Chemical Characterization of Oils and Meals from Wild Sunflower (*Helianthus petiolaris* Nutt)

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ABSTRACT: Chemical characterizations of oils and meals from the wild sunflower species (*Helianthus petiolaris* Nutt) and their comparison with those from cultivated sunflower (*H. annuus*) were performed. Seeds from spontaneous populations of *H. petiolaris* were harvested in Argentina in different years. The analytical parameters studied were as follows: (i) FA profile, PV, *p*-anisidine value, oxidative stability, phosphorus and phospholipid content, tocopherols, and polar compounds and waxes in the extracted oils; (ii) moisture, ash, crude fiber, metals, sugars, urease activity, starch, protein, available lysine, neutral detergent fiber, acid detergent fiber, lignin, and gross energy, and amino acid content in the residual meals. The products from wild sunflower seed, which yielded 27–30% oil by solvent extraction, showed some characteristics similar to the commercial products. Nevertheless, the oil had lower quality and stability owing to the high unsaturation levels and lower concentrations of antioxidant components, and the meal had a lower protein content. The phospholipid content was significantly lower than in industrial crude sunflower oils. Most of the important parameters in the meal such as available lysine, gross energy, and digestibility compared favorably with those for cultivated sunflower meals. The results showed the potential for using these meals for animal feed.

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KEY WORDS: Amino acids, chromatography, *Helianthus petiolaris*, meal, oil, quality, waxes, wild sunflower.

Helianthus petiolaris Nutt is an annual wild sunflower species native to the Americas. It grows in dense clumps on loose, sandy soils. In Argentina, it is widespread in the semiarid lands of Córdoba, San Luis, La Pampa, and Buenos Aires provinces, where cultivated sunflower (*H. annuus*) is also found. *Helianthus petiolaris* hybridizes with *H. annuus* either spontaneously or artificially. *Helianthus petiolaris* is the most important source of cytoplasmic male-sterility for sunflower hybrid seed production. It has also been used as a source of genes for resistance to diseases, drought resistance, and to improvement of oil quality in sunflower breeding programs (1,2).

Information about *H. petiolaris* oil and meal is relatively scarce. Interest has centered mainly around seed oil and protein content, FA composition, amino acid composition, and mineral content (3–6). Analyses of *H. petiolaris* populations from Canada and the United States found a seed oil content of 22.8–39.5%; the oil contained mainly oleic acid (15–33%) and linoleic acid (55–80%). Variability in the concentration and composition of the oil was attributed to environmental factors, plant multi-headed characteristic, and degree of maturation. From the FA composition, it has been concluded that this species could provide the genetic resource for improving oil and FA composition in cultivated sunflowers. However, there is a need to extend the knowledge to the minor components in the oil and to consider other parameters of quality and stability beyond those evaluated in previous works. A whole-seed crude protein content of 17.1% has been reported (6), and the analysis of protein composition indicated a high lysine level (2). This wild sunflower species may have potential for increasing the protein concentration of cultivated sunflower seeds. Concentrations of calcium, phosphorus, magnesium, and potassium in the seed also were determined (6).

The aim of this work was to perform a complete study on the chemical composition and quality of oil and residual meal from *H. petiolaris* Nutt and to compare these results with those from cultivated sunflower.

EXPERIMENTAL PROCEDURES

Plant material. Seeds from spontaneous populations of *H. petiolaris* in several regions of Argentina were harvested in different years: south of Córdoba, 1995 (Sample A); northeast of La Pampa and west of Buenos Aires, 1997 (Sample B); Northeast of La Pampa, 1998 (Sample C); and northeast of La Pampa, 1999 (Sample D). Seeds were cleaned and selected; vain, immature, or underdeveloped seeds were rejected.

Oil extraction. Whole seeds were ground and extracted with *n*-hexane (b.p. 68–72°C) in a Soxhlet apparatus following IUPAC Standard Method 1.122 (7). The solvent contained in extracted oils and residual meals was removed by a nitrogen stream. The oil was weighed in order to calculate the extraction yields. Solvent-free residual meals were ground in a mill to pass through a 1-mm screen. Oils and meals were stored at 5°C under nitrogen atmosphere for further analysis.

Oil characterization. Several analytical methods were used to evaluate oil composition, quality, and oxidative stability. Standard AOCS (8) and IUPAC (7) official methods

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were used to determine acidity or FFA (IUPAC 2.201), PV (IUPAC 2.501), *p*-anisidine value (AOCS Cd 18-90), total phosphorus content (AOCS Ca 12-55), iodine value (IUPAC 2.205), saponification value (IUPAC 2.202), and refractive index (IUPAC 2.102). The oxidative stability index (OSI), represented as induction time in hours, was measured with a Metrohm 679 Rancimat (Metrohm, Herisau, Switzerland) at 98°C and 20 L/h airflow. FA composition was determined by GC analysis according to IUPAC 2.301-2.302 standard methods (7). The FAME were separated on a 10% GP-DEGS-PS (L = 2 m; i.d. = 0.32 cm) tubular column and quantified by FID using a Varian 3700 gas chromatograph (Varian Associates Inc., Palo Alto, CA). Tocopherol content was measured by HPLC using AOCS method Ce 8-89 (8). A Varian Vista 5500 HPLC system (Varian Associates Inc.) with a fluorescence detector set at 290–330 nm and a LiChrosorb Si-60 (250 × 4 mm, 5 μm particle size) column (Merck, Darmstadt, Germany) was used. Quantitative determination of phospholipids was carried out by enrichment using diol solid phase extraction cartridges (J.T.Baker Inc., Phillipsburg, NJ) and subsequent analysis by HPLC (9). A Varian Vista 5500 HPLC system with a UV detector set at 206 nm and a LiChrosorb Si-60 (250 × 4 mm, 5 μm particle size) column was used. Wax composition was determined by separation with a silica gel chromatographic column and analysis by GCO. A Varian 3700 GLC with FID detector and on-column injection (Varian Associates Inc.), an HP5, 11 m × 0.32 mm (0.52 μm) capillary column (Hewlett-Packard, Palo Alto, CA) and a Millenium 2010 data processor (Millipore Corporation, Milford, MA) were used (10). Separation of polar compounds was carried out by column chromatography with silica gel and verified by TLC according to AOCS method Cd 20-91 (8). Polar compounds were analyzed by high-performance size-exclusion chromatography (11). A Waters HPLC, two 500 and 100 Å Ultrastyragel (0.77 × 30 cm) columns connected in series, a refractive index detector, and a Millenium 2010 Chromatography Manager were used.

Residual meal characterization. Standard AOCS (8) official methods were used to determine moisture (AOCS Ba 2a-38), ash (AOCS Ba 5a-49), crude fiber (AOCS Ba 6-84), phosphorus (AOCS Ca 12-55), and urease activity (AOCS Ba 9-58). Total sugars (reducing and nonreducing) and starch were determined according to AOAC 925.05-925.4-959.11 official methods (12) and the Nelson–Somogyi method (13). The Kjeldahl method with $CuSO_4/K_2SO_4$ catalyst was used to determine protein content (12,14). Neutral detergent fiber (NDF), acid detergent fiber, and acid detergent lignin performed the cell wall and the cell content (14). Gross energy was measured with a ballistic bomb calorimeter (CB-370 Gallenkamp) (14). Fe, Cu, Zn, and Mg were determined by flame atomic absorption spectrophotometry with a GBC 902 Atomic Absorption Spectrometer (GBC Scientific Equipment, Victoria, Australia). *In vitro* digestibility was determined by the procedure described by Tilley and Terry (14). Available lysine determinations were made by a modified Carpenter's procedure that uses 2,4-dinitrofluorobenzene (15). Amino acid analysis was performed by HPLC as 4-(dimethylamino)azobenzene–4′–sulphonyl chloride (DABS-cl) derivatives after 24 h hydrolysis with *6 N* hydrochloric acid at 120°C (16). A Waters 600E chromatograph (Waters Associates, Milford, MA), a Waters 996 photodiode array detector set at 436 nm, and a Supelcosil LC-DABS (150 × 4.6 mm i.d., 3 μm particle size) column (Supelco, Bellefonte, PA) were used.

RESULTS AND DISCUSSION

Wild sunflower seeds yielded oil content between 27 and 30% by extraction with hexane, a range similar to those reported for this species by Dorrell and Whelan (3) as well as Seiler (5), which amounted to 22.8–39% and 25–32%, respectively. In cultivated sunflower, an oil content of 30% or less can be found in the non-oilseed for confectionary use and of 40% or greater in the oilseed.

In Table 1 the main physicochemical characteristics of wild sunflower oils are summarized and compared with those of cultivated sunflower oils obtained by hexane extraction. The oil was yellowish in color and some general indexes such as refractive index, iodine value, and saponification value were within the expected ranges for cultivated sunflower. The FA content was high in oleic and linoleic acids, 12–29% and 64–80% respectively. The percentage of saturated FA, mainly stearic and palmitic acids, was less than 10%. The variability can be explained in terms of the different harvesting areas and years, since environmental conditions affect the maturation period of achenes in this multiheaded variety. A review of reports for different *H. petiolaris* populations from the United States and Canada produced the following ranges: 4–11% for saturated FA, 12–33% for oleic acid, and 55–84% for linoleic acid (3–5). The FA composition for *H. petiolaris* showed more unsaturation than that of cultivated sunflower from the same country (18,19).

Referring to minor compounds, the oils showed lower concentrations of natural antioxidants, such as tocopherols and phospholipids, than cultivated sunflower. The phospholipid and phosphorus contents were comparable to those for water-degummed sunflower oils, which had values between 0.10–0.21 wt% and 44–85 mg/kg, respectively (18). The relative proportions of the main phospholipids varied widely, i.e., PC (8.4–17.1%), PI (5.1–44.0%), PE (8.2–16.8%) and (26.2–78.3%). The phospholipid profile exhibited a high relative percentage of PA, as is the case with water-degummed sunflower oils (18). The main tocopherol in wild sunflower is α-tocopherol, although small amounts of β-tocopherol were also detected in sample A.

The main wax components were esters of between 33 and 48 carbon atoms. The wax chromatographic analysis afforded the following results in ester percentages: C_{33}, 3.4–4.6; C_{34}, 1.7–2.6; C_{36}, 6.7–7.1; C_{37}, 5.3–6.0; C_{38}, 4.9–5.3; C_{39}, 2.5–3.0; C_{40}, 7.8–9.3; C_{41}, 5.4–10.9; C_{42}, 8.6–10.2; C_{43}, 2.9–4.0; C_{44}, 9.4–11.8; C_{45}, 2.3–2.9; C_{46}, 10.8–12.8; C_{47}, 1.6–2.2; C_{48}, 14.0–15.6. The higher concentrations correspond to C_{44},

TABLE 1
General Physicochemical Characteristics of *Helianthus petiolaris* Oils

Analytical determination	Sample[a] A	B	C	D	Cultivated sunflower (17–19)
Refractive index(20°C)	1.4620	1.4727	1.4747	1.4749	1.467–1.469
Acidity (% oleic acid)	1.58	1.43	—	2.54	0.98–1.68
P-Anisidine value	—	6.26	—	3.9	1.46–2.07
PV (meq/kg)	—	11.7	—	4.1	3.1–11.7
Iodine value	136	147	141	134	110–143
Saponification value	178	183	195	191	188–194
OSI (h at 98°C)	2.67	5.37	3.10	5.44	14.7–22.4
FA (%)					
$C_{16:0}$	4.7	6.8	5.9	5.8	6–7
$C_{18:0}$	1.8	2.7	2.4	1.9	3–4
$C_{18:1}$	25.3	13.8	11.8	28.6	22–24
$C_{18:2}$	68.2	76.7	79.9	63.7	66–68
Phospholipids (wt%)	0.081	0.043	0.063	0.130	0.72–1.2
Phosphorus (mg/kg)	32.5	15.9	28.1	84.8	200–480
Tocopherols (mg/kg)	401	343	317	559	447–900
Total waxes (mg/kg)	—	678	—	1128	400–1100
Polar compounds (wt%)	—	11.4	—	13.6	6.3–9.8

[a]Arithmetic means of duplicate or triplicate determinations. OSI, oxidative stability index.

C_{46}, and C_{48}, whereas the most soluble fraction ($<C_{42}$) represented less than 50%. The wax content differed between the two samples analyzed, 680 and 1184 mg/kg, respectively, but wax profiles were similar, in agreement with previous studies on cultivated sunflower oil (10,18).

Wild sunflower oils showed a higher rate of deterioration, as measured by standard values (acidity, peroxide, *p*-anisidine) and polar compounds, and a lower OSI than cultivated sunflower oils. The analysis of polar compounds, mainly oxidized triglyceride monomers (OTG), diglycerides (DG), and FFA, is reported as a good measurement for early and advanced stages of deterioration (11,19). Sample B showed a higher level of OTG (56.8%) and relatively lower levels of DG (13.1%) and FFA (30.1%), indicating oxidative deterioration. On the other hand, sample D had a lower percentage of OTG (15.8%) than DG (26.5%) and FFA (57.7%), showing a prevailing hydrolytic deterioration. Peroxide, anisidine, and acidity values confirmed the preceding results. The higher concentration of unsaturated FA and the lower level of tocopherols and phospholipids, which have strong antioxidant effects, can explain this behavior. It is well known that phospholipids may act synergistically with tocopherols to inhibit the autoxidation of PUFA (19).

Table 2 summarizes the chemical composition of wild sunflower meals, which compares favorably with most oilseed meals. It had a reasonably high level of crude protein [20.6–23.1% dry basis (d.b.)], higher than that reported by Seiler (6) for the same species (17.1%) but lower than whole-seed cultivated sunflower meal, which ranges from 26 to 34% (20,21). High fiber contents are inversely related to high protein contents. Wild sunflower meals had higher contents of fiber, particularly cellulose and lignin, and lower contents of ash than cultivated sunflower meals (21–30% and 7.0-8.2 %) (20,21). Total sugar content was similar to that of cultivated sunflower meal, although the ratio of reducing to nonreducing sugars was very low and the concentration of hydrolyzable carbohydrates was relatively high. No urease activity was detected in the four samples. Calcium and phosphorus concentrations were lower than those previously reported by Seiler (6) for wild sunflower seeds (Ca, 1.9 g/kg; P, 4.9 g/kg), whereas magnesium concentrations were higher (2.4 g/kg). The Ca/P ratios obtained (0.57–0.72) were acceptable for utilization by ruminants since high Ca/P ratios can possibly cause nutritional disorders. Concentrations of other chemical compounds were within ranges reported for whole-seed sunflower meals.

Table 3 shows digestibility coefficients for the residual wild sunflower meal as compared with those for cultivated sunflower meal (21,22). The more readily utilized nutrients are inside

TABLE 2
Chemical Composition[a] of *Helianthus petiolaris* Seed Meals

Analytical determination	Samples A	B	C	D
Moisture (%)	5.0	8.8	5.6	8.5
Ash (% d.m.)	4.2	5.7	—	6.5
Protein (N × 6.25) (% d.m.)	23.1	20.6	21.5	22.8
Crude fiber (% d.m.)	24.5	26.8	39.4	24.2
Reducing sugars (% glucose)	0.2	0.14	0.2	0.2
Nonreducing sugars (% sucrose)	4.1	1.2	5.8	1.2
Hydrolyzable Carbohydrates (% starch)	7.0	9.7	7.9	10.3
Total phosphorus (g/kg)	1.8	1.3	1.3	0.7
Mg (g/kg)	3.8	3.4	—	3.3
Ca (g/kg)	1.3	1.0	1.0	0.4
Fe (ppm)	385.9	554.0	—	498.8
Cu (ppm)	64.9	70.0	—	20.5
Zn (ppm)	64.8	77.0	—	78.4

[a]Arithmetic means of duplicate or triplicate determinations. d.m., dry matter.

TABLE 3
Comparison of Digestibility Coefficients[a] Between Wild Sunflower Seed Meals and Cultivated Sunflower Whole-seed Meals

Analytical determinations	Wild sunflower samples			Cultivated sunflower
	B	C	D	(20–22)
Dry matter (%)	97.6	94.4	92.3	88–95
Crude protein (% d.b.)	20.6	21.5	22.8	26–34
Crude ffiber (% d.b.)	39.4	26.8	24.2	18–35
ADF (% d.b.)	40.9	41.6	47.7	22–33
NDF (% d.b.)	58.9	57.4	61.2	34–48
Lignin (% d.b.)	18.3	17.8	21.1	8–11
Hemicellulose (%)	18	15.8	13.5	12–17
Cellulose (%)	22.6	23.8	20.6	14–20
Digestibility (% d.b.)	44.1	45.7	36.7	43.6
Gross energy (kcal/kg)	4937	5105	4657	4193
Digestible energy (kcal/kg)	1940	2010	1610	1920–2337

[a]Arithmetic means of duplicate or triplicate determinations. % d.b., percentage based on dry matter; ADF, acid detergent fiber; NDF, neutral detergent fiber.

the cytoplasm and include protein, soluble carbohydrates, soluble minerals, and lipids. The NDF accounts for cell wall components. In our case, NDF was higher than for cultivated sunflower, and cellulose and hemicellulose values were slightly greater than in cultivated sunflower. The high fiber content indicates some lignification. Values for digestibility, gross energy, and digestible energy were comparable to those for cultivated sunflower.

The average amino acid composition (%) of wild sunflower seed meal was as follows: arginine, n.d.; histidine, 0.31, isoleucine, 0.43; leucine, 1.25; lysine, 2.34; methionine, n.d.; phenylalanine, 0.81; valine, 0.86; threonine, 0.27; aspartic acid, 3.63; asparagine, 0.46; glutamine, n.d.; serine, 0.90; glycine, 1.29; alanine, 0.99; proline, 1.03; cystine, 0.6; and tyrosine, 0.29. The values for essential amino acid composition were relatively lower than those for cultivated sunflower (22), except for lysine, which was twice the value with an availability of 94%. The nonessential amino acid concentration does not vary greatly with respect to the cultivated species (22). The most limiting amino acid in cultivated sunflower meal is lysine, and the lack of lysine reduces the nutritive value of the protein for growth.

This report provides quality and nutritional data about *H. petiolaris*, which are useful to assess its potential for practical applications. In general, most values found were comparable to those for cultivated sunflower oil and meal. The wild sunflower oil showed a higher deterioration and lower oxidative stability due to the higher concentration of unsaturated fatty acid and lower level of tocopherols and phospholipids, which have strong antioxidant effects. Wild sunflower could be used as an alternative raw material to obtain meal for animal feeding since its gross energy, digestibility, and available lysine compare favorably with the values typically found in commercial sunflower meal. The use of *H. petiolaris* in a sunflower-breeding program should also be considered as a means of raising the level of lysine in the commercial sunflower.

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Notes to Authors/Re: J10606

1. Abstract, 2nd sentence; Experimental Procedures, 1st paragraph, 1st sentence. What is a "spontaneous population"? Would this be the same as a "wild" or "indigenous" or "free-living" population?
2. Introduction, 2nd paragraph, 5th sentence. In "it has been concluded," should you include a literature citation? Or is this your conclusion?
3. Experimental Procedures, 1st paragraph, last sentence. What is a "vain seed"? This may be a problem of translation from Spanish to English.
4. Experimental Procedures, 4th paragraph, 4th sentence. Unclear what "Neutral detergent fiber (NDF), acid detergent fiber, and acid detergent lignin **performed** the cell wall and the cell content (14)" means.
5. Experimental Procedures, 4th paragraph, 7th sentence. Here you refer to "the procedure described by Tilley and Terry (14)," but reference 14 is a text edited by V.Y. Guiragossian *et al*. Are you referring to an article by Tilley and Terry in the text by Guiragossian *et al.*? If so, please include the title of the article, and indicate the inclusive page numbers.
6. Experimental Procedures, 4th paragraph. Verify change to "Amino acid analysis was performed by HPLC as 4-(dimethylamino)azobenzene-4′-sulfonyl chloride (DABS-Cl) derivatives . . . ."
7. Experimental Procedures, 4th paragraph, final sentence. Verify spelling change to Supelcosil LC-DABS.
8. Results and Discussion, 1st paragraph, 2nd sentence. Unclear what "In cultivated sunflower an oil content of 30% or less can be found in the non-oilseed" means.
9. Results and Discussion, 1st paragraph, 2nd sentence. How does "for confectionary use [= for use in making candy?]" relate to the rest of the sentence?
10. Results and Discussion, 5th paragraph, 1st sentence. Verify that change to "a higher rate of deterioration" has not changed your meaning.
11. Results and Discussion, 6th paragraph, 2nd sentence, vs. Table 2 vs. Table 3. In text you have used "d.b." [= dry basis?] whereas in the Table 2 you have used "d.m." [= dry matter?] but in Table 3 you used "d.b.". Should these three reference points be the same? If so, which do you wish to use?
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