Chapter 7
Evaluating the Phytochemical Potential of Camelina: An Emerging New Crop of Old World Origin

Mark A. Berhow, Steven F. Vaughn, Bryan R. Moser, Deniz Belenli and Umit Polat

Abstract Out on the next frontier of nutritional research will be the complete biochemical and physiological characterization of plant-derived foods that prevent or delay the development of chronic diseases in humans and animals. The chemical composition of many major crop products (seeds, flour, oil, leaves, etc.) have been determined, but the slow process of evaluating each compound alone or in mixtures for the biological function in nutrition and health of the animals that consume them has only just begun. Camelina, or false flax (*Camelina sativa* L. Crantz), is an emerging oil seed crop in North America mostly used as a biodiesel fuel. The seeds contain up to 45% oil, which is rich in polyunsaturated omega-3 and omega-6 fatty acids, as well as fat-soluble antioxidants such as the vitamin E-active tocopherols. Extraction of oil from camelina seeds by mechanical expeller yields a seed meal that consists of approximately 10% residual oil, 45% crude protein, 10% soluble sugars, 13% fiber, 5% minerals, and 10% phytochemical constituents such as glucosinolates, flavonols, lignans, phenolic acids as well as nucleic acids. The seed meal also contains a hydrophilic gum. While the oil fraction has been well characterized and its uses are growing, the seed meal has yet to be fully characterized for its potential use

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M. A. Berhow (✉) · S. F. Vaughn
United States Department of Agriculture, Agricultural Research Service,
National Center for Agricultural Utilization Research, Functional Foods Research Unit,
1815 N. University Street, Peoria, Illinois 61604, USA
e-mail: mark.berhow@ars.usda.gov

B. R. Moser
United States Department of Agriculture, Agricultural Research Service,
National Center for Agricultural Utilization Research, Bio-Oil Research Unit,
1815 N. University Street, Peoria, Illinois 61604, USA

D. Belenli · U. Polat
Faculty of Veterinary Medicine, Department of Biochemistry,
University of Uludag, 16059 Bursa, Turkey

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in animal feeds or in foods for humans. The phytochemical components of camelina potentially have strong benefits for use in functional food roles.

Keywords Camelina · Oil seed · Chemical composition · Glucosinolate · Flavonols · Phenolics

7.1 Complete Characterization of Plant Crop Products

The next frontier of nutritional research will be the characterization of plant-derived chemical components—and the resulting biochemical processes that they regulate in animals—that are critical in preventing or delaying the development of chronic diseases such as cancer, diabetes, heart and circulatory diseases, and other chronic conditions that develop in humans and animals. Seeds, leaves, bark, stems and flowers, as well as extracts from these plant organs, have historically been used to treat diseases and infirmaries in humans and animals. The development of the modern pharmaceutical industry owes its very existence to the characterization and purification of physiologically active chemical compounds from plants, fungi, and bacteria. Epidemiological observations have shown that regular consumption of a number of specific foods or plant extracts significantly reduces the occurrence, or slows the development of nearly all of the chronic disease conditions that afflict humankind.

The challenge in preventing chronic disease by nutritional phytochemicals is that their effect may not be due to a single chemical agent or even a set of chemically related compounds, unlike pharmacological chemicals which tend to specifically destroy disease-causing microbes or abnormal mammalian cell types. Disease prevention through nutrition may be the result of an optimal mix of phytochemical agents—from a single plant species or even a mixture of plant species—combined with a defined caloric intake and regular exercise that will result in the prevention or slowing of the development of chronic disease. Traditional Chinese and Indian medicines have been prescribed for exactly these purposes for thousands of years, yet we still do not understand how they work on a chemical or biochemical level. In order to fully understand these very complex processes, it is essential to have a complete chemical profile of the components that make up a particular food. As many of these compounds are produced by plants to mediate ecophysiological stress, microbial, or herbivore pressure, this would have the added benefit of being able to better evaluate the full chemical profile of particular plant species for more effective pest control.

Full chemical characterization of a given plant material is still a difficult endeavor even with all the advances in modern computers and analytical equipment. Also, the considerable variation in plant organ chemical composition due to genetic and environmental factors makes this complex analysis even more challenging. Analytical research has yielded fairly complete chemical characterization of the major food crops, especially the grains and soybean. These foods are fairly complex mixtures of primary metabolites and phytochemicals of which the minor components may

play key roles in determining the long-term bioactivity. Yet even in these major crops, very few of the minor phytochemical components are available as pure standards for further nutritional evaluation. Often, as in the case with soy, researchers have focused on just one family of phytochemicals, such as the isoflavones, and the resulting nutritional and disease research studies conducted on these compounds have often shown that isolated soy isoflavones are generally less effective than the whole foods.

It would be beneficial to have a relatively simple phytochemical model food system as an effective tool to use for compositional nutritional studies, such as *Arabidopsis* is being used in plant genetic studies. The nutritional model plant needs to be in crop production, as no rare and hard to obtain plant will be effective for such studies. One candidate is *Camelina sativa*, or simply camelina, which is an emerging bio-oil crop in North America from the Brassicaceae family (see Sect. 7.2) The aim of this review is to summarize all of the known chemical components of camelina seed meals with special emphasis on the analysis and determination of the phytochemical composition, with the aim of assessing the key chemical components to be evaluated for their roles in the prevention of chronic disease in animals. The oil composition has already been well characterized, so it will only be summarized here (Sect. 7.3), followed by a detailed discussion of the phytochemical compositions of camelina seed meal (Sect. 7.4) and the methods used for the analyses (Sect. 7.5).

7.2 Camelina: An Emerging New Crop in North America

Camelina sativa L. Crantz—also known as gold-of-pleasure, false flax, wild flax, linseed dodder, German sesame, and Siberian oilseed—is an annual member of the Brassicaceae (mustard family). It is a plant native to temperate northern Europe and central Asia, but was introduced to North America, possibly as a contaminant in flax seed. It was traditionally cultivated in Europe to produce vegetable oil and animal feed [1, 2]. There is ample archeological evidence to show that it has been grown for at least 3,000 years [3, 4]. Camelina was an important oil crop in eastern and central Europe, and has continued to be cultivated in a few parts of Europe for its seed, which was used in oil lamps and as edible oil. Camelina has a number of agronomic advantages: it can be grown in a variety of climatic and soil conditions as a spring/summer crop or as a biannual winter crop, and can be easily incorporated into crop rotations; it has a short growing season (85–100 days); it is compatible with existing farm practices and does not require specialized harvesting equipment; it tolerates cold weather, drought, and low-fertility/saline soils; it has few natural pests and so requires relatively little pesticide application [5–7]. It is currently grown in the northern USA and in Canada, and the USDA, AgCanada, several US states and Canadian provincial agencies, and at least two private companies have both breeding and genetic modification programs aimed at further improving camelina traits such as improved oil content, seed viability, expanded growing locations, and resistance to disease, pest, and weed competition.

Table 7.1 Yield of camelina compared to other commodity oil crops

Crop	Seed yield (kg ha-1 year-1)	Seed oil (mass %)	Oil yield (kg ha-1 year-1)
Camelina	1,500–3,000	28–47	420–1,410
Rapeseed	2,680–3,390	40–44	965–1,342
Soybean	2,140–2,840	18–22	347–562
Sunflower	1,440–1,700	39–49	505–750

Data for seed yield of rapeseed, soybean, and sunflower were taken from Oil World Annual 2009, 1, Oilseeds pp. 5–9. Data for seed oil content of camelina (and seed yield), rapeseed, soybean, and sunflower were taken from the 3rd edition of The Lipid Handbook.

Although camelina is harvested primarily for its seed, the rest of the plant could be used as a straw or as a source of cellulose and lignin [6]. Interest in the use of camelina as a functional food and as a source of biodiesel continues to grow. The seed is generally processed by cold pressing to remove 80–90 % of the oil, yielding crude oil and seed meal. Methods have been developed to refine the oil for both food and fuel uses, and the chemical composition has been extensively studied. Yields are anywhere from 336 to 2,240 kg per hectare with lipid contents of 25–45 weight percent (Table 7.1 and [8–10]). Oil yields are comparable to rapeseed, soybean, and sunflower. The interest in North America is partly due to its exceptionally high level of omega-3 fatty acids, which is uncommon in commodity vegetable sources. Over 50 % of the fatty acids in cold-pressed camelina oil are polyunsaturated (Table 7.2). Because of its apparent health benefits and its relative oil stability, camelina oil should be added to the growing list of functional foods. However, additional uses are still needed for processing coproducts to render camelina economically viable. Defatted camelina seed meal contains significant levels of proteins and carbohydrates as well as a number of phytochemicals including glucosinolates, which could be utilized in additional food, feed, and agricultural uses.

On a compositional level, a wealth of information has recently become available on the chemical components of camelina seeds. Applying modern analytical techniques can solve a few of the missing pieces of the phytochemical puzzle. In the next step, nearly all of the camelina chemical components can then be assembled to create artificial food that very closely resembles that of camelina oil and seed meal. Nutritional researchers will thus be able to evaluate the artificial mixture against natural camelina seed meal and further evaluate each of the components—either individually or as mixtures—to characterize its nutritional bioactivity.

7.3 Camelina Seed Oil

Crude camelina oil consists of about 45 % polyunsaturated fatty acids, 35 % monounsaturated fatty acids, 10 % saturated fatty acids, and up to 10 % free fatty acids, tocopherols, sterols, other terpenes and volatiles.

Table 7.2 Fatty acid composition of camelina oil ([a][16], [b][18])

	Carbon number	Weight percent[a]
Unsaturated [a, b]		
α-Linolenic acid	18:3	37%
Linoleic acid	18:2	15%
Oleic acid	18:1	15%
11Z-Eicosenoic acid	20:1	15%
Erucic acid	22:1	3%
cis-11,14-Eicosadienoic acid	20:2	2%
Eicosatrienoic acid	20:3	2%
Saturated [a, b]		
Palmitic acid	16:0	5%
Stearic acid	18:0	3%
Arachidic acid	20:0	1%
Minor[b]		
Total		2%
13Z,16Z-Docosadienoic acid	22:2	Trace
10Z-Heptadecenoic acid	17:1	0.5%
Palmitoleic acid	16:1	Trace
Nervonic acid	24:1	Variable
Heptadecanoic acid	17:0	0.2%
Heneiosanoic acid	21:0	Trace
Behenic acid	22:0	0.5%
Tricosanoic acid	23:0	Trace
Lignoceric acid	24:0	Trace

7.3.1 Camelina Oil Composition

The average concentrations are covered in detail in several published reports [9–22] and summarized in Table 7.2. α-Linolenic acid, an omega-3 fatty acid, is the most abundant fatty acid in camelina oil along with linoleic, oleic, and 11-eicosenoic acids. These fatty acids account for 80–85% of the oil. Camelina oil contains relatively high levels of erucic acid, but the amount is below the 5% threshold that is critical for food use [20, 23]. Extensive breeding programs are currently underway hope to lower the levels of this fatty acid in future crop lines.

The extractable oil fraction (Table 7.3 and [11, 16–18, 24]) includes the nonvolatile terpenes—sterols, tocopherols, small amounts of the un-cyclized terpenes squalene and phytol, and a few other degradation products—the levels of which depend on the amount of processing conducted on the sample. Of interest here, are the relatively high levels (for a plant) of cholesterol [25]. The tocopherol levels are relatively low compared to oils of other species such as soybean. The major tocopherols found in camelina are α-tocopherol, γ-tocopherol, and δ-tocopherol, with small amounts of β-tocopherol also identified. Tocotrienols have not generally been detected in camelina.

Small amounts of free fatty acids in the range of less than 0.1–0.8% are present in the extracted oil, which are probably released during the course of oil extraction, processing, and storage [16]. All plant seed oil fractions have a unique and often distinct

Table 7.3 Composition of unsaponifiables (sterols and nonvolatile terpenoids) comprising 0.5–0.8 weight percent of camelina oil ([a][16], [b][17], [c][24], [d][18])

Sterols	Tocopherols
ß-Sitosterol[a b c]	α-Tocopherol[a c]
Campesterol[a b c]	β-Tocopherol (trace)[a c]
Campestanol[c]	γ-Tocopherol[a c]
Cholesterol[a b c]	δ-Tocopherol (trace)[a c]
Brassiciasterol[a b c]	Retinoic acid (trace)[a]
γ-Sitosterol[a c]	Plastochromanol[d]
Sitostanol[c]	
δ-5-Avenosterol (trace)[a b]	Non-cyclized terpenes
δ-7-Avenosterol (trace)[c]	Squalene[a]
14-Methylergost-8-en-3-ol (trace)[a]	Phytol[a]
Stigmasterol (trace)[a]	Squalane (trace) [a]
Stigmasta-5,24-dienol[c]	
Obtusifoliol (trace)[a]	
Cycloartenol (trace)[bc]	
24-Methylenecycloartanol[c]	
α-1-Sitosterol (trace)[a]	
14-Methylfecosterol (trace)[a]	
6-Methylcholest-5-en-ol (trace)[a]	
Gramisterol + α-amyrin[c]	
β-Amyrin (trace)[a]	
Citrostadienol[c]	

odor made up of the volatile and semi-volatile compounds. Typically, this comprises a large number of alkyl and benzyl compounds, each present in relatively small amounts. The amounts and types of these compounds can vary considerably from cultivar to cultivar and depend on growth conditions, as well as the amount and type of processing in the oil extraction and preparation. Table 7.4 lists 30 volatile compounds found in freshly prepared camelina oil by headspace analysis, another reference identified 168 acids, alcohols, esters, ketones, aldehydes, alkanes, alkenes, aromatics, ethers, pyrazines, terpenes, and sulfur-containing compounds [16, 19]. Overall, this mixture of volatiles constitutes a very small fraction of the camelina oil, and their concentration in the oil continually decreases with storage time and at elevated temperatures.

7.3.2 Camelina Oil Uses

The unusually high content of polyunsaturated omega-3 fatty acids makes camelina oil useful as a nutritional food and for cosmetic applications although this has been underexploited so far.

The rise in production of camelina in North America has been fueled, so to speak, by its potential use as a feedstock for the production of biodiesel [6–8, 13–15]. Camelina oil has been successfully converted to biodiesel by a variety of catalytic and heating methods. The fuel properties are similar to those of biodiesel prepared

Table 7.4 Volatile compounds in camelina oil listed by retention time on a nonpolar GC column (less than 0.5 weight percent of fresh crude extracted oil) [19]

Compound	Compound
Acetic acid	*trans*-2-Heptenal
Ethylacetate	Benzaldehyde
trans-2-Butenyl	Sabinene
trans-3-Penten-2-one	1-Octen-3-ol
trans-2-Pentenal	6-Methyl-5-hepten-2-one
Butyric acid	β-Myrcene
Isovaleric acid	*trans, trans*-2,4-Heptadienal
trans-2-Hexenal	Octanal
Hexanol	3-Carene
2-Heptanone	p-Cymene
Styrene	Limonene
Heptenal	*trans*-3-Octen-2-one
trans, trans-2,4-Hexadienal	*trans, trans*-3,5-Octadiene-2-one
γ-Butyrolactone	Nonal
α-Pinene	Decanal

from soybean oil, but as camelina oil contains a high percentage of polyunsaturated fatty acid methyl esters, it requires antioxidant additives to meet fuel stability specifications, which is typical for most biodiesels. Camelina-based diesel blends provide fuel performance characteristics similar to those of the corresponding soybean-based blends. Camelina oil can also be converted to a wax ester that can be used as a biolubricant and an ingredient for cosmetics [6, 7].

7.4 Camelina Seed Meal

Extraction of the oil from camelina seeds is typically done by mechanical expellers which yield a seed meal that consists of approximately 10% residual oil, 45% protein, up to 15% carbohydrate/lignin insoluble fiber, up to 10% soluble carbohydrates, 5% minerals, approximately 0.2% nucleic acids, and 10% or more of a mixture of phytochemical components consisting mostly of glycosylated glucosinolates, flavonoids, phenolics, and terpenoids. Ground seed can also be extracted with solvents, such as hexane, or by newer "green" extraction technologies, such as supercritical carbon dioxide or high-pressure and temperature ethanol, to produce a powdered meal that contains less than 1% residual oil.

7.4.1 Carbohydrates

Carbohydrates found in camelina seed meal include mono-, di-, tri-, and tetra-saccharides, along with both oligo- and polysaccharides in the form of starch, pectin, and fiber of which a substantial part is composed of cellulose (Table 7.5 and [26]).

Table 7.5 Weight percent of carbohydrates, fiber, and phytic acid in solvent-defatted camelina seed meal

Sugar	Berhow	Zubr [26]
Glucose	0.02%	0.42%
Fructose	0.01%	0.04%
Sucrose	5.60%	5.50%
Raffinose	0.80%	0.64%
Stachyose	0.30%	0.36
Verbascose	0.08%	–
Total Soluble	8.60%	6.96%
Mucilage	–	6.70%
Starch (measured as glucose)	0.52%	1.21%
Pectin	–	0.96%
Acid-hydrolyzable uronic acid (pectin)	1.21%	–
Acid-hydrolyzable fructan	0.42%	–
Acid-hydrolyzable arabinose	4.61%	–
Acid-hydrolyzable galactose	4.24%	–
Acid-hydrolyzable glucose	5.96%	–
Acid-hydrolyzable xylose	1.68%	–
Insoluble sugars (total)	18.64%	–
Crude fiber	–	12.80%
Lignans[a]	–	7.40%
Acid insoluble residue	9.96%	–
Ash	0.47%	–
Inositol hexaphosphate[b]	–	2.25%
Inositol pentaphosphate[b]	–	0.10%

[a] Table 7.9
[b] [28]

The most interesting carbohydrate component is the mucilage that is formed after the addition of water, which forms a gel that can be isolated as a separate component that may be useful as a gum or tackifier [6, 26, 27]. Some of the soluble disaccharides and polysaccharides can contribute to caloric intake, while the insoluble fiber and phenolic lignin precursors have good effects on gastrointestinal processes and health. Camelina does not contain appreciable levels of beta-glucan [11]. A percentage of the digestible carbohydrates is bound to a variety of proteins and phytochemicals and may be nutritionally available or utilized by gut microflora. Camelina, like many other plant species, accumulates significant amounts of phytic acid, a polyphosphorylated inositol sugar [28–30]. This compound can decrease mineral and protein bioavailability; however, some protective effects have also been described.

7.4.2 Proteins

The proteins of camelina meals are the least characterized of the camelina seed components [10]. Unlike for soy, wheat, rice, and peanuts, there has been no careful characterization of the storage proteins of camelina seeds. The amino acid

Table 7.6 Weight percent of amino acids in camelina seeds [10]

Amino acid	% protein	% seeds
Alanine	4.61%	1.96%
Arginine	8.15%	3.46%
Aspartic acid	8.71%	3.70%
Cysteine	2.12%	0.90%
Glutamic acid	16.40%	6.97%
Glycine	5.44%	2.31%
Histidine[a]	2.60%	1.10%
Isoleucine[a]	3.96%	1.68%
Leucine[a]	6.63%	2.82%
Lysine[a]	4.95%	2.10%
Methionine[a]	1.72%	0.73%
Phenylalanine[a]	4.19%	1.78%
Proline	5.09%	2.16%
Serine	5.04%	2.14%
Threonine	4.25%	1.81%
Tryptophan[a]	1.15%	0.49%
Tyrosine	3.04%	1.29%
Valine[a]	5.42%	2.30%

[a] Dietary essential amino acid

composition has been examined [23], and a few studies have looked at trypsin inhibitor activity. In one study, the trypsin inhibitor activity was found to be between 16 and 21 units per mg on a dry weight basis [11], high enough to warrant some concerns. However, the activity could be alleviated by heat treatment, and sufficient variation exists to indicate that it could also be minimized through selective cultivar breeding. Camelina has at least 18 amino acids, of which nine are essential (Table 7.6 and [23]). No allergenic proteins or peptides have been detected in camelina.

7.4.3 Phytochemicals and Other Components

One of the more difficult aspects of plant chemical compositions to assess is the group of compounds aggregately termed "phytochemicals". Phytochemicals are generally defined as "secondary metabolites" or "natural products"—those compounds produced by individual plant species, not inherently required to reproduce and maintain living cells, unlike the primary metabolites, which enables the plant species to chemically mediate environmental stresses such as microbial infestation, herbivore feeding, water stress, light stress, etc. [31]. Phytochemicals are distinct and characteristic to each plant species, and may be produced at various times in a plant's life cycle and accumulate in specific organs or even cell types. As such, they can be present in fairly significant quantities or in very minor quantities. They have been classified by their biosynthetic pathways: the major classes are the terpenes (isoprenoids), the phenolics (phenylpropanoids and polyphenols), and the alkaloids;

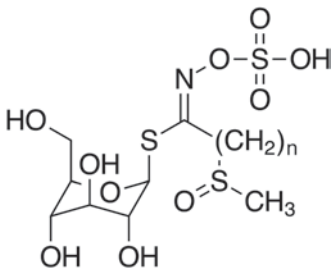

Fig. 7.1 Structure of glucosinolates found in camelina seeds. Glucoarabin (9-methylsulfinylnonyl-glucosinolate) $n=9$, glucocamelinin (10-methylsulfinyldecyl-glucosinolate) $n=10$, and 11-methylsulfinylundecyl-glucosinolate $n=11$

the minor ones which include sulfur-containing phytochemicals such as the glucosinolates, and other nitrogenous compounds such as the indoles and bioactive peptides. These minor phytochemical groups are often produced only by members of a few plant families. It is generally true that the types of phytochemicals found in a plant species/cultivar are always consistent, but the levels may vary considerably from cultivar to cultivar, from location to location, and from crop year to crop year. In many plant species—even the important crop species—not all of the phytochemicals have been completely characterized. This is true for camelina as well. Camelina seeds accumulate a suite of compounds presumably to facilitate germination and growth. These include terpenoids found in the oil fraction discussed above, lignans, tannins, flavonoids and other polyphenolics, and glucosinolates. Camelina seeds do not contain detectable levels of alkaloids, triterpenoid glycosides, or indoles.

7.4.3.1 Glucosinolates

Glucosinolates occur as secondary metabolites in many plants of the order Brassicales (especially in the Brassicaceae, as well as in members of the Capparidaceae and Caricaceae), with about 120 different glucosinolates known to occur naturally [32–34]. The plants contain the enzyme myrosinase, which in the presence of water liberates glucose. The remaining part of the molecule is quickly converted to either a thiocyanate, an isothiocyanate, or a nitrile; these are the active substances that serve as chemical defenses for the plant. Glucosinolates are well known for their toxic effects (mainly as goitrogenic agents) in both humans and animals at high doses. In contrast, at sub-toxic doses, their hydrolytic and metabolic products act as chemoprotective agents against chemically induced carcinogens by blocking the initiation of tumors in a variety of mammalian tissues. They exhibit their effect by inducing phase I and phase II enzymes, by inhibiting enzyme activation, modifying steroid hormone metabolism, and protecting against oxidative damages [32, 35–37].

Camelina accumulates significant levels of just three glucosinolates in its seeds: glucoarabin (9-methylsulfinylnonyl-glucosinolate), glucocamelinin (10-methylsulfinyldecyl-glucosinolate), and 11-methylsulfinylundecyl-glucosinolate (Fig. 7.1, Table 7.7) [38–40]. As with all phytochemicals, this accumulation is greatly

Table 7.7 Weight percent of glucosinolates in camelina seeds

	Berhow	Berhow	Shuster [40]
Glucosinolate	*Defatted*	*Whole*	*Whole*
Glucoarabin (GS9)	0.55%	0.47%	0.29%
Glucocamelinin (GS10)	1.35%	1.15%	0.80%
11-(Methylsulfinyl) undecyl-GS (GS11)	0.20%	0.17%	0.15%
Total	2.13%	1.79%	1.23%

affected by genotype and environmental growing conditions. The effect of the degradation products—the isothiocyanates, thiocyanates, and nitriles—from the camelina glucosinolates in diets and in agriculture has not been assessed, mainly due to a lack of purified standards for the necessary bioassays.

7.4.3.2 Flavonoids

Flavonoids and the (biosynthetically) related coumarins are ubiquitous in the plant kingdom, though most plant species accumulate significant levels of only a select few. No other class of phytochemicals has been credited with so many and such diverse functions in plant growth and survival as the flavonoids [41]. Not only are these compounds involved in defense against pathogens, herbivores, and other plants, but they also function in plant reproduction, mineral absorption, and a variety of symbiotic relationships with species as diverse as bacteria, insects, birds, and humans. Many flavonoids, along with benzoic acid derivatives discussed below, function as antioxidants, which allow these compounds to act as reducing agents, hydrogen donators, and singlet oxygen quenchers, an important role as natural antioxidants in foods [42].

In camelina plants, there are likely many flavonoids synthesized and utilized in leaves, stems, roots, and flowers, but in mature seeds only five are accumulated to detectable levels, the most significant of which are the glycosides of the flavonol quercetin [43–45]. Work in our lab has identified three forms of quercetin glycosides, resulting in a total concentration in the range of 10 mg/g (Fig. 7.2, Table 7.8). Other flavonoids that have been tentatively identified include an apigenin di-glycoside and a diosmetin di-glycoside, both found at relatively low levels. The total flavonoid amounts in a seed extract was reported to be 143 mg/g based on spectroscopic measurements, but these are likely inaccurate as many phenolics react with the chromogen reagent to produce absorbance at 640 nm [42]. Camelina has no reported levels of proanthocyanins or coumarins.

7.4.3.3 Mono- and Polyphenolics: Sinapine, Lignans, Tannins

The hydroxylated derivatives of benzoic and cinnamic acid are used by plants as the building blocks for flavonoids, coumarins, lignans, hydrolysable tannins, proanthocyanins, and a variety of other phenolic compounds. The phenolic compounds are

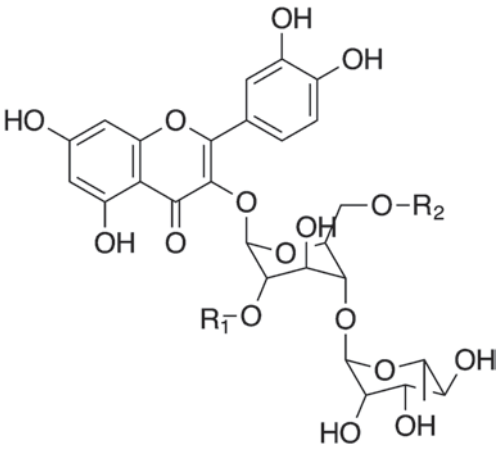

Fig. 7.2 Structure of quercetin glycosides in camelina seed meals. Rutin (quercetin-3-O-rutinoside) *R*1 = *R*2 = *H*; quercetin-2″-O-apiosyl-3-O-rutinoside *R*1 = apiose, *R*2 = *H*; quercetin-O-sinopyl-2″-O-apiosyl-3-O-rutinoside *R*1 = apiose, *R*2 (position not confirmed) = sinapic acid

the building blocks of many plant structural materials and they have a large array of biological activities including as antioxidants, as defense against pathogens and herbivores, as well as an array of dietary effects in humans and animals that consume them [30, 48].

Camelina seeds are reported to contain lignans, tannins, and some unbound phenolic acids (Table 7.9 and [28, 42, 45–48]). Camelina seeds contain sinapine, the choline ester of sinapic acid, at levels potentially as high as 60 mg/g [42]. Sinapine is present in many other Brassicaceae species and has several undesirable properties as a constituent in animal feeds. It is bitter tasting, thus rendering it less palatable to animals. It is possible that natural variability or breeding programs will lead to camelina cultivars with much lower levels of sinapine.

Table 7.8 Flavonoids and phenolics in whole camelina seeds

RT	Class	Name	Weight %
5	Phenolic	Chlorogenic acid derivative	0.07%
9	Phenolic	Sinapine	0.22%
12	Phenolic	Ellagic acid	0.11%
13	Phenolic	Sinapic acid	0.10%
16	Phenolic	Phenolic acid	0.06%
23.2	Flavonoid	Quercetin-2″-O-apiosyl-3-O-rutinoside	0.27%
23.4	Flavonoid	Flavone di-glycoside	0.11%
26	Flavonoid	Quercetin-3-O-rutinoside (rutin)	0.52%
27	Flavonoid	Kaempferol-O-rutinoside	0.07%
28.8	Flavonoid	Quercetin-O-sinopyl-2″-O-apiosyl-3-O-rutinoside	0.05%
29.2	Flavonoid	Isorhamnetin-O-rutinoside	0.08%
Total			1.61%

RT retention time in HPLC elution profile in minutes running a 250 × 4.6 C-18 reverse-phase column at 1 mL per min. The column was developed over 50 min with a liner gradient from 20% methanol and 80% 0.01M phosphoric acid to 100% methanol

Table 7.9 Sinapine, lignans, and tannins in whole camelina seeds [47, 48]

Compound	Weight percent
Sinapine[a]	0.50%
Protocatechuic acid	–
p-Hydroxybenzoic	–
Ellagic acid	–
(+)-Secoisolariciresinol	1.52%
(+)-Pinoresinol	0.98%
(+)-Syringaresinol	0.71%
(−)-Lariciresinol	2.18%
(−)-7-hydroxymatairesinol	0.04%
(+)-Medioresinol	0.65%
Cyclolariciresinol	0.12%
Secoisolariciresinol-sesquilignan	0.04%
Matairesinol	0.03%
Lariciresinol-sesquilignan	0.02%
Tannins[b]	0.20%
Total	6.58%

[a] [42] notes as high as 5%
[b] Tannins expressed as gallic acid equivalents

Table 7.10 Vitamins in camelina full fat seed meal [26]

Vitamin	Weight %
Thiamin (B1)	0.0019%
Riboflavin (B2)	0.0004%
Niacin (B3)	0.0194%
Panthothenic acid (B5)	0.0011%
Pyridoxine (B6)	0.0002%
Biotin (B7)	0.0001%
Folate (B9)	0.0003%

7.4.3.4 Vitamins

Camelina seeds contain detectable levels of several vitamins. Besides vitamin E in the oil fraction, the seeds contain several of the B vitamins (Table 7.10) [26] and are considered to be a good source of thiamin (B1), niacin (B3), and panthothenic acid (B5).

7.4.3.5 Minerals

Camelina has appreciable levels of several essential dietary minerals (Table 7.11) [26]. There is ancillary evidence that, like other Brassicaceae species, it is capable of sequestering heavy metals such as cadmium. This should be considered when growing camelina in areas that have high levels of toxic minerals in the soil.

Table 7.11 Minerals in camelina full fat seed meal [26]

Mineral	Weight %
Calcium	1.00 %
Magnesium	0.51 %
Sodium	0.06 %
Potassium	1.60 %
Chlorine	0.04 %
Phosphorus	1.40 %
Sulfur	0.24 %
Iron	0.0329 %
Copper	0.0099 %
Manganese	0.0040 %
Nickel	0.0002 %
Zinc	0.0069 %

7.4.4 Camelina Meal Uses

Due to the glucosinolate content, camelina meal has had only limited evaluation as a feed ingredient, but its use is slowly increasing. Recent research results have shown that adding defatted camelina meal to chicken [49], sheep [50], and cattle feeds [51, 52] has no discernable ill effects for meat, egg, and milk production animals. Press cake meals typically contain 10 % residual oil, which is high in omega-3 fatty acids and is somewhat enriched in phytosterols and tocopherols relative to expressed oil.

These observations, combined with the favorable amino acid composition of the protein, indicate that camelina meal is an excellent animal feed ingredient from a nutritional perspective. Current breeding work in the USA, Canada, and Finland, coupled with the analytical methodology discussed in this review, indicate that it is possible to reduce or eliminate some of the taste components of camelina that currently prevent higher percentages in feed blends. The unique character of the camelina glucosinolates, especially since they are similar in structure when converted to isothiocyanate form to the anticancer constituent sulforaphane identified in broccoli, makes camelina meal even more interesting as a functional food and as a source of nutraceuticals [33, 38].

7.5 Phytochemical Characterization Methods

Analytical methods and equipment are now well established and numerous chromatographic and spectrophotometric methods have been published for the analysis of all of the major classes of compounds found in camelina. In general, it can be concluded that (1) most spectrophotometric methods are inherently inaccurate; (2) most chromatographic methods are generally accurate and reproducible (as long as good calibration standards are available). Inaccuracies with the chromatographic

methods generally come from poor and inconsistent sample preparation methods which either do not allow for the maximum extraction of the components of interest, and/or enhances the loss of the compounds of interest through binding to clean-up and concentration methods or alteration/degradation by chemical and physical effects. The best general sample preparation method is to start with dry samples, grind them into as fine and uniformly-sized powder as possible with minimal addition of heat, extract with the maximum ratio of solvent to solid as possible, using heat or sonication to aid the process, then run the analysis on the samples with as little postextraction cleanup as possible.

For camelina, analysis should be performed on whole seeds, which can be used to compare to analytical results obtained on processed samples. Seeds can be ground with a variety of mills—for instance, coffee grinders. Because the seeds are small, sieving is not generally required.

For accurate measurement of the oil and seed meal components, they should be separated by extraction with a nonpolar solvent such as hexane. Care should be taken to prevent volatile loss if one is interested in headspace analysis [19]. Unrefined oil can be quantified after derivatization to fatty acid methyl esters using conventional GC-FID methodology [15]. Analysis of sterols, tocopherols, and terpenes is accomplished by HPLC after recovery of unsaponifiables [17, 24, 53]. The defatted seed meal may be extracted using a variety of polar solvents (water, methanol, DMSO, ethyl acetate, dichloromethane, etc.) to isolate polar constituents for further quantification using chromatographic methods such as HPLC. Some indirect spectrophotometric methods or digestion methods are needed to obtain values for starch, pectin, cellulose amounts [26], or for amino acid [23] and lignin composition [48]. Quantitative analysis of the water-soluble oligosaccharides from camelina meal is achieved by high-performance anion-exchange chromatography-pulsed amperometric detection [54–56]. The bound carbohydrates in the remaining sample can then be hydrolyzed with trifluroacetic acid and per-acetylated for subsequent GC analysis [57].

Free phenolics, flavonoids, and many other glycosylated phytochemicals can be measured by a variety of reverse phase HPLC methodologies as long as the proper absorption wavelength and standards are used [58, 59]. The emergence of accurate mass spectrometry as a benchtop detection system makes the identification of known chemical species even more straightforward. Glucosinolate analysis is more complicated. The original methodology was to analyze desulfonated forms of the glucosinolates [33, 40, 47], but if good ion-pairing agents are used, reverse phase chromatographic analysis can be carried out on intact glucosinolates extracted from the meal [33, 38, 60].

Currently there remain a few uncharacterized components of camelina seeds. Comprehensive evaluation of the seed proteins would essentially complete the compositional profile of camelina. For phytochemicals, we have used the latest accurate mass LC-MS analysis to quickly characterize the potential chemical formulas for a series of unknown compounds. We were able to tentatively identify several phenolics in the methanol extracts of defatted camelina seeds including

rutin (quercetin-3-O-rutinoside), and two derivatives of rutin—quercetin-2″-O-apiosyl-3-O-rutinoside and quercetin-O-sinopyl-2″-O-apiosyl-3-O-rutinoside. The identification of the latter two compounds was accomplished in a single CID/HCD mass fragmentation experiment in which interpretation of the daughter ions allowed for the identification of the aglycone and the various substitute fragments (Fig. 7.3). This result—coupled with DEPT NMR analysis of a partially purified compound and the observation that the isolated compound degraded to rutin—resulted in the identification of these phytochemicals in camelina.

The original M-H^- accurate mass ion of the peak at retention time 23.8 min was 741.18518 providing a chemical formula for the intact compound of $C_{32}H_{38}O_{20}$. The upper trace in Fig. 7.3 shows the UV absorbance at 280 nm, the second trace shows the SIM trace for the negative ion *m/z* 741, the third panel shows the identification of the daughter mass ions produced after collision-induced-fragmentation at 30% energy level on the original ion *m/z* 741. *M* = negative ion of the original compound, Api = loss of apiose mass, Rha = loss of rhamnose mass, Glu = loss of glucose mass. The aglycone of the compound is quercetin (M-H^- *m/z* 301), which also gives rise to an ion M-$2H^-$ (*m/z* 300) in the fragmentation. The ions resulting from the losses of apiose, rhamnose, and glucose were identified in the spectrum.

The attachment point of the apiose sugar was determined to be the 2″-position of the glucose moiety from the observation that fragmentation formed ions that were the result of the loss of two-thirds of the glucose (and the rhamnose attached to it), but the apiose moiety remained attached to one of those fragments (*m/z* 475) [61]. The identity of the unknown third 5-carbon sugar as apiose was determined by DEPT NMR on a partially purified isolate prepared from the methanol extract of camelina defatted seed meal.

Similarly, a peak with a later retention time of 27 min had a negative mass ion of 947.24225, which provided a chemical formula for the intact compound of $C_{43}H_{48}O_{24}$. It was identified by accurate mass fragmentation showing the loss of sinapic acid to form an ion *m/z* 741, which was further fragmented into ions *m/z* 609 and *m/z* 300, matching the fragmentation pattern found for quercetin-2″-O-apiosyl-3-O-rutinoside. This compound was tentatively identified as quercetin-O-sinopyl-2″-O-apiosyl-3-O-rutinoside.

The rapid advances in chromatography equipment have made the reliable and reproducible measurement of a wide range of plant chemical components possible. These accurate measurements on a limited scale can be coupled to more rapid nondestructive spectrophotometric analytical methods such as pulsed NMR and near infrared (NIR) spectroscopy, which will allow for the rapid and nondestructive analysis of thousands of samples for a wide range of physical and chemical composition parameters.

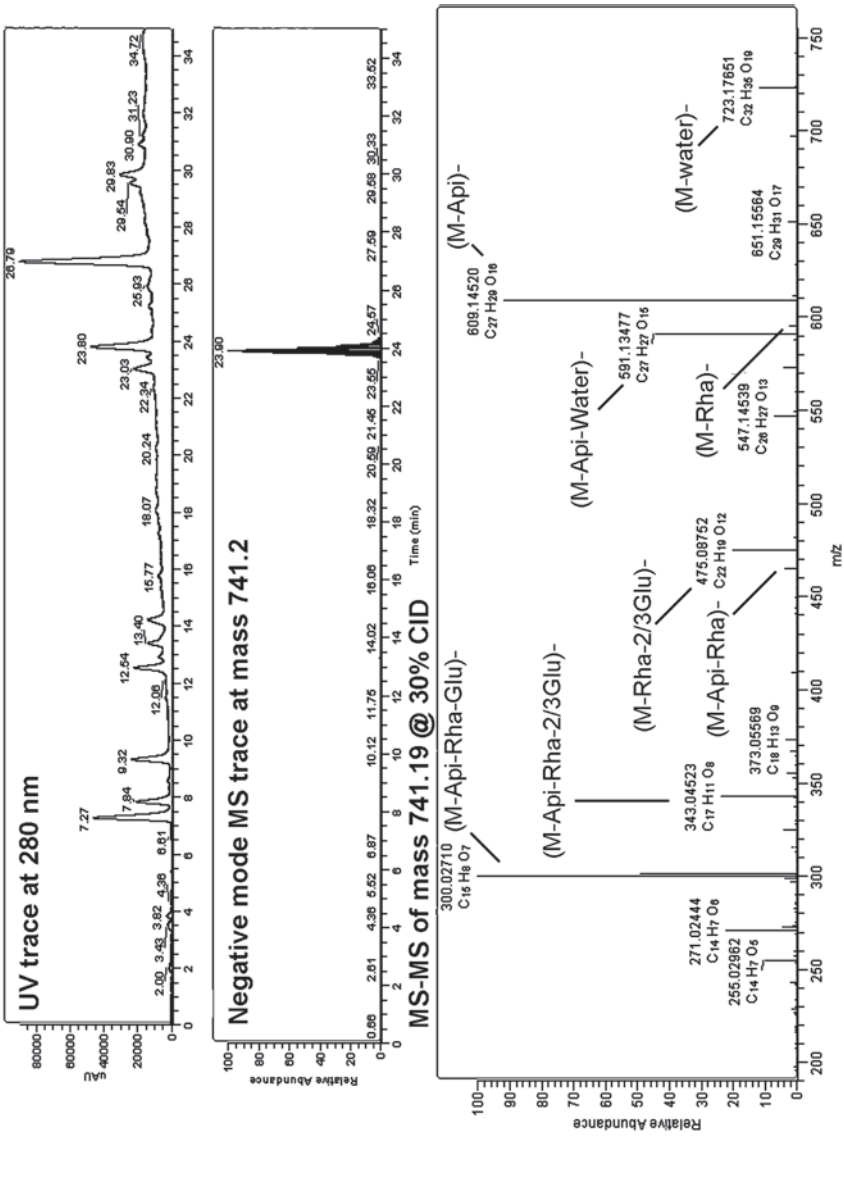

Fig. 7.3 Accurate mass MS–MS analysis used to characterize the unknown flavonol peak at retention time 23.8 min. The extract was run on a Thermo Scientific Accela UHPLC system and mass spectra were obtained on LTQ Orbitrap Discovery Mass Spectrometer (a linear ion trap (LTQ XL) MS, coupled to a high precision electrostatic ion trap (Orbitrap) MS with HCD cell, using an Ion Max electrospray ionization (ESI) source

References

1. Gugel RK, Falk KC (2006) Agronomic and seed quality evaluation of Camelina sativa in western Canada. Can J Plant Sci 86:1047–1058
2. Zeman N (2007) Crazy for Camelina. Biodiesel Magazine, February 2007. http://biodieselmagazine.com/articles/1438/crazy-for-camelina
3. Jones G, Valamoti SM (2005) Lallemantia, an imported or introduced oil plant in Bronze Age northern Greece. Veg Hist Archaeobotany 14:571–577
4. Jones G, Valamoti SM (2006) Erratum: Lallemantia, an imported or introduced oil plant in Bronze Age northern Greece. Veg Hist Archaeobotany 16:71
5. Ehrensing DT, Guy SO (2008) Camelina, Oregon State University Extension Service EM 8953-E, January 2008: Corvallis http://wwwir.library.oregonstate.edu/xmlui/bitstream/handle/1957/20552/em8953-e.pdf
6. McVay KA, Lamb PF (2008) Camelina production in montana. Montana State University Extension Office, Bozeman. http://wwwmsuextension.org/publications.asp
7. Putnam DH, Budin JT, Field LA, Breene WM (1993) Camelina: a promising low-input oilseed. In: Janick J, Simon JE (ed) New Crops. Wiley, New York, pp 314–322
8. Moser BR (2010) Camelina (*Camelina sativa* L.) oil as a biofuels feedstock: golden opportunity or false hope? Lipid Technol 22:270–273
9. Vollmann J, Moritz T, Kargl C, Baumgartner S, Wagentristl H (2007) Agronomic evaluation of camelina genotypes selected for seed quality characteristics. Ind Crop Prod 26:270–277
10. Zubr J (2003). Qualitative variation of *Camelina sativa* seed from different locations. Ind Crop Prod 17:161–169
11. Budin JT, Breene WM, Putnam DH (1995) Some compositional properties of Camelina (Camelina sativa L. Crantz) Seeds and Oils. J Am Oil Chem Soc 72:309–315
12. Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007) Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. Eur J Lipid Sci Technol 109:710–732
13. Frohlich A, Rice B (2005) Evaluation of Camelina sativa oil as a feedstock for biodiesel production. Ind Crop Prod 21:25–31
14. Moser BR (2012) Biodiesel from alternative feedstocks: Camelina and field pennycress. Biofuels 3:193–209
15. Moser BR, Vaughn SF (2010) Evaluation of alkyl esters from Camelina sativa oil as biodiesel and as blend components in ultra low-sulfur diesel fuel. Bioresour Technol 101:646–653
16. Sampath A (2009) Chemical characterization of Camelina seed Oil. In: Food Science Rutgers, the State University of New Jersey, New Brunswick, p 193
17. Szterk A, Roszko M, Sosińska E, Derewiaka D, Lewicki PP (2010) Chemical composition and oxidative stability of selected plant oils. J Am Oil Chem Soc 87:637–645
18. Zubr J, Matthaus B (2002) Effects of growth conditions on fatty acids and tocopherols in Camelina sativa oil. Ind Crop Prod 15:155–162
19. Krist S, Stuebiger G, Bail S, Unterweger H (2006) Analysis of volatile compounds and triacylglycerol composition of fatty seed oil gained from flax and false flax. Eur J Lipid Sci Technol 108:48–60
20. Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007) Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. Eur J Lipid Sci Technol 109:710–732
21. Hutcheon C, Ditt RF, Beilstein M, Comai L, Schroeder J, Goldstein E, Shewmaker CK, Nguyen T, De RJ, Kiser J (2010) Polyploid genome of Camelina sativa revealed by isolation of fatty acid synthesis genes. BMC Plant Biol 10:233
22. Peiretti PG, Meineri G (2007) Fatty acids, chemical composition and organic matter digestibility of seeds and vegetative parts of false flax (Camelina sativa L.) after different lengths of growth. Anim Feed Sci Technol 133:341–350
23. Zubr J (2003) Dietary fatty acids and amino acids of Camelina sativa seeds. J Food Qual 26:451–462

24. Schwartz H, Ollilainen V, Piironen V, Lampi AM (2008) Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. J Food Compos Anal 21:152–161
25. Shukla VKS, Dutta PC, Artz WE (2002) Camelina oil and its unusual cholesterol content. JAOCS, J Am Oil Chem Soc 79:965–969
26. Zubr J (2010) Carbohydrates, vitamins, and minerals of Camelina sativa seed. Nutr Food Sci 40:523–531
27. Vaughn SF, Kenar JA, Felker FC, Berhow MA, Cermak SC, Evangelista RL, Fanta GF, Behle RW, Lee E (2013) Evaluation of alternatives to guar gum as tackifiers for hydromulch and as clumping agents for biodegradable cat litter. Ind Crop Prod 43:798–801
28. Matthaus B (2004) Antinutritional factors in different oilseeds usable as renewable resources compared with rapeseed. In: Muzquiz M et al (eds) Recent advances of research in antinutritional factors in legume seeds and oilseeds. Academic Publishers, Wageningen, pp 63–67
29. Matthaus B, Angelini L (2005) Anti-nutritive constituents in oilseed crops from Italy. Ind Crop Prod 21:89–99
30. Matthaus B, Zubr J (2000) Variability of specific components in Camelina sativa oilseed cakes. Ind Crop Prod 12:9–18
31. Finn R (2010) Nature's chemicals: the natural products that shaped our world. Oxford University Press, New York
32. Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51
33. Clarke DB (2010) Glucosinolates, structures and analysis in food. Anal Method 2:310–325
34. Daxenbichler ME, Spencer GF, Carlson DG, Rose GB, Brinker AM, Powell RG (1991) Glucosinolate composition of seeds from 297 Species of wild plants. Phytochemistry 30:2623–2638
35. Anilakumar KR, Khanum F, Bawa AS. (2006) Dietary role of glucosinolate derivatives: a review. J Food Sci Technol 43:8–17
36. Talalay P, Fahey JW (2001) Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. J Nutr 131:3027S–3033S
37. Vig AP, Rampal G, Thind TS, Arora S (2009) Bio-protective effects of glucosinolates—a review. Food Sci Technol 42:1561–1572
38. Berhow MA, Polat U, Glinski JA, Glensk M, Vaughn SF, Isbell T, Ayala-Diaz I, Marek L, Gardner C (2013) Optimized analysis and quantification of glucosinolates from Camelina sativa seeds by reverse-phase liquid chromatography. Ind Crop Prod 43:119–125
39. Lange R, Schumann W, Petrzika M, Busch H, Marquard R (1995) Glucosinolates in linseed dodder. Fett 97:146–152
40. Schuster A, Friedt W (1998) Glucosinolate content and composition as parameters of quality of Camelina seed. Ind Crop Prod 7:297–302
41. Andersen OM, Markham KR. (2006) Flavonoids: chemistry, biochemistry and applications. CRC Press, Taylor
42. Matthaus B (2002) Antioxidant activity of extracts obtained from residues of different oilseeds. J Agric Food Chem 50:3444–3452
43. Onyilagha J, Bala A, Hallett R, Gruber M, Soroka J, Westcott N (2003) Leaf flavonoids of the cruciferous species, Camelina sativa, Crambe spp., Thlaspi arvense, and several other genera of the family Brassicaceae. Biochemical Systematics and. Ecology 31:1309–1322
44. Onyilagha JC, Gruber MY, Hallett RH, Holowachuk J, Buckner A, Soroka JJ (2012) Constitutive flavonoids deter flea beetle insect feeding in Camelina sativa L. Biochem Syst Ecol 42:128–133
45. Terpinc P, Polak T, Makuc D, Ulrih NP, Abramovic H (2003) The occurrence and characterization of phenolic compounds in Camelina sativa seed, cake and oil. Biochem Syst Ecol 31:1309–1322
46. Matthaus B, Zubr J (2000) Bioactive compounds in oil-cakes of Camelina sativa (L.) Crantz. Agro-Food-Ind Hi-Tech 11:20–24
47. Matthaus B, Zubr J (2000) Variability of specific components in Camelina sativa oilseed cakes. Ind Crop Prod 12:9–18

48. Smeds AI, Eklund PC, Willfor SM (2012) Content, composition, and stereochemical characterisation of lignans in berries and seeds. Food Chem 134:1991–1998
49. Kakani R, Fowler J, Haq AU, Murphy EJ, Rosenburger TA, Berhow MA, Bailey CA (2012) Camelina meal increases egg n-3 fatty acid content without altering egg quality or production in laying hens. Lipids 47:519–526
50. Mierlita D, Daraban S, Lup F, Chereji A (2011) The effect of grazing management and camelina seed supplementation in the diet on milk performance and milk fatty acid composition of dairy ewes. J Food Agric Environ 9:368–373
51. Halmemies-Beauchet-Filleau A, Kokkonen T, Lampi AM, Toivonen V, Shingfield KJ, Vanhatalo A (2011) Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage. J Dairy Sci 94:4413–4430
52. Moloney AP, Woods VB, Crowley JG (1998) A note on the nutritive value of camelina meal for beef cattle. Irish J Agric Food Res 37:243–247
53. Gruszka J, Kruk J (2007) RP-LC for determination of plastochromanol, tocotrienols and tocopherols in plant oils. Chromatographia 66:909–913
54. Giannoccaro E, Wang YJ, Chen P (2006) Effects of solvent, temperature, time, solvent-to-sample ratio, sample size, and defatting on the extraction of soluble sugars in soybean. J Food Sci 71:C59–C64
55. Giannoccaro E, Wang YJ, Chen P (2008) Comparison of two HPLC systems and an enzymatic method for quantification of soybean sugars. Food Chem 106:324–330
56. Hou A, Chen P, Shi A, Zhang B, Wang Y-J (2009) Sugar variation in soybean seed assessed with a rapid extraction and quantification method. Int J Agron 2009:1–8
57. Price NPJ (2004) Acylic sugar derivatives for GC/MS analysis of 13C-enrichment during carbohydrate metabolism. Anal Chem 76:6566–6574
58. Berhow MA (2002) Modern analytical techniques for flavonoid determination. In: Buslig BS, Manthey JA (eds) Flavonoids in the Living Cell. Kluwer Academic/Plenum Publishers, New York, pp 61–76
59. Berhow MA, Kong S-B, Duval SM (2006) Complete quantification of group A and group B Saponins in Soybeans. J Agric Food Chem 54:2035–2044
60. Betz JM, Fox WD (1994) High-performance liquid chromatographic determination of glucosinolates in Brassica vegetables. In: Huang M-T et al (eds) Food Chemicals for Cancer Prevention I: Fruits and Vegetables, ACS Symposia series 546. ACS publications, Washington, DC, pp 181–195
61. Vulkas V, Guttman A (2010) Structural characterization of Flavonoid Glycosides by multistage mass spectrometry. Mass Spectrom Rev 29:1–16