dietary fiber, Fe, and phenols.

KEYWORDS: *sunflower, ray florets, disc florets, phenolic compounds, RP-HPLC-DAD/ESI-TOF-MS*

INTRODUCTION

Helianthus annuus L. (Compositate), commonly known as sunflower, is a tall herb that blooms in late summer and autumn.[1] Sunflower, which originated from North America, is one of the leading worldwide oil seed crops.[1] The seeds of sunflower are used as food material, whereas other parts of the plant, notably the petioles and flowers (at their early stage of blossoming), were reported to be used in preparing delicacies.[2] Flowers and seeds of sunflower are also used in traditional remedies for cancer in Venezuela and in white wine maufacturing.[2] There are two types of flowers (ray florets and disc florets) on the capitulum of sunflower. Ray florets (Rf), located in the outermost whorl of the capitulum, are characterized by one plane of reflectional symmetry with three elongated petals.[2−4] They are sterile, retaining only filamentous remnants of the aborted stamens and large flat ovaries with no ovules. Disc florets (Df), also described as "disk florets" or "tubular florets", come in arcs from the center of the capitulum to form unique left- and right-turning spirals.[2−4] Typically, Rf is yellow, but Df is pale yellow-green when immature or dried and brown or maroon when ripe. According to statistics from the National Sunflower Association (NSA), sunflower is fourth in oil seeds worldwide, with a production of 38 million metric tons of seeds in 2011−2012. This represents about 25 million ha of cultivated land of sunflower primarily for the production of sunflower seeds.[5] Usually, 50 kg production of dried sunflower yields 1 and 5 kg of dried Rf and Df. There were approximately 0.76 and 3.8 million metric tons of Rf and Df produced in 2011. However, most of it was discarded as byproducts of agriculture.

Recently, polyphenols have generated a lot of interest in food research.[6] Phenolic compounds are secondary plant metabolites with antioxidant and anti-inflammatory activity. The phenolic compounds can be divided into two groups with regard to their extractability: free phenolic compounds solubilized by aqueous organic solvents and bound phenolic compounds that remain in the residues.[7,8] Some flowers are a source of food, rich in nutritional compositions, such as carbohydrates, vitamins, minerals, and antioxidants.[9] Many studies have reported that flowers contain a lot of polyphenols, which can be divided into three groups on the basis of phenolics composition: phenolic acids flower (banana flower[10]), flavonoids flower (chrysanthemum[11] and roses[12]), anthocyanins flower (tulips[13]). Published research on the flower of sunflower focused on its essential oils, triterpene glycosides constituents, and functionality.[14−18] The nutritional composition and phenolic compounds in the florets of sunflower have not been reported. Thus, the objective of our study was (1) to comparatively study nutrients in the ray and disc florets of sunflower and (2) to identify and quantify phenolic compounds in the florets of sunflower.

MATERIALS AND METHODS

Materials and Chemicals. Ray florets and disc florets were collected from the sunflower capitulum in August 2011 in Jingtai County, Gansu Province, China. The samples were air-dried in the dark, ground with an YF-103 blender (Zhengjiang, China), and sieved through a 60-mesh screen, resulting in floret powder. Samples were stored at room temperature in a brown desiccator with oxygen scavenger until analysis.

Acetonitrile and formic acid of HPLC grade were purchased from Merck (Darmstadt, Germany). Pepsin 1:10000 (No. P7000, >250 U/mg), pancreatin (No. P7545, 8 × USP), and α-amylase (No. A3306)

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were purchased from Sigma (St. Louis, MO, USA); 50% boron trifluoride–methanol complex (BF_3) and nonadecanoic acid were purchased from Adamas (Basel, Switzerland). Isoquercitrin, chlorogenic acid, ferulic acid, caffeic acid, and *p*-coumaric acid (>98.0%) were commercially purchased from Chengdu Biopurify Phytochemicals (Chengdu, China). All other reagents and solvents used were of analytical grade. All aqueous solutions were prepared using freshly double-distilled water. Water was treated by a Milli-Q water purification system (TGI Pure Water Systems, USA).

Proximate Composition Analysis. Moisture, ash, protein, and lipid were determined using AOAC methods.[19] Soluble dietary fiber, insoluble dietary fiber, and total dietary fiber were determined by a rapid enzymatic assay.[20] Reducing and total sugars were determined by the Fehling method.[21]

Mineral Elements and Metals Analysis. K, Na, Ca, Mg, Fe, Cu, Zn, and Mn were determined using an 1100 B atomic absorption spectrophotometer (Perkin-Elmer, Germany). Hg, As, Cr, and Pb were analyzed using an inductively coupled plasma-atomic emission spectrometer (Spectro Analytical Instruments, USA), while P was measured using a colorimetric molybdenum vanadate method.[19]

Vitamin Analysis. Vitamin C was assayed by reaction with 2,6-dichlorophenol–indophenol.[19] Vitamin B_1 and vitamin B_2 were determined using a fluorometric method.[19] Vitamin E was extracted with ethanol and determined by an HPLC–FLD method.[22]

Fatty Acid Analysis. The powdered samples (Rf 1 g and Df 2 g) were extracted with chloroform–methanol (2:1, v/v). To each specimen 1 mg of an internal standard (nonadecanoic acid) was added. After transmethylation with 14% BF_3 in methanol, the extracted lipid in hexane was subjected to fatty acid composition analysis with a QP2010 GC/MS instrument (Shimadzu, Japan).[23]

Amino Acid Analysis. Amino acid composition was determined with an L-8800 automatic amino acid analyzer (Hitachi, Japan).[24] The L-8800 Hitachi automated amino acid analyzer system was equipped with two #2662 ion-exchange columns (4.6 × 60 mm) and a UV detector. For separation, buffer flow rate, pump pressure, and column temperature of the first column were set at 0.45 mL/min, 12.260 MPa, and 70 °C, respectively. For postcolumn derivatization of amino acids, ninhydrin solution was supplied at 0.35 mL/min to the second column held at 135 °C by a pump with a pressure of 0.780 MPa.

Extraction of Free and Bound Phenolic Compounds. Free phenolic compounds of the powder (1 g) were extracted by blending with 50 mL of 80% chilled acetone for 10 min.[22] After centrifugation at 2500*g* for 10 min, the supernatant was removed and the residue was re-extracted. Supernatants were pooled and evaporated at 45 °C until less than 10 mL. The remainder was made up to 25 mL with methanol, filtered through a 0.45 μm nylon membrane, and frozen at −80 °C until analysis.

With the residue from free phenolic compound extraction, bound phenolic compounds were extracted by mixing with 40 mL of 1.2 N HCl–methanol and 2 mg of ascorbic acid as antioxidant.[25] After incubation at 35 °C for 16 h with continuous stirring, the resulting suspension was filtered through a Whatman 3 filter paper. Filtrates were evaporated at 45 °C to dryness. The residue was redissolved in 25 mL of methanol, filtered through a 0.45 μm nylon membrane, and frozen at −80 °C until analysis.

RP-HPLC-DAD Analysis of Phenolic Compounds. The analysis of phenolic compounds was performed with a LC-20A HPLC instrument (Shimadzu, Japan) equipped with a diode array detector (DAD). A reverse phase Thermo BDS C_{18} column (250 × 4.6 mm i.d.) with 5 μm particle diameter was applied. The chromatographic conditions were set as follows: flow rate of 0.7 mL/min, sample injection volume of 10 μL, column temperature of 40 °C, and mobile phase A (0.2% formic acid) and mobile phase B (100% acetonitrile). The gradient profile was optimized as follows: 0–5 min, 10% B; 5–50 min, 10–40% B; 50–55 min, 40–90% B; 55–62 min, 90% B; 62–65 min, 90–10% B; 65–75 min, 10% B. Phenolic compounds were monitored at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids). Retention times of standards were chlorogenic acid, 15.51 min; caffeic acid, 18.71 min; *p*-coumaric acid, 25.61 min; ferulic acid, 28.18 min; isoquercitrin, 29.61 min. Additionally, UV/vis spectra were recorded in the range 200–600 nm at a acquisition rate of 1.25 scans/s (peak width 0.2 min).

For calibration, standard stock solutions (400 μg/mL) were diluted with methanol to obtain a series of standard solutions (1, 5, 25, 50, 100, 200, 400 μg/mL). The method was validated with high values in correlation coefficients of calibration curves (R^2 > 0.9998) and recovery (>86%). When a reference compound was not available, the calibration of a structurally related substance was applied with a molecular weight correction factor.[26]

RP-HPLC-DAD/ESI-TOF-MS Analysis of Phenolic Compound Extracts. For the identification of phenolic compounds, an Agilent 1200 HPLC (Agilent Technologies, USA) was used, equipped with a binary pump, microvacuum degasser, auto plate-sampler, column compartment, and diode array detector, and coupled to an Agilent 6210 TOF-MS (Agilent Technologies, USA). The RP-HPLC was operated as described above. The effluent from the RP-HPLC column was split using a T-type phase separator before being introduced into the mass spectrometer (split ratio = 1:3). The electrospray source of the MS was operated in a negative mode. The operating parameters were as follows: drying gas (N_2) flow rate, 12.0 L/min; drying gas temperature, 320 °C; nebulizer, 32 psig; capillary, 3600 V; Oct RFV, 220 V; fragmentor voltage, 135 V; skimmer, 62 V. Mass spectra were recorded across the *m*/*z* range 100–1200. The operation, acquisition, and analysis of data were monitored by using LC-TOF-MS MassHunter workstation software (Version B.02.00, Agilent Technologies).

Statistical Analysis. Results are presented as mean value ± standard deviation for triplicate determinations. ANOVA and Tukey's comparison tests were performed to identify differences between values using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA). Significant differences were declared at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate Chemical Composition. The proximate compositions of Rf and Df are presented in Table 1. Although

Table 1. Proximate Chemical Composition Content of Ray and Disc Florets (g/100 g dw, except moisture g/100 g ww) (n = 3)[a]

	ray florets		disc florets	
component	av	SD	av	SD
moisture	5.88	0.20	6.93 a	0.05
crude ash	12.03 a	0.06	10.15	0.06
crude protein	9.95	0.37	9.87	0.15
crude lipid	8.26 a	0.06	5.82	0.03
reducing sugar	10.47 a	0.34	2.65	0.29
total sugar	12.29 a	0.09	4.45	0.43
soluble dietary fiber	4.49	0.44	7.15 a	0.61
insoluble dietary fiber	38.21	0.60	52.75 a	0.68
total dietary fiber	42.90	0.32	58.97 a	0.76

[a]A value that is significantly greater ($p < 0.05$) than its paired value is denoted with an "a". Paired results with no letter have no significant difference.

Rf and Df had similar values of crude protein, Rf had higher levels of crude ash, crude lipid, reducing sugar, and total sugar than Df ($p < 0.05$), especially in crude lipid, reducing sugar, and total sugar. On the contrary, for dietary fiber including insoluble, soluble, and total dietary fiber, Df had significantly higher values than Rf ($p < 0.05$). The dietary fiber in both Rf and Df was mainly identified as insoluble, which is similar to the results obtained with banana flower.[10] The total dietary fiber in Rf and Df was determined as 42.90 and 58.97 g/100 g dw, respectively, which suggested that the flower of sunflower was a good source of dietary fiber.

Mineral Elements, Metals, and Vitamins. The results of mineral element, metal, and vitamin analyses of Rf and Df are shown in Table 2. Although Rf had higher levels of nonmetal mineral elements (P) than Df, Df was significantly richer in transition metals (Fe, Mn, Cr, and Pb) and alkali metals (Na) than Rf. K was identified as the most abundant mineral element in both Rf and Df, with levels beyond 1900 mg/100 g dw. The huge value in the ratio of K/Na makes sunflower florets useful in maintaining body electrolytic balance. Although Rf had higher levels of Ca and P than Df, Df was significantly richer in Fe and Mn than Rf. Df had higher levels of Fe than other edible flowers, such as flowers of *Antirrhinum majus*, *Chrysanthemum frutescens*, and *Viola* x *wittrockiana*.[27] Regarding harmful minerals, although Df had higher levels of Cr and Pb than Rf, Cr, Pb, Hg, and As were present in very low concentrations, which were far less than their possible poisonous concentrations for human beings. The results suggested that Df of sunflower was a promising source of Fe for humans. In terms of vitamins B_2, C, and E, Rf was superior to Df. The lower value of vitamin C was possibly ascribed to sample dehydration.

Table 2. Content of Minerals, Metals, and Vitamins in Ray and Disc Florets (mg/100 g dw, except Hg and As μg/100 g dw) (n = 3)[a]

		ray florets		disc florets	
analyte	standard quantitative range	av	SD	av	SD
K	250−2500	1983.83	12.76	2006.76	35.37
Na	25−250	59.67	2.29	72.21 a	7.18
Ca	62.5−875	748.26 a	24.71	622.81	26.89
Mg	62.5−375	202.55	1.40	261.92 a	16.35
P	125−750	335.67 a	3.19	127.81	0.28
Fe	2.5−40	12.84	1.59	34.81 a	1.70
Cu	1.25−7.5	2.33	0.12	2.70	0.54
Zn	1.25−7.5	2.26	0.36	2.99	0.38
Mn	1.25−8.75	1.98	0.20	5.56 a	0.08
Cr	0.1−1.4	0.24	0.09	1.11 a	0.18
Pb	0.1−1.4	0.29	0.02	0.82 a	0.25
Hg	1−20	1.34	0.58	1.69	0.59
As	10−100	37.57	6.97	30.35	3.35
vitamin B_1	0.02−1	0.54	0.09	0.53	0.06
vitamin B_2	0.25−1	0.92 a	0.03	0.75	0.03
vitamin C	10−100	68.08 a	2.45	43.56	0.80
vitamin E	2−24	6.31 a	0.76	3.79	0.65

[a]A value that is significantly greater ($p < 0.05$) than its paired value is denoted with an "a". Paired results with no letter have no significant difference.

Fatty Acids. The fatty acid compositions of Rf and Df are presented in Table 3. Eleven fatty acids were identified in the flowers of sunflower. Saturated fatty acids accounted for two-thirds of the total fatty acids in both Rf and Df. For saturated fatty acids in Rf and Df, palmitic acid was the most abundant, followed by stearic acid. For unsaturated fatty acids, linoleic and linolenic acids were more abundant than oleic acid. These three acids constituted over 90% of the unsaturated fatty acids in Rf and Df.

Amino Acids. Table 4 shows the amino acid composition of Rf and Df. The eight essential amino acids, namely, Lys, Phe, Met, Thr, Ile, Leu, Val, and Try, were found in both Rf and Df. The total amounts of essential amino acids in Rf and Df were 3.21 and 3.09 g/100 g dw, respectively. The most abundant essential amino acid was valine (0.67 g/100 g dw) and leucine (0.65 g/100 g dw) in Rf and Df, respectively. Glutamate was the most prominent nonessential amino acid in Rf and Df, as observed in other food proteins. The chemical scores of essential amino acids with respect to the reference protein of FAO/WHO[28] are shown in Table 5. Tryptophan obtained the highest score among the essential amino acids in both Rf and Df followed by the branched amino acids (isoleucine and valine). Lysine had the lowest score in both Rf and Df, and it was declared as the limiting amino acid. Previous research attested that branched-chain amino acids are the major nitrogen sources for glutamine and alanine synthesis in muscle.[29] Therefore, protein isolates from sunflower florets could be a potential nutritional supplement.

Identification of Phenolic Compounds. The HPLC chromatograms of free and bound phenolic compounds in Rf and Df are shown in Figure 1. The majority of compounds were efficiently separated. No significant peaks were apparent in DAD chromatograms before 5 min or after 55 min. However, base peak chromatograms (Figure S3A−D) show another major group of compounds from the mobile phase and column

Table 3. Fatty Acid Content of Ray and Disc Florets (mg/100 g dw) (n = 3)[a]

		ray florets		disc florets	
fatty acid	standard quantitative range	av	SD	av	SD
lauric acid ($C_{12:0}$)	5−400	28.13 a	0.50	15.72	1.02
myristic acid ($C_{14:0}$)	5−400	133.06 a	8.95	44.74	0.74
palmitic acid ($C_{16:0}$)	5−400	383.16 a	40.51	131.49	3.24
margaric acid ($C_{17:0}$)	5−400	15.15	4.03	12.25	2.26
stearic acid ($C_{18:0}$)	5−400	135.01 a	12.93	49.50	1.14
oleic acid ($C_{18:1}$)	5−400	27.99	6.74	17.11	0.84
linoleic acid ($C_{18:2}$)	5−400	220.42 a	26.71	101.52	1.86
linolenic acid ($C_{18:3}$)	5−400	172.39 a	15.54	78.13	2.42
arachidic acid ($C_{20:0}$)	5−400	51.43	5.15	39.11	6.56
behenic acid ($C_{22:0}$)	5−400	49.14	7.39	47.21	11.11
lignoceric acid ($C_{24:0}$)	5−400	25.68	5.46	40.41 a	0.88
total saturated		820.76 a(66.1%)	59.62	380.42 (65.9%)	17.74
total unsaturated		420.8 a (33.9%)	47.15	196.75 (34.1%)	3.77

[a]A value that is significantly greater ($p < 0.05$) than its paired value is denoted with an "a". Paired results with no letter have no significant difference.

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Table 4. Amino Acid Content of Ray and Disc Florets (g/100 g dw) (n = 3)[a]

amino acids	standard quantitative range	ray florets av	ray florets SD	disc florets av	disc florets SD
lysine (Lys)	0.09−2.19	0.41	0.01	0.38	0.02
phenylalanine (Phe)	0.10−2.48	0.41	0.01	0.44	0.01
methionine (Met)	0.09−2.24	0.11	0.00	0.14	0.02
threonine (Thr)	0.07−1.79	0.31	0.05	0.34	0.03
isoleucine (Ile)	0.08−1.97	0.52 a	0.01	0.45	0.01
leucine (Leu)	0.08−1.97	0.56	0.03	0.65 a	0.02
valine (Val)	0.07−1.76	0.67 a	0.02	0.48	0.01
tryptophan (Trp)	0.12−3.06	0.22	0.01	0.21	0.01
total essential amino acids		3.21	0.05	3.09	0.03
aspartic acid (Asp)	0.08−2.00	0.74	0.01	0.71	0.04
serine (Ser)	0.06−1.58	0.33	0.05	0.34	0.03
glutamate (Glu)	0.09−2.21	1.08 a	0.03	0.76	0.03
glycine (Gly)	0.05−1.13	0.42	0.00	0.47 a	0.02
alanine (Ala)	0.05−1.34	0.39	0.01	0.50 a	0.05
cystine (Cys)	0.14−3.60	0.18	0.01	0.19	0.01
tyrosine (Tyr)	0.11−2.72	0.18	0.01	0.22 a	0.01
proline (Pro)	0.07−1.73	0.40	0.03	0.41	0.02
arginine (Arg)	0.10−2.61	0.29	0.04	0.35	0.00
histidine (His)	0.09−2.33	0.27	0.04	0.24	0.02
total nonessential anmino acids		4.27	0.15	4.17	0.02
total amino acids		7.47	0.15	7.27	0.05

[a]A value that is significantly greater ($p < 0.05$) than its paired value is denoted with an "a". Paired results with no letter have no significant difference.

Table 5. Essential Amino Acid Composition of Compared with the FAO/WHO Pattern (g/16Ng = g of amino acid per 100g protein)

amino acids	ray florets g/16Ng	disc florets g/16Ng	FAO/WHO pattern	ray florets percentage (%)	disc florets percentage (%)
isoleucine	5.21	4.52	4.0	130	113
leucine	5.64	6.62	7.0	81	95
lysine	4.10	3.89	5.5	75	71
methionine + cystine	2.85	3.25	3.5	81	93
phenylalanine + tyrosine	5.84	6.72	6.0	97	112
threonine	3.12	3.43	4.0	78	86
tryptophan	2.20	2.17	1.0	220	217
valine	6.72	4.83	5.0	134	97

(Figure S3E), approximately eluting between 55 and 68 min (isocratic from 55 to 62 min at 90% MeCN, return to initial conditions from 65 to 75 min, 10% MeCN). Most constituents showed similar UV spectra with maximum absorbance at 320−330 nm and a shoulder around 300−310 nm, characterizing them as hydroxycinnamic acid derivatives.[30,31] The LC-TOF-MS data for all identified compounds are presented in Table 6. Chlorogenic acid (compound **3**) and isoquercitrin (compound **8**) were identified by comparing their UV spectra and retention times to the results obtained with corresponding standards. Moreover, the quasi-molecular ion (m/z 353.0959) of chlorogenic acid (**3**) yielded a product ion at m/z 191.0625, representing the quinic acid moiety, which was in agreement with previous findings.[30] The quasi-molecular ion (m/z 463.0979) of isoquercitrin (**8**) yielded a product ion at m/z 355.1120, which was in agreement with the result obtained by Verardo et al.[32]

Compounds **1** and **2**, showing an $[M - H]^-$ ion at m/z 341.0966 and 341.0965, respectively, were observed at 12.120 and 14.606 min. We tentatively assigned these two substances as isomeric caffeic acid hexose.[32,33] Mass spectrometric analysis of compound **4** showed a quasi-molecular ion at m/z 325.1008 and a fragment ion at m/z 163.0461 (162 u, loss of hexose) resulting from *p*-coumaric acid. Therefore, compound **4** was tentatively identified as *p*-coumaric acid hexose.

Compounds **5**, **6**, and **7**, showing an $[M - H]^-$ ion at m/z 367, were observed at 19.387, 24.383, and 26.491 min, respectively. They were found to be various feruloylquinic acid (FQA) derivatives with similar UV spectra. The mass spectrometric detection of quasi-molecular ions allowed identification of hydroxycinnamic acid and to preliminarily distinguish its isomers with the aid of the data from previously published literature.[30,31,33−35] On the basis of information from the literature, the compounds **5**, **6**, and **7** were identified as 3-*O*-feruloyquinic acid (3-FQA), 5-*O*-feruloyquinic acid (5-FQA), and 4-*O*-feruloyquinic acid (4-FQA), respectively, by their quasi-molecular ions and elution order on the C_{18} column. Also, compounds **9**, **10**, **11**, and **12**, exhibiting $[M - H]^-$ quasi-molecular ions at m/z 515, were found to be dicaffeoylquinic acid (diCQA) derivatives, which were tentatively identified as 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA), 1,5-di-*O*-caffeoylquinic acid (1,5-diCQA), 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA), and 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA), respectively.

Compound **13** showed an identical $[M - H]^-$ quasi-molecular ion at m/z 193.0567 and similar UV spectrum to ferulic acid. However, compound **13** could not be identified as ferulic acid, since the experimentally determined retention time (36.75 min) of ferulic acid did not match the retention times of those peaks exhibiting an $[M - H]^-$ ion at m/z 193.0567. According to the literature, we tentatively assigned compound **13** as isoferulic acid.[36]

Compounds **14**, **15**, and **16**, exhibiting $[M - H]^-$ quasi-molecular ions at m/z 529 and similar UV spectra, were found to be three isomers of caffeoyl-feruloylquinic acid (CFQA). However, six CFQA isomers were previouly characterized in coffee samples and eluted on a C_{18} column as three pairs. In one pair, the first eluted component always presented a much lower concentration than the latter.[30,31] Unfortunately, the three isomers of CFQA in the present study could not be distinguished and identified. This may, therefore, require further study.

In summary, the main phenolic compounds in sunflower florets were derivatives of hydroxycinnamic acids (Tables 6 and 7). A standard of ferulic acid was analyzed, but a peak match was not found in the chromatograms. Most of the identified hydroxycinnamic acid derivatives were esterified with quinic acid. Fourteen hydroxycinnamic acid derivatives, namely, two caffeic acid hexose isomers, three feruloyquinic acid isomers, four caffeoylquinic acid isomers, three caffeoylferuloyquinic acid isomers, isoferulic acid, and isoquercitrin, were tentatively identified. In other words, phenolic compounds in sunflowers mainly manifested in the form of depsides.

Quantification of Phenolic Compounds. Table 7 showed the free and bound phenolic content in sunflower florets. For free phenolic compounds, nine compounds were

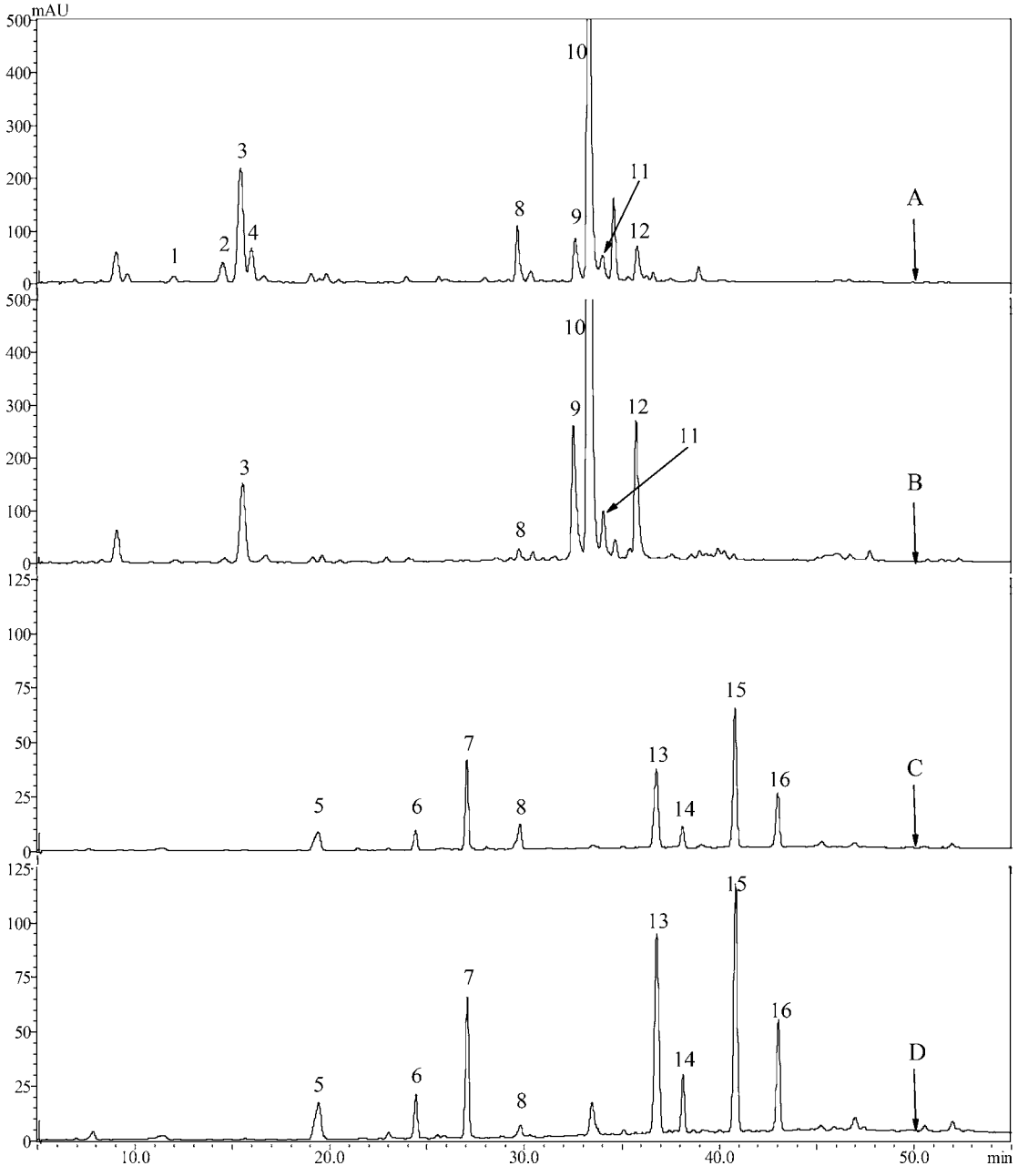

Figure 1. High-performance liquid chromatograms of (A) free phenolic compounds of ray floret; (B) free phenolic compounds of disc floret; (C) bound phenolic compounds of ray floret; and (D) bound phenolic compounds of disc floret, at 320 nm. The numbers of the peaks in this figure coincide with the compound numbers in Tables 6 and 7.

found with Rf and only six with Df. Compared to free phenolic compounds in Rf, two caffeic acid hexose isomers (**1**, **2**) and *p*-coumaric acid hexose (**4**) were not found in Df. Free phenolic contents in Rf and Df were 1685 and 2513 mg/100 g dw, respectively. Free phenolic compounds respectively represented 89.69% and 88.71% of the total phenolics in Rf and Df. 1,5-diCQA (**10**) was identified as the most abundant free phenolic compound in both Rf and Df, at 892 and 1626 mg/100 g dw. Isoquercitrin (**8**) stood as the second free phenolic compound in Rf followed by CGA (**3**) and 3,4-diCQA (**9**). In Df, the relative abundance of free phenolic compounds decreases in the order 4,5-diCQA (**12**), 3,4-diCQA (**9**), and CGA (**3**). It is important to point out that the level of CGA (**3**) in Rf was higher than that in daylily flowers[37] and *Prunus mume* flowers,[38] but lower than that in sunflower kernels.[35]

The bound phenolic fraction accounted for only a little more than 10% of the total phenolics in Rf and Df. A similar percentage had been reported for *C. siamea* and *L. leucocephalade* flowers. However, bound phenolics were far more abundant than free phenolics in *T. minor*, *P. obtuse*, and *M. arboreus* flowers.[39] Unlike the significantly different profile of free phenolic compounds, identical components (eight compounds) were found in bound phenolics from Rf and Df. However, the concentrations of these components differed significantly in Rf and Df. CFQA (**15**) was prominent in Df, and isoquercitrin replaced it in Rf. Interestingly, isoquercitrin (**8**) was observed in both free and bound phenolics from Rf and

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Table 6. Retention Times, UV Spectra, Mass Spectrometic Data, and Indentification of Phenolic Compounds Extracted from Ray and Disc Florets

peak	retention time (min)	identity	HPLC-DAD λ_{max} (nm)	$[M - H]^-$ m/z	fragment ion	ref
1	12.120	caffeic acid hexose	291, 325	341.0966	164.0781	32, 33
2	14.606	caffeic acid hexose	315	341.0965		32, 33
3	15.506	chlorogenic acid	303sh,[a] 325	353.0959	191.0625	30
4	16.070	*p*-coumaric acid hexose	282	325.1008	163.0461	
5	19.387	3-*O*-feruloylquinic acid	303sh, 323	367.1121	179.0412	30, 31, 33−35
6	24.383	5-*O*-feruloylquinic acid	226, 303sh, 326	367.1121		30, 31, 33−35
7	26.491	4-*O*-feruloylquinic acid	235, 302sh, 327	367.1117		30, 31, 33−35
8	29.594	isoquercitrin	254, 369	463.0979	355.1120	38
9	32.563	3,4-di-*O*-caffeoylquinic acid	242, 302sh, 323	515.1298	131.0887, 173.0887	30, 31, 33−35
10	33.251	1,5-di-*O*-caffeoylquinic acid	241, 301sh, 328	515.1295		30, 31, 33−35
11	33.968	3,5-di-*O*-caffeoylquinic acid	244, 301sh, 326	515.1302	145.0930	30, 31, 33−35
12	35.742	4,5-di-*O*-caffeoylquinic acid	244, 301sh, 326	515.1289		30, 31, 33−35
13	36.745	isoferulic acid	242, 301sh, 324	193.0567		36
14	38.095	caffeoylferuloyquinic acid	243, 305sh, 324	529.1460		30, 31
15	40.789	caffeoylferuloyquinic acid	242, 305sh, 328	529.1148		30, 31
16	42.979	caffeoylferuloyquinic acid	243, 303sh, 328	529.1451		30, 31

[a]sh = shoulder. Underline "_" is the λ_{max} of each compound.

Table 7. Free and Bound Phenolic Compounds in Ray and Disc Florets (mg/100 g dw) (n = 3)[a]

			ray florets, free		ray florets, bound		disc florets, free		disc florets, bound	
peak	compound	standard quantitative range	av	SD	av	SD	av	SD	av	SD
1	caffeic acid hexose	4.75−950	12.2	0.9	nd[b]	nd	nd	nd	nd	nd
2	caffeic acid hexose	4.75−950	38.0	1.9	nd	nd	nd	nd	nd	nd
3	chlorogenic acid	2.5−1250	209.7 a	3.3	nd	nd	127.3	0.2	nd	nd
4	*p*-coumaric acid hexose	4.97−994	64.8	1.4	nd	nd	nd	nd	nd	nd
5	3-*O*-feruloylquinic acid	2.6−1300	nd	nd	7.9	0.2	nd	nd	21.6 a	1.4
6	5-*O*-feruloylquinic acid	2.6−1300	nd	nd	4.4	0.3	nd	nd	10.4 a	0.5
7	4-*O*-feruloylquinic acid	2.6−1300	nd	nd	17.4	1.3	nd	nd	33.0 a	1.2
8	isoquercitrin	2.5−500	226.4 b	2.75	79.7 c	5.4	53.0 d	1.0	68.4 c	6.4
9	3,4-di-*O*-caffeoylquinic acid	3.65−1822	100.9	2.6	nd	nd	278.6 a	6.9	nd	nd
10	1,5-di-*O*-caffeoylquinic acid	3.65−1822	892.7	12.2	nd	nd	1626.6 a	26.1	nd	nd
11	3,5-di-*O*-caffeoylquinic acid	3.65−1822	60.1	3.2	nd	nd	88.6 a	2.9	nd	nd
12	4,5-di-*O*-caffeoylquinic acid	3.65−1822	80.2	1.4	nd	nd	340.0 a	20.9	nd	nd
13	isoferulic acid	2.5−500	nd	nd	13.6	1.23	nd	nd	37.7 a	2.6
14	caffeoylferuloyquinic acid	3.74−1871	nd	nd	6.9	0.9	nd	nd	20.8 a	2.3
15	caffeoylferuloyquinic acid	3.74−1871	nd	nd	46.2	5.5	nd	nd	90.4 a	8.0
16	caffeoylferuloyquinic acid	3.74−1871	nd	nd	17.7	2.0	nd	nd	37.7 a	4.3
	total phenolic		1684.9 b	5.9	193.6 c	16.3	2513.4 d	43.8	319.9 e	20.1
			1878.52 ± 18.11				2833.31 ± 36.02			

[a]A value that is significantly greater ($p < 0.05$) than its paired value is denoted by "a". Different lower case letters in the same line indicate significantly different values ($p < 0.05$). Paired results with no letter designation have no significant difference.. [b]nd = not detected.

Df, but it was present in lower levels than that in other flowers, such as tree peony flowers.[40]

Total phenolics were higher in Df (2833.31 mg/100g) than in Rf (1878.52 mg/100g). Zeng et al.[41] reported that total phenolics in Rf were 1.652 mg of catechin equivalents (CE)/g on a wet basis. Although the total phenolics in sunflower florets were lower than that in sunflower kernels,[35] *Prunus mume*, and *Opuntia* flowers,[38,42] it was higher than that in *T. erecta*, *C. sulphureus*, *A. leptopus*, and *B. glabra* flowers.[43] The results presented here clearly demonstrated that florets of sunflower are a suitable material for polyphenol extraction.

The nutritional composition and phenolic compounds of sunflower florets were first investigated in this study. The results showed that florets contain a high amount of dietary fiber. Df had a higher level of Fe and thus could be used as an iron deficiency anemia supplement. The florets are rich in branched-chain amino acids, which suggests they are good nutritional supplements for sportsmen. Sixteen phenolic compounds, mainly depsides, were identified and quantified. Free phenolic compounds accounted for almost 90% of the total phenolics in sunflower florets, with 1,5-diCQA being the most prominent one. In conclusion, on the basis of chemical composition, we assume that sunflower florets can be utilized in formulating functional foods with potential health benefits, such as regulating the gut eco-environment with dietary fiber, as a phenolic antioxidant, and as an Fe supplement. To further this research, potential biological effects of sunflower florets should be intensively investigated with in vitro or in vivo methods.

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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