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Analytical Methods

Identification and quantification of phenolic compounds from sunflower (*Helianthus annuus* L.) kernels and shells by HPLC-DAD/ESI-MS^n

Georg M. Weisz, Dietmar R. Kammerer *, Reinhold Carle

Hohenheim University, Institute of Food Science and Biotechnology, Chair Plant Foodstuff Technology, Garbenstraße 25, D-70599 Stuttgart, Germany

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ABSTRACT

Phenolic compounds were extracted from defatted sunflower (*Helianthus annuus* L.) kernels and shells and characterised by HPLC with diode array and electrospray ionisation (ESI) mass spectrometric detection in the negative mode. Quantification of individual compounds was carried out by external calibration. Among the eleven compounds analysed 5-*O*-caffeoylquinic acid was predominant amounting up to 59.1 mg/100 g in the shells and 3050.5 mg/100 g in the kernels. The specific fragmentation patterns of mono- and dihydroxycinnamoylquinic acids allowed the unambiguous distinction of several stereoisomers which have not been described for sunflower seeds and seed shells so far. The total phenolic content of about 4200 mg/100 g on a dry matter basis revealed defatted sunflower meal to be a promising source of phenolic compounds that might be recovered and used as natural antioxidants. Furthermore, the press residues originating from sunflower oil extraction were shown to be still rich in phenolic antioxidants, thus, providing the opportunity to valorize these by-products in terms of sustainable agricultural production.

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1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops. Besides palm, soy and rapeseed oil, sunflower oil is ranking fourth with a worldwide production of about 10.6 million metric tons (mt) in 2006 (FAO-STAT, 2008). Sunflowers have been known since the 26th century B.C. and have their origin in the lowlands of Mesoamerica (Pope et al., 2001). Nowadays, two main types of sunflowers are grown, the oilseed and non-oilseed or confectionary types.

The residues resulting from oil extraction are a valuable and nutritious by-product with high protein contents that range from 40% to 50% of the defatted kernels depending on the type of the extraction process (González-Pérez & Vereijken, 2007). Therefore, meal prepared from the press residues is primarily used as ruminant feed. In contrast to legumes, sunflowers prove to be a protein source of great interest for human nutrition, especially due to their sensory but also nutritional and functional properties (Sodini & Canella, 1977). Besides the high protein contents, the residues originating from oil extraction are rich in phenolic compounds which account for 1–4% of the total mass with chlorogenic acid being the predominant component (Leung, Fenton, & Clandinin, 1981; Milić, Stojanović, Vučurević, & Turčić, 1968; Pedrosa et al., 2000). The occurrence of high amounts of phenolic compounds significantly affects the quality of sunflower proteins, e.g., by reducing the digestibility, or causing undesirable browning and structural modifications which adversely alter the functional properties of the proteins and their behaviour in various food systems. Both polyphenol oxidase activities and conventional protein extraction which is performed under alkaline conditions result in the oxidation of polyphenols and thus, their conversion into *o*-quinones as in the case of caffeoyl derivatives or further *o*-dihydroxy structures. These highly reactive compounds may form covalent bonds with thiol or amino groups of proteins. The nutritive value of sunflower proteins is generally limited by their genuine lysine deficiency and is further lowered as a consequence of the afore-mentioned reactions, since the condensation products cannot be metabolized by humans (Synge, 1975). For those reasons, sunflower proteins have not been used so far on an industrial scale for human nutrition.

In contrast to these adverse effects of phenolic compounds on the functional and nutritional properties of proteins, numerous polyphenols such as caffeic, chlorogenic and ferulic acids have been shown in many studies to exert a high antioxidative potential, which might be beneficial both from a technofunctional and biofunctional point of view (Maier, Schieber, Kammerer, & Carle, 2009; Moure et al., 2001; Velioglu, Mazza, Gao, & Oomah, 1998). De Leonardis, Macciola, and Di Rocco (2003) demonstrated the capability of sunflower polyphenols to be used as effective antioxidants for sunflower oil. In a subsequent study, a procedure for recovering phenolic antioxidants from sunflower seed shells by solvent extraction was developed on laboratory scale (De Leonardis, Macciola, & Di Domenico, 2005). This seed shell extract was

* Corresponding author. Tel.: +49 (0)711 459 22995; fax: +49 (0)711 459 24110.
E-mail address: dietmark@uni-hohenheim.de (D.R. Kammerer).

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reported to be composed of protocatechuic, chlorogenic, caffeic, syringic, ferulic and *o*-cinnamic acids.

Several studies for the determination of phenolic compounds in sunflowers have been reported (Aramendia et al., 2000; Dabrowski & Sosulski, 1984; Milić et al., 1968; Pedrosa et al., 2000; Sabir, Sosulski, & Kernan, 1974). However, peak assignment still remains doubtful in some of these studies, and a method for unambiguous compound characterisation making use of mass spectrometric detection with specific fragmentation of individual molecular ions is still lacking. For this reason, an LC/MS method should be developed, based on a method described by Schütz, Kammerer, Carle, and Schieber (2005), Schütz, Kammerer, Carle, and Schieber (2004), to more thoroughly characterise the phenolic profile in sunflower kernels and shells of various origins by the mass-to-charge ratios of individual phenolic compounds. Additionally, collision induced dissociation (CID) experiments should be used to differentiate between stereoisomeric phenolic compounds. Besides the mass spectrometric determination of phenolic compounds their contents should be quantified both in kernels and shells of oilseed and non-oilseed sunflowers and in a residue originating from an oil extraction process to assess the potential of various matrices as a source of phenolic antioxidants.

2. Materials and methods

2.1. Plant material

The plant material analysed in the present study consisted of whole sunflower seeds, dehulled seeds and a press residue from an industrial oil production. Furthermore, seeds of oilseed and non-oilseed types were compared in terms of their phenolic profiles and contents. Details of the samples are given in Table 1.

2.2. Solvents and reagents

Solvents and reagents were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. 1,3-Di-*O*-caffeoylquinic acid (1,3-diCQA) (cynarin) was from Roth (Karlsruhe, Germany); 5-*O*-caffeoylquinic acid (5-CQA) was obtained from Extrasynthèse (Lyon, France), caffeic acid (CA) was purchased from Fluka (Buchs, Switzerland). Deionised water was used throughout.

2.3. Sample preparation

Whole sunflower seeds were manually separated into kernels and shells. These were minced separately in a laboratory grinder (Typ A 10, IKA Werke GmbH & Co. KG, Staufen, Germany) for 2 min, avoiding high temperature by cooling the grinder with water. Aliquots of 5 g of the meal obtained were defatted with 500 mL *n*-hexane in a soxhlet extractor and left to dry over night at room temperature. An aliquot of 1 g of each sample was extracted twice by stirring in 280 mL aqueous methanol (60%, v/v) for 1 h at ambient temperature. After centrifugation at 3000 *g* and filtration through Whatman 595½ filter papers the two extracts were combined and evaporated to dryness *in vacuo* at 30 °C. The residue obtained was dissolved in 4 mL of 50% aqueous methanol, membrane filtered (Macherey-Nagel Chromafil® RC-45/15 MS) and used for HPLC-DAD-MS^n analysis.

2.4. Recovery studies

Recovery studies were performed in separate experiments by adding suitable amounts of 5-CQA stock solutions (1000 mg/L) during extraction of 'Dovgan' kernels and shells, respectively. The samples were treated as described above. The determinations of the recovery studies were performed in duplicate.

2.5. HPLC analysis

Polyphenol analyses were carried out using a series 1100 HPLC (Hewlett–Packard, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detection system. The column used was a 150 mm × 3.0 mm inner diameter (i.d.), 4 μm particle size, C18 Hydro-Synergi from Phenomenex (Torrance, CA), with a security guard 4.0 mm × 2.0 mm i.d. C18 ODS column, operated at 25 °C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 10% B to 17.2% B (18 min), 17.2% B to 23% B (12 min), 23% B isocratic (10 min), 23% B to 31.3% B (13 min), 31.3% B to 46% B (12 min), 46% B to 55% B (5 min), 55% B to 100% B (5 min), 100% B isocratic (8 min), 100% B to 10% B (2 min), 10% B isocratic (5 min). Total run time was 90 min. The injection volume for all samples was 5 μL. Phenolic compounds were monitored separately at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids), at a flow rate of 0.4 mL/min. Additionally, UV/Vis spectra were recorded in the range of 200–600 nm at a spectral acquisition rate of 1.25 scans/s (peak width 0.2 min).

For calibration appropriate volumes of standard stock solutions (1000 mg/L) were diluted with methanol, and ten concentration levels (0.5, 2, 5, 10, 20, 40, 100, 200, 500 and 1000 mg/L) were analysed. Individual compounds were quantified using a calibration curve of the corresponding standard compound. When reference compounds were not available, the calibration of structurally related substances was used, including a molecular weight correction factor (Chandra, Rana, & Li, 2001). All determinations were performed in duplicate.

2.6. LC-MS analysis

Analyses were performed with the HPLC system described above coupled on-line with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an electrospray ionisation (ESI) source. Data acquisition and processing were

Table 1
Specification of the sample material used for polyphenols analysis.

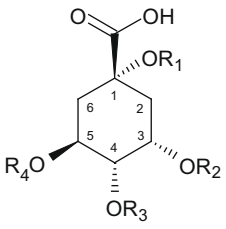
Sample name	Type	Sample matrix	Origin	Crop year	Source
Gusto	Non-oilseed	Dehulled seeds	Unknown	Unknown	Gusto AG, Hohenpolding, Germany
Naturalita	Non-oilseed	Dehulled seeds	Unknown	Unknown	Maryland Trockenfrucht Vertriebs GmbH, Henstedt-Ulzburg, Germany
Dovgan	Non-oilseed	Whole seeds	Unknown	Unknown	DOVGAN GmbH, Hamburg, Germany
Schilfer	Oilseed	Residue from oil production	Unknown	2006	Teutoburger Ölmühle GmbH & Co. KG, Ibbenbüren, Germany
Geiger	Oilseed	Whole seeds	Germany	2006	Carl Geiger GmbH & Co.KG, Marbach, Germany
Italy	Oilseed	Whole seeds	Italy	2006	Teutoburger Ölmühle GmbH & Co. KG, Ibbenbüren, Germany
France	Oilseed	Whole seeds	France	2006	Teutoburger Ölmühle GmbH & Co. KG, Ibbenbüren, Germany
Germany	Oilseed	Whole seeds	Germany	2006	Teutoburger Ölmühle GmbH & Co. KG, Ibbenbüren, Germany

performed using Esquire Control software. Negative ion mass spectra of the column eluate were recorded in the range *m/z* 50–1000. Nitrogen was used both as drying gas at a flow rate of 9.0 L/min and as nebulizing gas at a pressure of 45.0 psi. The nebulizer temperature was set at 365 °C, and a potential of 4500 V was used on the capillary. Helium was used as collision gas for CID at a pressure of 4.9×10^{-6} mbar. 5-CQA was used for the optimisation of ionisation and fragmentation parameters.

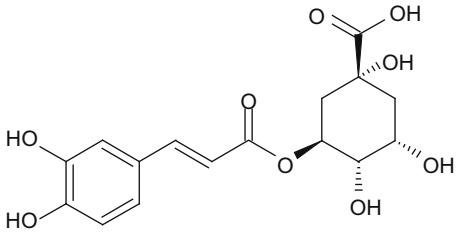
3. Results and discussion

3.1. Methodology

A C18 stationary phase with hydrophilic endcapping, which has been demonstrated to be suitable for the determination of phenolic compounds from various matrices such as apples, mangos, black carrots, grapes and artichokes (Kammerer, Carle, & Schieber, 2004a; Kammerer, Claus, Carle, & Schieber, 2004b; Schieber, Berardini, & Carle, 2003; Schieber, Keller, & Carle, 2001; Schütz et al., 2004), was used for the analysis of hydroxycinnamoyl derivatives of sunflower (Fig. 1). As can be seen from Fig. 2 baseline separation was achieved for nearly all of the major phenolic compounds of sunflower characterised in the present study. In preliminary trials, exhaustive extraction of the polyphenols was investigated using different extraction solvents such as acetone (100%, 80% and 60%), methanol (100%, 80% and 60%) and ethanol (100%, 80% and 60%). Since aqueous methanol (60%, v/v) gave highest yields (data not shown), it was used for all subsequent polyphenol extractions. In our previous investigations of phenolic compounds in various matrices C18-Sep-Pak cartridges proved to be a useful tool for the purification and fractionation of polyphenols, thus, improving peak separation and compound quantification by HPLC (Schütz

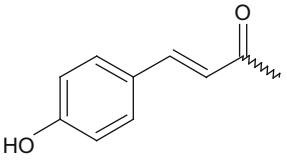


R_1, R_2, R_3 and R_4 = H: quinic acid

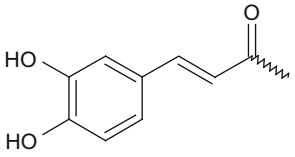


5-*O*-caffeoylquinic acid

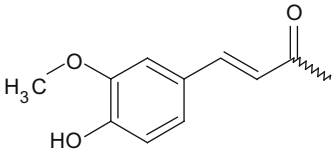
Structures and abbreviations of hydroxycinnamoyl substituents



p-coumaroyl (*p*Co)



caffeoyl (C)



feruloyl (F)

Name and abbreviation	Peak number	R_1	R_2	R_3	R_4
3-*O*-caffeoylquinic acid (3-CQA)	1	H	C	H	H
5-*O*-caffeoylquinic acid (5-CQA)	2	H	H	H	C
4-*O*-caffeoylquinic acid (4-CQA)	3	H	H	C	H
5-*O*-*p*-coumaroylquinic acid	6	H	H	H	*p*Co
5-*O*-feruloylquinic acid	7	H	H	H	F
3,4-di-*O*-caffeoylquinic acid (3,4-diCQA)	9	H	C	C	H
3,5-di-*O*-caffeoylquinic acid (3,5-diCQA)	10	H	C	H	C
4,5-di-*O*-caffeoylquinic acid (4,5-diCQA)	11	H	H	C	C

Fig. 1. Structures of selected phenolic acids detected in sunflower seeds.

et al., 2004). Since coextracted components did not interfere in the present study, thus, not affecting peak separation, and since coeluting phenolic compounds were not observed, the use of C18 cartridges was unnecessary for pretreating sunflower extracts before HPLC analysis. For this reason, further sample preparations were performed without solid phase extraction.

3.2. Recovery studies

Recovery studies for hydroxycinnamic acids, especially 5-CQA, were performed by standard addition during extraction of 'Dovgan' kernels and shells, yielding recovery rates of 92% and 83%, respectively. The lower value for 5-CQA recovery from the shells might be due to polyphenol adsorption onto the lignocellulosic shell material which is lacking in the kernels. These studies were carried out to demonstrate the suitability of the extraction method presented here, however, the recovery rates were not considered in the calculation of the phenolic contents of the kernels and shells.

3.3. Identification of phenolic compounds by HPLC/MS

Most constituents showed similar UV spectra with maximum absorbance at 320–330 nm and a shoulder around 300–310 nm, characterising them as hydroxycinnamic acid derivatives. Since only CA, ferulic acid (FA), 5-CQA and 1,3-diCQA were available as reference compounds, HPLC coupled to mass spectrometry proved to be extremely useful for peak assignment and further characterisation of individual compounds. In particular, the electrospray ionisation (ESI) has been widely applied in polyphenol analysis. While it provides information on their molecular masses due to

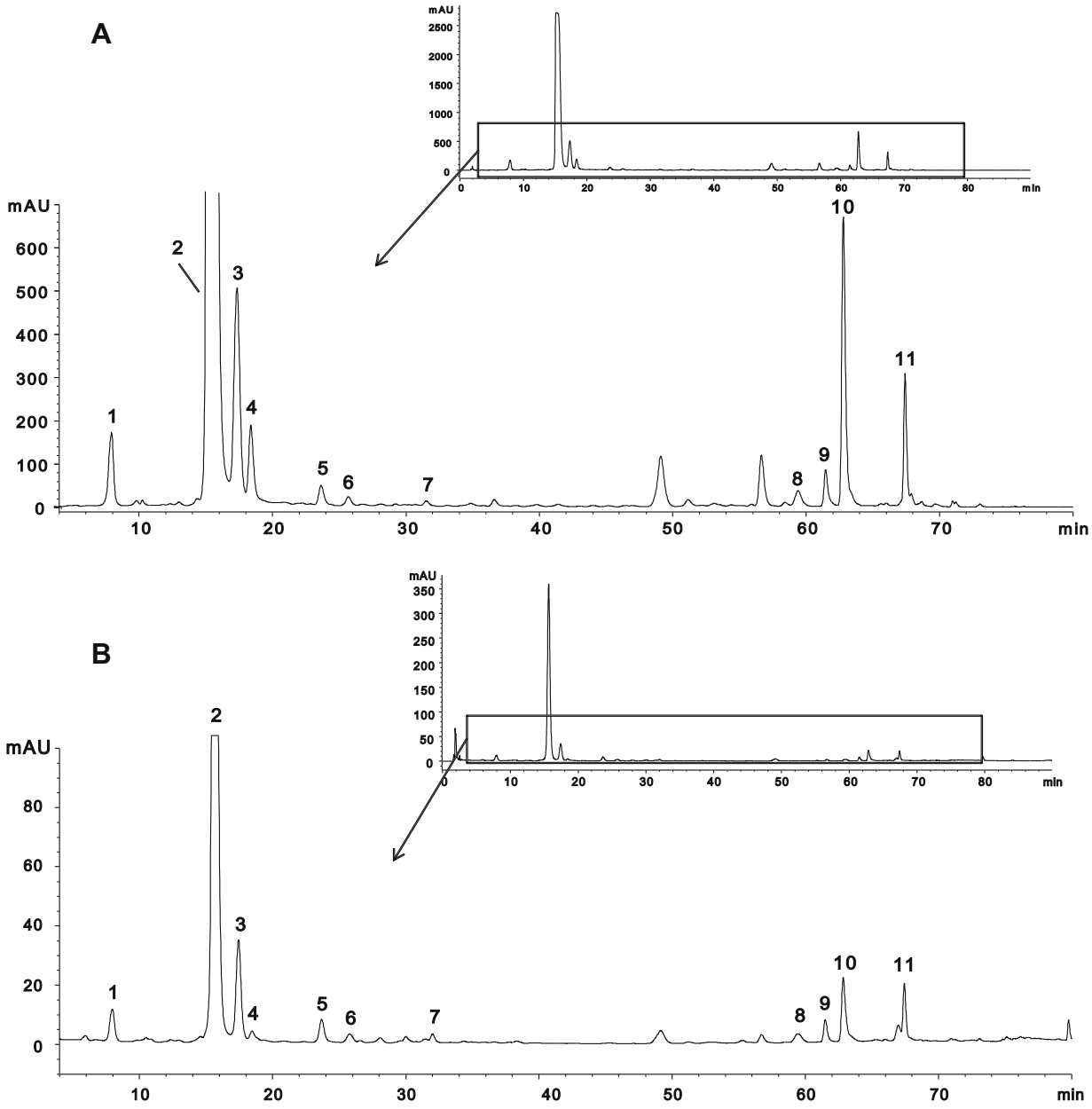


Fig. 2. Separation of caffeoylquinic acids and further phenolic compounds in sunflower kernels (A) and shells (B) by HPLC-DAD (320 nm); peak assignment: (1) 3-*O*-caffeoylqunic acid, (2) 5-*O*-caffeoylquinic acid, (3) 4-*O*-caffeoylquinic acid, (4) caffeic acid, (5) caffeoylquinic acid, (6) 5-*O*-*p*-coumaroylquinic acid, (7) 5-*O*-feruloylquinic acid, (8) ferulic acid, (9) 3,4-di-*O*-caffeoylquinic acid, (10) 3,5-di-*O*-caffeoylquinic acid, and (11) 4,5-di-*O*-caffeoylquinic acid.

prominent $[M-H]^-$ ions in the negative ionisation mode, the fragmentation patterns obtained by CID of isolated $[M-H]^-$ ions provide valuable information for the differentiation of isomeric substances (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford, Knight, & Kuhnert, 2005). The LC/MS data of all identified compounds are presented in Table 2.

In accordance with previous studies on the phenolic profile and contents of sunflower seeds (Aramendia et al., 2000; Dreher & Holm, 1983; Felice, King, & Kissinger 1976; Milić et al., 1968; Pedrosa et al., 2000), 5-CQA was identified as the predominant phenolic acid (compound **2**). Identification of this compound was based on the comparison of the UV spectra and retention times with those of the reference substance. Furthermore, fragmentation of the parent ion *m/z* 353 yielded product ions at *m/z* 191 and 179, with the quinic acid moiety representing the base peak and the caffeoyl moiety being present only with minor intensities, which is in agreement with literature findings (Clifford et al., 2003) (Table 2). Two further substances showing an $[M-H]^-$ ion at *m/z* 353 (compounds **1** and **3**) were unambiguously assigned to 3-CQA (neochlorogenic acid) and 4-CQA (cryptochlorogenic acid). The fragmentation behaviour of both constituents was well in accordance with literature data (Clifford et al., 2003). While collision induced dissociation of the parent ion of 3-CQA resulted in a base peak corresponding to the quinic acid moiety and a comparatively intense signal from caffeic acid (*m/z* 179), a $[\text{quinic acid}–H–H_2O]^-$ ion at *m/z* 173 as base peak was detected for 4-CQA with a prominent signal at *m/z* 179 resulting from caffeic acid in the MS^2 experiment. The 5-CQA and 3-CQA isomers were devoid of the $[\text{quinic acid}–H–H_2O]^-$ ion, which has been explained by their particular stereochemistry, not allowing 1,2-acyl participation during fragmentation. Thus, water is only significantly eliminated from the 4-isomer (Clifford et al., 2003). Additionally, this peak assignment was corroborated by the elution order which is in accordance with previous studies (Clifford et al., 2003; Clifford et al., 2005; Kammerer et al., 2004a).

A further component (compound **5**) was detected exhibiting an $[M-H]^-$ ion at *m/z* 353 and a fragmentation pattern in the MS^2 experiment similar to that of 5-CQA. The spectral characteristics of compound **5** are indicative of a *cis*-isomer of a CQA (Kammerer et al., 2004a). However, it is also conceivable that this substance is either a 1-CQA isomer or contains less common quinic acid moieties such as *muco*-quinic or *iso*-quinic acid (Clifford, 2003). Identification of compounds **4** (caffeic acid) and **8** (ferulic acid) (Fig. 2) was based on the comparison of their UV spectra and retention times with those of reference substances. Peak assignment was confirmed by their mass spectrometric behaviour exhibiting $[M-H]^-$ ions at *m/z* 179 and 193, respectively, and of a carboxyl moiety and methyl radical (44 + 15 Da), respectively (Table 2). In agreement with Clifford et al. (2003), the fragmentation patterns of peak **6** and **7** could be assigned to 5-*O*-*p*-coumaroylquinic acid (5-*p*CoQA) and 5-*O*-feruloylquinic acid (5-FQA), respectively. Compound **6** exhibited an $[M-H]^-$ parent ion at *m/z* 337. CID of that component led to the formation of a predominant fragment at *m/z* 191 originating from the quinic acid moiety. Compound **7** eluting after 31.5 min and exhibiting an $[M-H]^-$ ion at *m/z* 367 showed the release of the predominant fragment ion at *m/z* 191, which is characteristic of 5-FQA. These latter peak assignments are supported by the elution order of the 5-hydroxycinnamoylquinic acids (5-CQA, 5-*p*CoQA, 5-FQA) which is in accordance with previous studies (Clifford et al., 2003; Kammerer et al., 2004a).

Although previous studies revealed the occurrence of dicaffeoylquinic acids (diCQA) in sunflower seeds using MS techniques for peak assignment (Aramendia et al., 2000; Pedrosa et al., 2000), their unambiguous identification has not been achieved. In the present investigation compounds **9**, **10**, **11** exhibiting $[M-H]^-$ parent ions at *m/z* 515 were found to be diCQA derivatives. The mass spectrometric detection of molecular ions and the interpretation of their fragmentation pattern in the MS^2 and MS^3 experiment allows to identify individual hydroxycinnamic acids and to precisely distinguish between isomeric compounds since different fragmentation patterns can be attributed to stereochemical relationships (Clifford et al., 2003; Clifford et al., 2005). Based on these peculiarities thoroughly described in the literature the compounds eluting at 61.4, 62.7 and 67.2 min were identified as 3,4-diCQA, 3,5-diCQA and 4,5-diCQA, respectively, based on their fragmentation behaviour in the MS^2 and MS^3 experiments. Even though the relative abundance of some of the fragments slightly differed from those described earlier (Clifford et al., 2003; Clifford et al., 2005), it should be mentioned that MS^n spectra are not completely portable between laboratories. Furthermore, the detection of cynarin, which was reported first in artichokes (Schütz et al., 2004), also belonging to the Asteraceae, might be assumed. However, the occurrence of 1,3-diCQA in sunflower kernels and shells could be excluded, since the retention time of the standard compound did not match any of those peaks exhibiting an $[M-H]^-$ ion at *m/z* 515.

Table 2
Retention times, UV spectra and characteristic ions of phenolic compounds detected in sunflower kernels and shells.

Compound	Retention time (min)	Identity	HPLC-DAD λ_{max} (nm)	$[M-H]^-$ *m/z*	HPLC-ESI(-)-MS^n experiment *m/z* (% base peak)
1	8.0	3-*O*-Caffeoylquinic acid	240, 303sh, 324	353	MS^2 [353]: 191 (100), 179 (46), 192 (11), 180 (7), 135 (4),134 (3)
2	15.4	5-*O*-Caffeoylquinic acid (chlorogenic acid)	242, 305sh, 326	353	MS^2 [353]: 191 (100), 179 (3)MS^3 [353 → 191]: 119 (100)
3	17.5	4-*O*-Caffeoylquinic acid	237, 303sh, 326	353	MS^2 [353]: 173 (100), 179 (44), 191 (31), 161 (3), 174 (2)
4	18.5	Caffeic acid	238, 302sh, 326	179	MS^2 [179]: 135 (100)
5	23.7	Caffeoylquinic acid	233, 314	353	MS^2 [353]: 191 (100)
6	25.7	5-*O*-*p*-Coumaroylquinic acid	231, 310	337	MS^2 [337]: 191 (100), 163 (7)
7	31.5	5-*O*-Feruloylquinic acid	243, 326	367	MS^2 [367]: 191 (100), 173 (5), 111 (4), 193 (4), 274 (3), 336 (3)
8	59.3	Ferulic acid	296sh, 323	193	MS^2 [193]: 134 (100)
9	61.4	3,4-Di-*O*-caffeoylquinic acid	242, 303sh, 325	515	MS^2 [515]: 353 (100), 173 (23), 179 (18), 498 (14), 191 (11), 354 (9), 335 (6), 203 (4), 299 (3) MS^3 [515 → 353]: 173 (100), 179 (51), 191 (40), 131 (25)
10	62.7	3,5-Di-*O*-caffeoylquinic acid	241, 303sh, 327	515	MS^2 [515]: 353 (100), 191 (17), 179 (7)MS^3 [515 → 353]: 191 (100), 179 (55), 135 (13), 173 (5)
11	67.3	4,5-Di-*O*-caffeoylquinic acid	243, 303sh, 327	515	MS^2 [515]: 353 (100), 173 (22), 203 (16), 179 (13), 299 (8), 255 (7), 191 (5), 335 (3), 317 (3) MS^3 [515 → 353]: 173 (100), 179 (80), 191 (19), 135 (9)

Abbreviation: sh, shoulder.

Except from the amount of individual polyphenols, phenolic profiles of sunflower kernels and shells did not significantly differ (Fig. 2).

3.4. Quantification of individual compounds in sunflower kernels and shells

Even though the contents of phenolic compounds in sunflower seeds have been determined in various studies (Dabrowski & Sosulski, 1984; Dreher & Holm, 1983; Leung et al., 1981; Milić et al., 1968; Pedrosa et al., 2000), comparison with the data obtained in the present work is hardly possible due to differing analytical methodologies, the development of novel sophisticated techniques and differences in the sample material and origin. A detailed list of phenolic amounts is presented in Tables 3a and b showing the contents of 11 individual compounds of non-oilseed and oilseed sunflower kernels and shells and for one press residue originating from the oil recovery process. The total phenolic content (TPC) as determined by summarising individual amounts of all constituents ranged from 2938.8 mg/100 g to 4175.9 mg/100 g dry matter (DM) for the dehulled kernels and from 40.8 mg/100 g to 86.0 mg/100 g DM for the corresponding shells implying a variation of around 30% for the TPC from sunflower kernels and 52% for the shells. Expectedly, the TPCs of the sunflower kernels were up to 100 times higher than those determined in the shells. The kernels of non-oilseed sunflowers only slightly differed in a range of 3291.9–3611.0 mg/100 g DM (Table 3a), whereas oilseed kernels exhibited concentrations ranging from 3938.8 to 4175.9 mg/100 g DM (Table 3b). As already pointed out, 5-CQA was the predominant compound in all samples. This CQA and its isomers 3- and 4-CQA, respectively, represented 62.1% up to 92.9% of the TPC. The proportions of non-esterified phenolic acids, coumaric and ferulic acid derivatives, CQAs and diCQAs relative to the total phenolic contents are given in Table 4. These data indicate that the proportions of the phenolic subclasses are similar in kernels and shells, although quantitative amounts markedly differed.

The phenolic acids, caffeic and ferulic acids, and the quinic acid derivatives only represented minor amounts ranging from 0.8% to 3.6% for non-esterified phenolic acids and from 0.4% to 5.9% for coumaric and ferulic acid derivatives, respectively. As has already been stated, sunflower polyphenolics are mainly composed of CQAs followed by diCQAs. The proportions of CQAs were slightly higher as can be demonstrated for the 'Geiger' sample with relative amounts of 87.8% and 73.4% in the kernels and shells, respectively. In contrast the proportion of diCQAs was reversed accounting for 10.4% of total phenolics in the kernels and 19.1% in the shells. Among the diCQAs the 3,5-diCQA was the predominant compound with approximately 50% followed by 4,5-diCQA (33%).

When comparing sunflower types from different European origin (Tables 3) the lowest contents of 2938 mg/100 g DM were found in the Italian types and the highest concentration (4176 mg/100 g DM) in the seeds of French samples. However, these results may not be used to draw conclusions concerning the effects of climate and agricultural conditions on the phenolic contents of the sunflower seeds, since detailed information on growing area, period or climate were not available. Thus, these data cannot be correlated with any agronomical factors, and the contents are presumably cultivar-dependent. The data reported in the literature for the phenolic contents of sunflower kernels range from ~0.13 g/100 g (Aramendia et al., 2000; Pedrosa et al., 2000) to ~2.9 g/100 g (Dabrowski & Sosulski, 1984). However, a direct comparison with the results obtained in the present study is difficult since contents were sometimes given based on non-defatted kernels and the methodologies were significantly different The commercial samples 'Gusto', 'Naturalita' and 'Dovgan' of the non-oilseed group showed TPCs of 3611, 3292 and 3556 mg/100 g DM, respectively (Table 3a). The sample originating from oil extraction (Table 3b) contained about 2940 mg/100 g DM phenolic compounds. This by-product comprised shells as a consequence of the partial dehulling operation prior to the oil recovery. Therefore, it might be supposed that the phenolic contents of the kernels are even higher. These high polyphenolic amounts in oil production residues corroborate that the oil recovery process does not markedly lower the phenolic contents in the solid residue since the phenolic acids are hardly soluble in the lipid phase, thus, underlining their high potential as a suitable and cost-effective resource to recover phenolic antioxidants. A process for the extraction of food antioxidants from sunflower shells has been described in a previous study by De Leonardis et al., 2005, however, the results presented here clearly demonstrate that the residue originating from sunflower oil recovery are a much more suitable material for polyphenol extraction due to their high amounts and consumer acceptance demanding sustainable agricultural production. Since sunflower kernels contain about 50% oil, the residues of oil production amount to approximately 10.6 million

Table 3a
Contents of individual phenolic compounds in non-oilseed sunflower kernels and shells (mg/100 g of DM).

Identity	Non-oilseed kernels and shells			
	Gusto Kernels	Naturalita Kernels	Dovgan Kernels	Dovgan Shells
Caffeic acid	20.5 ± 1.6	21.3 ± 1.6	27.8 ± 3.1	0.6 ± 0.1
Ferulic acid	7.6 ± 3.6	10.5 ± 1.0	9.2 ± 3.2	0.9 ± 0.3
Non-esterified phenolic acids	*28.1 ± 4.0*	*31.8 ± 1.9*	*37.0 ± 4.4*	*1.5 ± 0.3*
5-*O*-*p*-Coumaroylquinic acid	11.3 ± 2.4	10.2 ± 0.8	10.6 ± 3.3	1.1 ± 0.0
5-*O*-Feruloylquinic acid	16.5 ± 1.5	17.3 ± 0.7	12.2 ± 1.2	1.0 ± 0.0
Coumaric and ferulic acid derivatives	*27.9 ± 2.8*	*27.5 ± 1.1*	*22.8 ± 3.5*	*2.1 ± 0.0*
3-*O*-Caffeoylquinic acid	480.4 ± 21.6	268.4 ± 28.0	515.9 ± 6.8	1.9 ± 0.2
4-*O*-Caffeoylquinic acid	58.2 ± 0.8	117.1 ± 3.5	91.2 ± 5.1	1.8 ± 0.2
5-*O*-Caffeoylqinic acid (chlorogenic acid)	2795.7 ± 167.4	2364.2 ± 159.4	2473.1 ± 45.9	20.2 ± 2.2
Caffeoylquinic acid	24.7 ± 3.3	23.3 ± 3.0	24.1 ± 1.5	1.4 ± 0.1
Monocaffeoylquinic acids	*3358.8 ± 168.8*	*2773.0 ± 161.9*	*3104.3 ± 46.7*	*25.3 ± 2.3*
3,4-Di-*O*-caffeoylquinic acid	14.9 ± 5.8	31.4 ± 0.9	29.9 ± 4.4	1.1 ± 0.1
3,5-Di-*O*-caffeoylquinic acid	135.0 ± 3.0	200.7 ± 11.5	227.6 ± 5.3	5.1 ± 0.3
4,5-Di-*O*-caffeoylquinic acid	46.3 ± 2.7	227.5 ± 13.1	133.8 ± 3.8	3.7 ± 0.4
Dicaffeoylquinic acids	*196.2 ± 7.0*	*459.6 ± 17.5*	*391.3 ± 7.8*	*11.2 ± 0.7*
Total amount	*3611.0 ± 169.1*	*3291.9 ± 162.9*	*3555.5 ± 47.7*	*40.8 ± 2.4*

Table 3b
Contents of individual phenolic compounds in oilseed sunflower kernels and shells (mg/100 g of DM).

Identity	Oilseed kernels and shells							
	Schilfer Oil extraction residue	Geiger Kernels	Geiger Shells	Italy Kernels	Italy Shells	France Kernels	France Shells	Germany Kernels	Germany shells
Caffeic acid	27.0 ± 0.0	26.7 ± 1.1	0.5 ± 0.1	19.2 ± 2.1	1.0 ± 0.1	22.4 ± 4.2	0.5 ± 0.0	23.1 ± 0.4	0.6 ± 0.0
Ferulic acid	7.0 ± 2.0	12.4 ± 2.0	0.3 ± 0.0	7.2 ± 0.4	1.0 ± 0.2	11.0 ± 0.8	0.5 ± 0.1	91.5 ± 9.1	0.7 ± 0.3
Non-esterified phenolic acids	*34.1 ± 2.0*	*39.0 ± 2.3*	*0.8 ± 0.1*	*26.4 ± 2.1*	*2.0 ± 0.2*	*33.4 ± 4.3*	*1.0 ± 0.1*	*114.7 ± 9.1*	*1.4 ± 0.3*
5-O-*p*-Coumaroylquinic acid	8.9 ± 0.1	11.3 ± 1.0	2.1 ± 0.1	5.2 ± 0.6	1.2 ± 0.1	11.2 ± 1.3	1.9 ± 0.2	10.0 ± 1.0	1.7 ± 0.1
5-O-Feruloylquinic acid	17.0 ± 1.4	11.3 ± 1.0	0.5 ± 0.1	6.2 ± 0.4	1.2 ± 0.2	19.0 ± 0.8	0.5 ± 0.0	3.8 ± 0.1	0.8 ± 0.1
Coumaric and ferulic acid derivatives	*25.9 ± 1.4*	*22.6 ± 1.4*	*2.6 ± 0.2*	*11.5 ± 0.8*	*2.4 ± 0.2*	*30.2 ± 1.5*	*2.5 ± 0.2*	*13.8 ± 1.0*	*2.5 ± 0.2*
3-O-Caffeoylquinic acid	316.2 ± 0.1	439.9 ± 8.6	2.9 ± 0.4	423.7 ± 35.8	4.2 ± 0.2	394.1 ± 71.6	3.4 ± 0.1	247.5 ± 6.3	4.8 ± 0.4
4-O-Caffeoylquinic acid	53.1 ± 2.7	87.5 ± 4.1	2.1 ± 0.4	57.5 ± 1.8	2.4 ± 0.1	86.2 ± 3.7	3.0 ± 1.2	86.3 ± 3.6	4.7 ± 1.0
5-O-Caffeoylquinic acid (chlorogenic acid)	2086.4 ± 132.4	2467.0 ± 13.9	26.6 ± 1.4	1945.7 ± 141.0	47.5 ± 2.2	3050.5 ± 282.0	45.0 ± 0.3	2271.4 ± 11.7	59.1 ± 3.8
Caffeoylquinic acid	15.6 ± 1.2	36.5 ± 2.2	1.5 ± 0.1	37.8 ± 5.6	3.7 ± 0.0	51.4 ± 11.3	4.8 ± 2.8	38.7 ± 0.7	3.3 ± 0.1
Monocaffeoylquinic acids	*2471.2 ± 132.4*	*3030.9 ± 17.0*	*33.1 ± 1.5*	*2464.7 ± 145.6*	*57.8 ± 2.2*	*3582.2 ± 291.2*	*56.3 ± 3.0*	*2644.0 ± 13.8*	*72.0 ± 3.9*
3,4-Di-O-caffeoylquinic acid	23.5 ± 1.0	28.8 ± 0.3	1.2 ± 0.1	26.6 ± 1.5	1.1 ± 0.0	27.4 ± 3.0	1.0 ± 0.2	29.0 ± 0.2	1.4 ± 0.2
3,5-Di-O-caffeoylquinic acid	260.1 ± 19.3	211.2 ± 1.1	4.8 ± 0.6	274.7 ± 23.6	8.1 ± 0.0	332.5 ± 47.2	4.4 ± 0.5	212.8 ± 4.4	4.6 ± 0.2
4,5-Di-O-caffeoylquinic acid	124.9 ± 9.8	120.9 ± 0.2	2.6 ± 0.0	135.0 ± 6.3	3.7 ± 0.0	170.3 ± 12.6	2.6 ± 0.2	131.4 ± 3.7	4.3 ± 0.4
Dicaffeoylquinic acids	*408.6 ± 21.7*	*360.9 ± 1.1*	*8.6 ± 0.7*	*436.2 ± 24.5*	*13.0 ± 0.0*	*530.2 ± 48.9*	*8.1 ± 0.6*	*373.2 ± 5.8*	*10.2 ± 0.5*
Total amount	*2939.7 ± 134.2*	*3453.5 ± 17.3*	*45.1 ± 1.6*	*2938.8 ± 147.7*	*75.2 ± 2.2*	*4175.9 ± 295.3*	*67.8 ± 3.1*	3145.6 ± 17.5	*86.0 ± 4.0*

Table 4
Proportion of four subclasses of phenolic compounds relative to the total phenolic contents in sunflower kernels and shells.

Sample	Non-esterified phenolic acids [%]	Coumaric and ferulic acid derivatives [%]	Monocaffeoyl-quinic acids [%]	Dicaffeoyl-quinic acids [%]
Gusto [kernels]	0.8 ± 0.1	0.9 ± 0.1	92.9 ± 0.1	5.5 ± 0.2
Naturalita [kernels]	1.0 ± 0.1	0.8 ± 0.0	84.4 ± 5.5	14.0 ± 0.7
Dovgan [kernels]	1.2 ± 0.1	0.6 ± 0.1	86.8 ± 1.2	11.4 ± 0.2
Dovgan [shells]	3.7 ± 0.6	5.0 ± 0.3	62.1 ± 5.9	29.2 ± 2.1
Schilfer [oil extraction residue]	*1.2 ± 0.1*	*0.9 ± 0.0*	*84.0 ± 4.8*	*14.0 ± 0.7*
Geiger [kernels]	1.1 ± 0.1	0.7 ± 0.0	87.8 ± 0.6	10.4 ± 0.0
Geiger [shells]	1.8 ± 0.2	5.9 ± 0.4	73.4 ± 3.9	19.1 ± 1.3
Italy [kernels]	0.9 ± 0.1	0.4 ± 0.0	84.1 ± 5.7	14.9 ± 0.9
Italy [shells]	2.7 ± 0.2	3.2 ± 0.2	76.9 ± 2.7	17.2 ± 0.3
France [kernels]	0.9 ± 0.1	0.4 ± 0.0	84.1 ± 5.7	14.9 ± 0.9
France [shells]	1.5 ± 0.1	3.6 ± 0.3	83.0 ± 3.9	11.9 ± 0.8
Germany [kernels]	3.6 ± 0.2	0.4 ± 0.0	84.1 ± 0.4	11.5 ± 0.2
Germany [shells]	1.6 ± 0.3	2.9 ± 0.2	83.8 ± 5.1	11.9 ± 0.6

mt, as can be estimated from the annual sunflower oil production (FAO-STAT, 2008). Assuming a TPC of 3%, as determined in the present study, the recovery of up to 300,000 mt from the by-products of the sunflower oil extraction would be possible, which might then be used for natural ingredients of functional or enriched foods or as natural food antioxidant components.

The contributions of individual phenolic compounds to antioxidant capacity substantially differ (Goupy, Dufour, Loonis, & Dangles, 2003). Therefore, comprehensive information on phenolic composition and contents in kernels and shells is particularly relevant considering phenolic sunflower seed extracts. The data presented are also valuable from a nutritional point of view since *o*-dihydroxyphenols, especially caffeic and chlorogenic acids when oxidised to *o*-quinones, might covalently bind to amino and thiol groups of amino acids. Commonly, the ε-amino group of lysine and the thioether group of methionine are most reactive which results in their reduced availability to the monogastric digestive system (Sabir et al., 1974). Therefore, to retain its nutritional value the exhaustive removal of phenolic compounds needs to be assured when producing protein concentrates or isolates from sunflower seeds. The development of such a process is the subject of our on-going research.

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