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Optimized analysis and quantification of glucosinolates from *Camelina sativa* seeds by reverse-phase liquid chromatography☆

Mark A. Berhow[a,*], Umit Polat[b], Jan A. Glinski[c], Michal Glensk[c,d], Steven F. Vaughn[a], Terry Isbell[a], Ivan Ayala-Diaz[f], Laura Marek[f], Candice Gardner[e]

[a] United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Functional Foods Research and Bio-Oils Research, 1815 N. University Street, Peoria, IL 61604, USA
[b] Department of Biochemistry, Faculty of Veterinary Medicine, Uludag University, Bursa 16059, Turkey
[c] Planta Analytica LLC, 39 Rose Street, Danbury, CT 06810, USA
[d] Department of Pharmacognosy, Wroclaw Medical University, Nankiera Sq. 1, 50-140 Wroclaw, Poland
[e] United States Department of Agriculture, Agricultural Research Service, Plant Introduction Research, North Central Regional Plant Introduction Station, Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[f] Agronomy Department, Iowa State University, Ames, IA 50011, USA

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ABSTRACT

Gold-of-pleasure or false flax (*Camelina sativa* L. Crantz) is being developed as an alternative oil crop for biodiesel and for food use. The seed meal, which contains three relatively unique glucosinolates, is being evaluated for approval for use as an ingredient in animal feeds and for other uses. The objective of this research was to develop reproducible methods for the isolation of large quantities of pure camelina glucosinolates (glucoarabin, glucocamelinin, and 11-(methylsulfinyl)-undecylglucosinolate) and develop efficient methods for quantifying these compounds. The separation and purification of the camelina glucosinolates were achieved using a combination of reverse phase chromatography, counter-current chromatography and ion exchange chromatography. An efficient reverse phase HPLC separation method was used to quantitate the glucosinolate content in camelina seed and plant extracts. The quantitation methodology was used to measure glucosinolate levels in seeds from 30 cultivars grown in a U.S. field trial and measure glucosinolates levels in sprouted camelina seeds.

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1. Introduction

Camelina sativa L. Crantz, known as gold-of-pleasure, false flax, wild flax, linseed dodder, camelina, German sesame, and Siberian oilseed, is a flowering plant in the Brassicaceae. It is native to Northern Europe and Central Asia, but has been introduced to North America, possibly as a weed in flax. It has been traditionally cultivated in Europe as an oilseed crop to produce vegetable oil and animal feed. There is ample archeological evidence to show it has been grown in Europe for at least 3000 years (Jones and Valamoti, 2005). Camelina was an important oil crop in eastern and central Europe, and has continued to be cultivated for its seed, which was used in oil lamps and as an edible oil. Interest in the use of camelina as a functional food and as a biodiesel feedstock continues to grow (Zeman, 2007; Moser and Vaughn, 2010). Camelina is a good alternative crop because it is much less weather dependent, has more consistent yields, and is cheaper to produce than other new crops (Moloney et al., 1998). Camelina has very low requirements for tillage and weed control (Putnam et al., 1993; Vollmann et al., 2007; Urbaniak et al., 2008). This could potentially allow this unique vegetable oil to be produced more cheaply than those from traditional oil crops, and it would be particularly attractive to biodiesel producers looking for a feedstock cheap enough to allow them to compete with petroleum diesel and gasoline. Significant new crop research is currently being conducted in the northern United States and in a number of Canadian provinces. The oil contains exceptionally high levels of omega-3 fatty acids, which is uncommon in vegetable sources (Budin et al., 1995; Abramovic and Abram, 2005; Abramovic et al., 2007; Schwartz et al., 2008) and over 50% of the fatty acids in cold-pressed Camelina oil are polyunsaturated (Budin et al., 1995; Abramovic et al., 2007).

Finding additional uses for the seed meal press-cake will make the crop more economically competitive. Camelina could be added to the growing list of functional foods. Current research efforts centers on its high levels of omega-3 fatty acids, as well as rich levels of antioxidants such as tocopherols which make the oil naturally

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* Corresponding author. Tel.: +1 309 681 6347.
E-mail address: mark.berhow@ares.usda (M.A. Berhow).

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stable (Budin et al., 1995; Abramovic et al., 2007; Dubois et al., 2007). Defatted camelina seed meal contains significant levels of proteins and carbohydrates as well as a number of phytochemicals including glucosinolates, which could be utilized for additional food, feed, and agricultural uses (Gugel and Falk, 2006; Zubr, 2010). If the meal can be used as quality feed and/or food ingredients, this will significantly increase the market value of the seed meal. Camelina seed meal contains 5–10% residual fat (which contains fairly high levels of omega 3 fatty acids), high-quality protein, and some potentially functional phytochemicals, which can be exploited to develop new feed and food uses.

Glucosinolates occur as secondary metabolites of many plants of the order Brassicales, especially in the family Brassicaceae, as well as in members of the Capparidaceae and Caricaceae families (Fenwick et al., 1983; Daxenbichler et al., 1991; Fahey et al., 2001; Clarke, 2010). Plants use chemicals derived from glucosinolates as natural pesticides and as defense against herbivores; these substances are also responsible for the bitter or sharp taste of many cruciferous vegetables (Fahey et al., 2003; Clarke, 2010). About 120 different glucosinolates are known to occur naturally in plants (Fahey et al., 2003; Clarke, 2010). The plants contain the enzyme myrosinase, which in the presence of water cleaves off the glucose group. The remaining molecule then quickly converts to a thiocyanate, an isothiocyanate or a nitrile; these are the active substances that serve as defensive compounds for the plant (Spencer and Daxenbichler, 1980; Vaughn and Berhow, 2005). To prevent damage to the plant itself, the myrosinase and glucosinolates are stored in separate compartments of the cell and come together only under conditions of stress or injury. Several degradation products of hydroxyl-substituted glucosinolates have been shown to be goitergenic in both man and animals (Hoist and Williamson, 2004; Anilakumar et al., 2006). In contrast, at subtoxic 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 (Shapiro et al., 2001; Talalay and Fahey, 2001; Fahey et al., 2003).

C. sativa accumulates significant levels of three glucosinolates in its seeds (Fig. 1): glucoarabin (9-(methylsulfinyl)nonylglucosinolate – GS9), glucocamelinin (10-(methylsulfinyl)decylglucosinolate – GS10), and 11-(methylsulfinyl)undecylglucosinolate (GS11) (Daxenbichler et al., 1991; Lange et al., 1995; Schuster and Friedt, 1998; Fahey et al., 2001; Vaughn and Berhow, 2005). The levels of glucosinolates accumulated in seeds are affected by genotype and environmental conditions (Farnham et al., 2005). Camelina glucosinolates may potentially be anti-cancer nutriceuticals in both animal and human diets. The structure of the camelina glucosinolates, containing terminal methylsulfinyl groups with varying-length aliphatic chains connecting to the glucosinolate functional group, are similar to that of glucoraphanin (4-(methylsulfinyl)butylglucosinolate), the difference being only the length of the aliphatic connecting chain. In theory, the degradation products of GS9, GS10, and GS11 should behave in a similar fashion to that of sulforaphane, the degradation product of glucoraphanin, which is an anticancer compound produced in broccoli and other crucifer vegetables (Shapiro et al., 2001; Talalay and Fahey, 2001; Fahey et al., 2003).

In order to prove this theory, quantities of purified camelina need to be produced. Preparative chromatography and countercurrent chromatography have been used to purify a variety of glucosinolates from plant extracts on a prep scale including glucoraphanin from broccoli and sinalbin from white mustard (Fahey et al., 2003; Toribio et al., 2009). These methods make it possible to purify larger quantities of glucosinolates for biological studies. The biological effects of the degradation products – the isothiocyanate, thiocyanate and nitrile forms – from the camelina glucosinolates in diets and in agriculture has not been assessed. In this report we have developed methodology to separate and purify the camelina glucosinolates in mg quantities and developed a robust method to analyze glucosinolates in camelina seeds, meal, and sprouts.

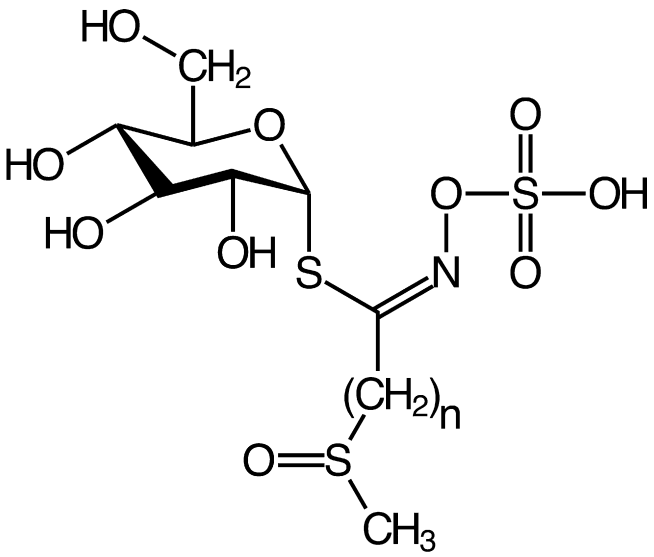

Fig. 1. Basic structure of the glucosinolates found in *Camelina sativa*. GS9 – glucoarabin (9-(methylsulfinyl)nonyl-glucosinolate), $n = 9$; GS10 – glucocamelinin (10-(methylsulfinyl)decyl-glucosinolate), $n = 10$; and GS11 – 11-(methylsulfinyl)undecyl-glucosinolate, $n = 11$.

2. Materials and methods

2.1. Reagents and chemicals

Sinigrin was purchased from Sigma–Aldrich Chemical Company (St. Louis, MO). All other chemicals and solvents were of analytical grade.

2.2. Camelina cultivars and sprouting studies

Camelina accessions were planted in individual cages and grown and harvested for seed bank increase in 2009 at NCRPIS field plots. One to two gram seed samples of each of 30 cultivars were used for comparative glucosinolate analysis.

2 g of camelina seeds were placed in a series of 10 cm diameter glass Petri plates on top of three sheets of filter paper. The plates were wetted with distilled water, covered with a glass top, and placed in a BioChambers plant growth chamber (Winnipeg, Manitoba) set at 24 °C during the day cycle and 20 °C during the night cycle, with a light dark cycle of 16 h of light (300 μE PPF PAR) and 8 h of dark. One set of plates was removed each day for the seven-day sprouting period. The remaining plates were wetted with additional water every 24 h. The sprouts were removed from the filter paper; placed in tared jar; weighed; freeze dried; then weighed again to determine an approximate weight/dry weight ratio.

2.3. Analytical sample preparation and extraction

Seeds were ground to a fine powder with a commercial coffee grinder. Weighed samples were placed in filter paper packets and defatted overnight in a Soxhlet extractor with hexane. After drying in the hood, the percent hexane extractables were determined by the difference in weight. For sprouting studies, sprouts