Lignans in Flaxseed

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Abstract: Lignans are phytoestrogens which are present in a wide variety of plants. Epidemiological studies indicate that phytoestrogen-rich diets reduce risk of various hormone-dependent cancers, heart disease, and osteroporosis. One of the reachest dietary sources of lignans are flaxseeds, with glycosides of secoisolariciresinol (SECO) and matairesinol (MAT) as the major components. In this study LC-MS/MS method for the determination of plant lignans SECO and MAT in flaxseed was developed for analysis of a wide range of samples: (*i*) nine cultivars of oil flax treated with two types of fertilisers containing humic acids and (*ii*) fibre flax cultivar Venica fertilized with preparations containing various amounts of zinc. The levels of major phytoestrogen, SECO, were in range 2312–6994 mg/kg in oil flax and 1570–3100 mg/kg in fibre flax. The content of MAT was significantly lower, ranging from 3 to 9 in oil flax and 7–27 mg/kg in fibre flax.

Keywords: secoisolariciresinol; matairesinol; flaxseed; LC-MS/MS

INTRODUCTION

Lignans belong to a group of plant phenols which are characterised by coupling of two phenylpropanoid units. They were identified in woody tissues of trees already in the 19th century. Several hundreds of lignans have been documented since then in cereals, oilseeds, nuts, legumes and fruits (Willför *et al*. 2006).

Considering the health promoting potential of food components, lignans are becoming an interesting topic because of their putative beneficial health effects, such as antitumor, antioxidant, both estrogenic, and antiestrogenic activity (Sicilia *et al*. 2003), the protection against coronary heart disease (Smeds *et al*. 2007) etc. The reachest dietary source of lignans are flaxseeds, in which glycosides of secoisolariciresinol (SECO) (Figure 1) and matairesinol (MAT) (Figure 2) are the major components accompanied with traces of pinoresinol, lariciresinol and isolariciresinol (Sicilia *et al*. 2003). The content of SECO in flaxseeds has been reported to vary from 2900 to 12 600 mg/kg, levels of MAT were found in range 5.5–58.6 mg/kg (Willför *et al*. 2006). After ingestion, plant lignans are converted by the human intestinal bacteria

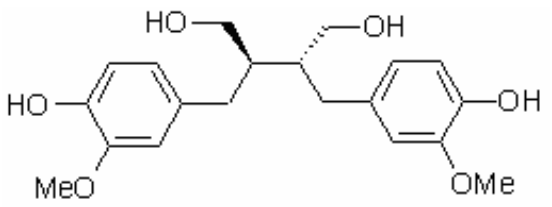

Figure 1. Chemical structure of secoisolariciresinol

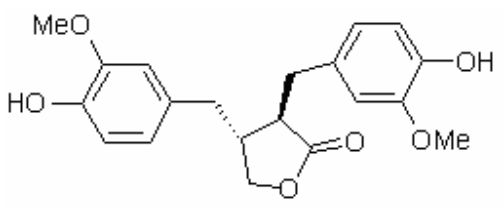

Figure 2. Chemical structure of matairesinol

Table 1. Fertilisers used for treatment of fibre flax cultivar Venica (*l/ha)

Number of experiment	Fertiliser	Amount of zinc (kg/ha)	Number of experiment	Fertiliser	Amount of zinc (kg/ha)
1	Control	0	9	Zintrak	2.15
2	Zinran	5	10	SuperZink Retard	1.5
3	Zinran	10	11	SuperZink Retard	2
4	$ZnSO_4$	5	12	$Zn(NO_3)_2$	5
5	$ZnSO_4$	10	13	$Zn(NO_3)_2$	10
6	Zinran	2.1	14	Agrozinfos	5*
7	Zinran	3	15	Zinkosol forte	2*
8	Zintrak	1.5			

to the enterodiol and enterolactone, known as mammalian lignans. These metabolites are considered to be responsible for the biological effects in humans (Charlet *et al*. 2002).

The aim of this study was to evaluate the influence of flaxseeds variety and way of farming on the content of SECO and MAT in order to identify the crop with the highest content of these health-promoting components.

MATERIALS AND METHODS

Material. Various flaxseed samples were obtained from Agritec, Plant Research Ltd. (Šumperk, Czech Republic): (*i*) Alaska, Astral, Atalante, Bajkal, Baladin, Flanders, Lola, Oural, and Recital represented oil flax cultivars (harvest 2007), which were treated with two types of fertilisers, Trisol fructus and Trisol activator at the rate of 2 l/ha. (These preparations consisted of humic acids, expected to support germination and growth.); (*ii*) fibre flax cultivar Venica was treated with several types of fertilisers containing zinc in different amounts and forms (Table 1). The crop from two harvest years (2006, 2007) was available.

Standards. Standards of secoisolariciresinol, ≥ 95.0%, (CAS No. 29388-59-8), and mataresinol, ≥ 85.0%, (CAS No. 580-72-3) were purchased from Sigma-Aldrich (Germany).

Chemicals. Acetic acid (glacial, 99.99%) and enzyme β-glucuronidase/sulfatase (type H2, from Helix Pomatia, 114 00 U/ml of β-glucuronidase and 3290 U/ml of sulfatase) were purchased from Sigma-Aldrich (Germany). Methanol (for LC), diethylether, *n*-hexane (for GC) were obtained from Merck KgaA (Germany). Sodium hydroxide was purchased from Penta (Czech Republic).

Sample preparation. 0.5 g of defatted milled flaxseeds were incubated with 12 ml of 0.3M sodium hydroxide in methanol/water (70/30, v/v) for 1 h at 60°C. After hydrolysis, the hydrolysate was neutralised with glacial acetic acid and centrifuged. An aliquot of 0.5 ml was evaporated to dryness, dissolved in 3 ml of sodium acetate buffer (0.1M, pH 5.0) with 400 µl of enzyme *Helix pomatia* *β*-glucuronidase/sulfatase and incubated overnight at 37°C. The enzymatic hydrolysate was extracted twice with 3 ml of diethylether, two organic phases were combined and evaporated to dryness. The dried sample was redissolved in 0.5 ml of methanol.

HPLC analysis were performed on an Alliance chromatography separation Module 2695 (Waters, USA) with separation on Discovery C 18 column (50 mm × 3.0 mm i. d., 5 µm) (Supelco, Germany). The mobile phase consisted of 0.5% acetic acid in water and 0.5% acetic acid in methanol, gradient elution was used. Detection was performed with a Quattro Premier XE (Waters, UK) employing an electrospray ionisation source. For detection characteristic precursor > product combinations were applied: secoisolariciresinol (361.2 > 165.0), matairesinol (357.1 > 82.7). Analytes were quantified by the standard addition method.

Methods performance characteristic. In validation study, parameters of the method were evaluated. The limits of detection (LOD) were determined by five repetitive analysis of standard solutions. The repeatability was determined by six times analysis of flaxseed extracts and was expressed as relative standard deviation (RSD, %).

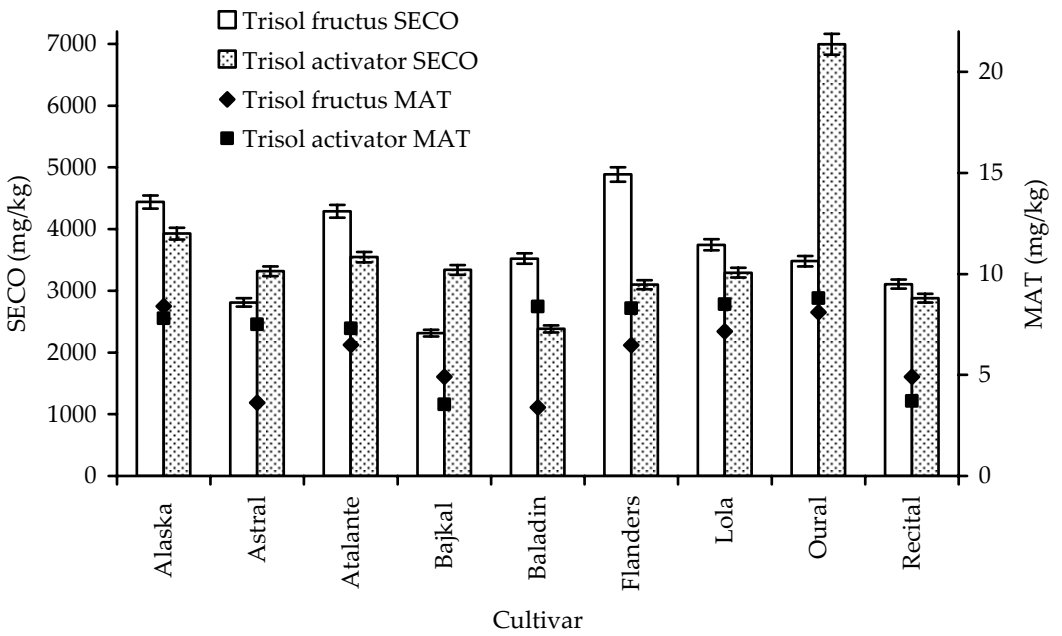

Figure 3. SECO and MAT in oil flax seeds treated with Trisol fructus or Trisol activator

RESULTS AND DISCUSSION

The content of SECO and MAT was determined in 48 flaxseed samples represented by oil and fibre flax cultivars. Before analysis, the target compounds were released from glycosidic bounds by alkaline and enzymatic hydrolysis and subsequently determined by validated LC-MS/MS method with LOD for MAT and SECO 0.06 mg/kg and 0.04 mg/kg, respectively, and with repeatability 5.5% for MAT and 2.4% for SECO.

The relationship between type of fertilisation and the levels of SECO and MAT in oil flaxseeds

The results of analysis of oil flax cultivars are shown in Figure 3. The levels of MAT in flaxseeds treated with Trisol fructus and Trisol activator ranged from 3.4 to 8.4 mg/kg and from 3.5 to 8.8 mg/kg, respectively. The highest increase in the amount of MAT was noticed in samples Baladin and Astral. Nevertheless no statistically

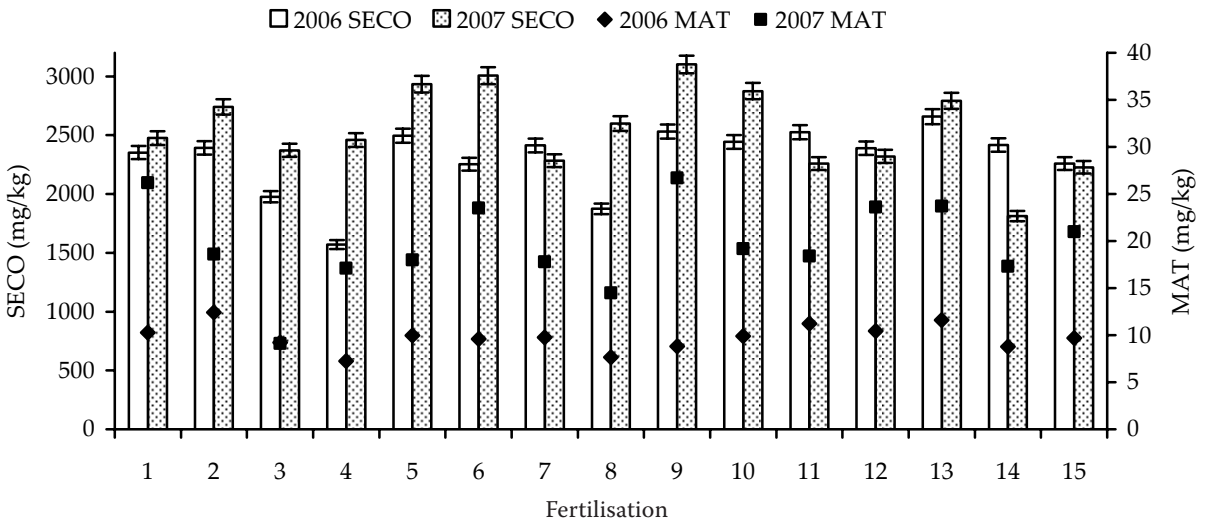

Figure 4. SECO and MAT in fibre flax Venica treated with zinc fertilisers (Table 1), two harvest years comparison

significant difference (*t*-test at 95% confidence level) was found between fertilisation with Trisol fructus and Trisol activator in the levels of MAT. Similarly in case of SECO, no general conclusion on the influence of tested fertilisers can be drawn on obtained data. The levels of SECO ranged from 2312.1 to 4886.0 mg/kg in samples treated with Trisol fructus and from 2381.8 to 6993.5 mg/kg in flaxseeds treated with Trisol activator. The largest difference in SECO content was found in Oural cultivar.

The relationship between type of fertilisation and the levels of SECO and MAT in fibre flax cultivar Venica

The results of SECO and MAT analysis of fibre flax Venica are summarised in Figure 4. While levels of MAT in 2006 were in range 7.2–12.4 mg/kg, significantly higher mean concentrations ranging from 9.1 to 26.7 mg/kg were found in 2007. The most pronounced increase amount were observed with Zintrak 2.15 kg/ha. Alike MAT, also content of SECO was significantly higher with concentration ranging from 1569.3 to 2658.3 mg/kg in 2006 compared to range 1812.2–3100.9 mg/kg in 2007.

CONCLUSIONS

The levels of major flaxseed phytoetrogens, SECO and MAT, found in this study corresponded with literature data (Willför *et al*. 2006). Relatively higher levels of these biological active compounds were found in oil flax samples. No general conclusion could be formulated neither on the influence of tested fertilizers, Trisol fructus and Trisol activator, nor on the impact of fertilizers containing zinc. More data from several harvest years are needed to find the relationship between SECO and MAT content and growing conditions.

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