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Determination of matairesinol in flax seed by HPLC with coulometric electrode array detection

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Dedicated to Emer. Univ. Prof. Dr. Gerald Kainz on the occasion of his 80th birthday.

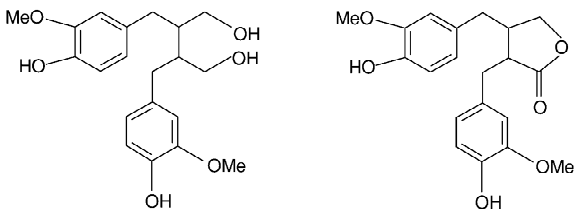
Abstract

A HPLC method coupled with coulometric electrode array detection for the determination of matairesinol in flax seed is described. The defatted sample was spiked with bisphenol A (internal standard), refluxed for 75 min in a mixture of ethanol–bidistilled water–12 *M* hydrochloric acid (2:2:1, v/v/v) to extract matairesinol conjugates and to hydrolyze them simultaneously. The extract was diluted with mobile phase [250 ml acetonitrile–750 ml buffer (730 ml bidistilled water, 20 ml glacial acetic acid adjusted to pH 3 with 5 *M* sodium hydroxide)] and injected into the HPLC system. Matairesinol was separated from other compounds on a reversed-phase column (Lichrospher 60 RP-Select B, 250×4 mm, 5 μm) and detected in a coulometric electrode array detector using a flow-rate of 0.8 ml/min. The potentials of the eight electrodes were set on +150, +200, +250, +300, +350, +400, +550 and +600 mV against modified palladium electrodes. The content of matairesinol determined in seven samples varies between 7 and 28.5 μg/g. The limit of quantitation is 5 μg/g.

Keywords: Matairesinol

1. Introduction

Dietary phytoestrogens like the lignans secoisolariciresinol and matairesinol (Fig. 1) are converted to enterodiol and enterolactone in the gastrointestinal tract. These metabolites are known for their estrogenic and antiestrogenic properties [1]. Lately flax seed gained popularity as health food, dietary supplement and as ingredient in bread and breakfast cereals leading to increased consumption of lignans [2]. Therefore reliable data on the lignan content are necessary to assess the health implications on humans.



I II

Fig. 1. Structure of secoisolariciresinol (I) and matairesinol (II).

Analytical methods based on HPLC with UV and mass spectrometric detection were developed especially for the determination of secoisolariciresinol, which is the major lignan in flax seed [3]. Matairesinol and secoisolariciresinol could be determined in flax seed, coffee, tea, cereals and berries

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by isotope dilution gaschromatography–mass spectrometry [4–9]. After enzymatic and acid hydrolysis two extractions and two ion chromatographic steps were necessary to purify the samples. Meagher et al. [10] have used HPLC with UV detection to determine lignans in flax seed meals, but this detection method lacks sensitivity and selectivity [11] and the lignans were additionally characterized after derivatisation by gas chromatography–mass spectrometry. Recently Setchell et al. [11] and Nurmi and Adlercreutz [12] have shown that HPLC with coulometric electrode array detection is suited for determination of secoisolariciresinol in processed flax seed samples and plasma, because the *o*-hydroxy-methoxy group located in both aromatic rings can easily be oxidized at a glassy carbon working electrode [13].

The present study was carried out to investigate whether this method is also suitable for determination of matairesinol, which is present in foods in very low concentrations [5]. It was also of interest to investigate whether the selectivity and sensitivity of the coulometric electrode array detection would allow simpler sample preparation.

2. Materials and methods

2.1. Materials

Seven flax seed samples were kindly provided by Flachsmann (Wädenswil, Switzerland). Matairesinol was purchased from Kristiina Wähälä (University of Helsinki, Finland) and bisphenol A (4,4′-isopropylidenediphenol) was supplied by Sigma (St. Louis, MO, USA). Methanol, ethanol, acetonitrile, *n*-hexane, glacial acetic acid, fuming hydrochloric acid (p.A. quality) were from Merck (Darmstadt, Germany).

2.2. Stock and standard solutions

Four mg matairesinol and 21 mg bisphenol A were dissolved each in 100 ml ethanol. Both solutions were stored at 4 °C in the dark. Appropriate standard solutions were prepared by diluting the stock solutions with mobile phase.

2.3. Mobile phase

Five *M* sodium hydroxide was added to 730 ml bidistilled water and 20 ml glacial acetic acid until pH 3 was reached. This solution was mixed with 250 ml acetonitrile. The mobile phase was degassed and filtered through a 0.2 μm membrane filter from Millipore (Bedford, MA, USA).

2.4. HPLC with coulometric electrode array detection

The HPLC system consisted of two pumps, model 420 (ESA, Chelmsford, USA) and a Rheodyne 7125 injector (Cotati, CA, USA) adapted with a 20 μl loop. A Lichrospher 60 RP-Select B column (250×4 mm, 5 μm) equipped with a precolumn (10×4 mm, same material) was used. For detection a coulometric electrode array system adapted with eight electrodes was combined with a controlling and data processing unit (ESA, Chelmsford, USA) and a printer (Hewlett-Packard, Boise, USA).

2.5. Linearity and limit of detection

Twenty microlitres of the standard solutions containing 2–8000 μg/l matairesinol and 10.5–42.000 μg/l bisphenol A were injected and separated isocratically on the reversed-phase column under following conditions: flow-rate, 0.8 ml/min; column temperature, 30 °C. The working potentials of the eight cells were adjusted to +150, +200, +250, +300, +350, +400, +550 and +600 mV against modified palladium electrodes. The response of each working electrode was stored, evaluated by data analysis software (Coul Array for Windows, ESA Chelmsford, USA) and printed as a multichannel chromatogram.

Calibration curves were obtained by plotting the peak heights of the optimal channels (+250 mV for matairesinol and +600 mV for bisphenol A) as a function of the concentration. The detection limits were calculated from diluted standards using a signal noise ratio (S/N) of 3:1.

A standard mixture containing 160 μg/l matairesinol and 840 μg/l bisphenol A (internal standard) was injected six times within 1 day, the

peak heights were measured and the relative standard deviation was calculated.

2.6. Analysis of flax seed samples

2.6.1. Defatting

The flax seed sample (2.5 g) was crashed and defatted with 40 ml *n*-hexane for 1 h under refluxing. The liquid was decanted and centrifuged (15 min at 1200 rpm). The clear liquid was discarded. The residue was additionally defatted as described above and then dried under vacuum at 40 °C for 30 min.

2.6.2. Extraction efficiency and acid hydrolysis

Five samples each with 0.1 g of the defatted powder were mixed with 30 ml ethanol–bidistilled water–12 *M* hydrochloric acid (2:2.5:0.5, v/v/v) and the refluxing periods of this mixture containing 1.2 *M* hydrochloric acid were varied between 5 and 180 min. After cooling to room temperature the pH was adjusted to 3–4 with 5 *M* sodium hydroxide and the solution was filtered through a folded filter (Schleicher & Schüll, Dassel, Germany) and then filled up with mobile phase to 50 ml. This procedure was repeated changing the hydrochloric acid content from 2.4 to 3.6 and 5.0 *M*. Twenty μl of the filtered hydrolysate (0.22 μm, low protein binding duropore membrane, Millipore, Bedford, MA, USA) were injected, chromatographed and detected in the coulometric electrode array detector.

The optimal hydrolysis conditions (2.4 *M* hydrochloric acid, 75 min) were used for the determination of lignans described in Section 2.6.3.

2.6.3. Qualitative and quantitative determination

A total of 0.1 g of the defatted powder was mixed with 30 ml ethanol–bidistilled water–12 *M* hydrochloric acid (2:2:1, v/v/v) and 200 μl bisphenol A standard solution (210 mg/l) were added. After a refluxing period of 75 min the mixture was cooled to room temperature and a pH between 3 and 4 was adjusted with 5 *M* sodium hydroxide. The solution was filtered through a folded filter (Schleicher & Schüll, Dassel, Germany) and then filled up with mobile phase to 50 ml. For qualitative and quantitative determination, 20 μl of the filtered hydrolysate (0.22 μm, low protein binding duropore membrane, Millipore, Bedford, MA, USA) were injected, chromatographed and detected in the coulometric electrode array detector. Matairesinol was identified by matching the retention times and the current–voltage curves with those of the standard solution. The voltammograms were obtained by plotting the signals of the working electrodes (channels) against their potentials. Matairesinol was quantified using a calibration curve by measurement of the peak height ratios (internal standard). The relative standard deviation was obtained from the results of seven double determinations. The limit of quantitation was calculated ($S/N=5$) for 0.1 g flax seed diluted after extraction and hydrolysis to 50 ml.

2.6.4. Determination of recovery

A standard mixture containing 160 μg/l matairesinol and 840 μg/l bisphenol A was hydrolysed under optimal conditions (2.4 *M* hydrochloric acid, hydrolysis time: 75 min) and the resulting mixture treated as described above. The recovery was determined from the results of four determinations using calibration curves.

After adding 200 μl bisphenol A standard solution (210 mg/l) to each flax seed sample (0.1 g) it was analyzed by the method described above. The recovery based on bisphenol A was evaluated using a calibration curve and the relative standard deviation of the percentage recovery was calculated from the results of seven double determinations.

3. Results and discussion

3.1. Qualitative analysis

From preliminary investigations of commercial flax seed extracts it was found that matairesinol can be separated from other compounds (Fig. 2) with a mobile phase containing 25% acetonitrile, 2% glacial acetic acid and 73% bidistilled water adjusted to pH 3 with sodium hydroxide. The presence of matairesinol could be confirmed by comparing the relative retention times and the current–voltage curves (Fig. 3) of matairesinol in the sample and standard solution. The optimal potentials for detecting of matairesinol (+250 mV) and bisphenol A (+600 mV) were derived from these curves. Its shape deviates from the standard sigmoid current–

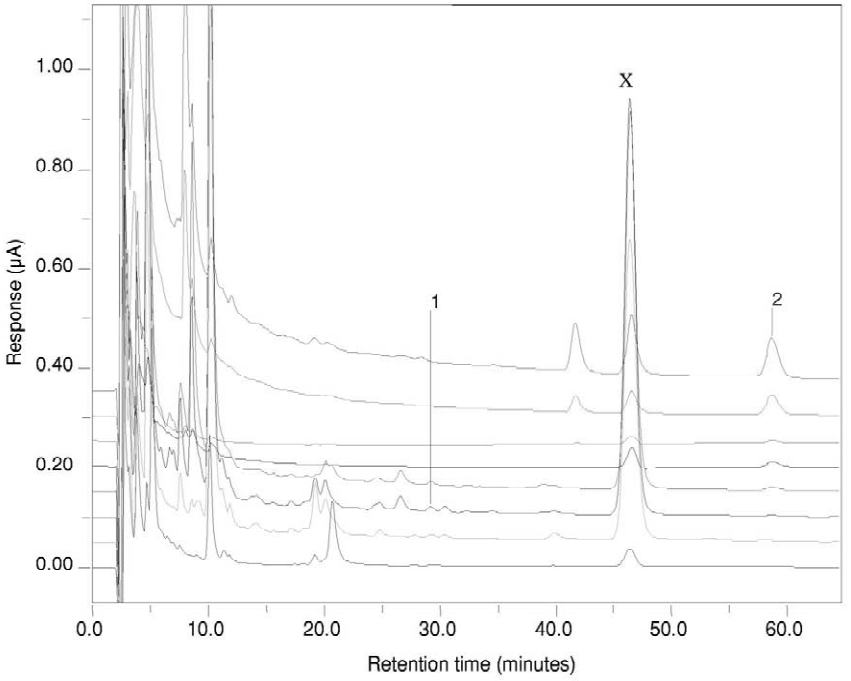

Fig. 2. Electrode array chromatograms of a flax seed extract: (1) matairesinol; (2) bisphenol A; (X) unknown compound.

voltage curve because the concentration of the compound is changing at each working electrode.

3.2. Quantitative analysis

Linear calibration plots were consistently obtained for peak heights over 2–8000 μg matairesinol/l. The correlation coefficient (R=0.9997) was calculated from the results of six standard measurements and the limit of detection was found with 2 μg/l. The precision of six repeated analysis of the pure matairesinol standard (160 μg/l) was 2.2%. The recovery of matairesinol (160 μg/l) and bisphenol A (840 μg/l) processed through the whole procedure were 93.1±2.8 and 96.8±1.3% (n=4), respectively.

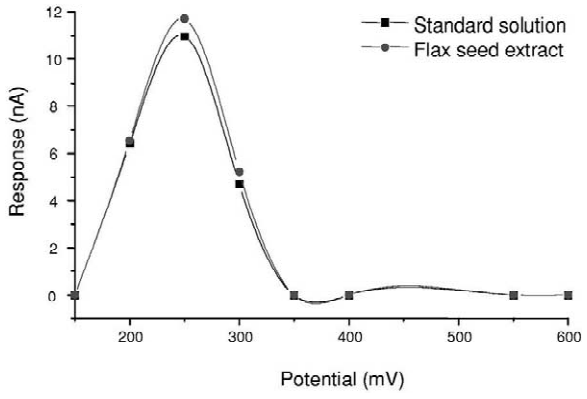

Fig. 3. Current–voltage curves of matairesinol.

Matairesinol occurs mainly as monoglucoside [14] in flax seed and has to be extracted with an appropriate solvent. Using 96% ethanol fat was released but no matairesinol was found in the alcoholic extract before and after acid hydrolysis. An ethanol–bidistilled water mixture (1:1, v/v) is suitable for the extraction of the matairesinol conjugates.

The method developed combines defatting with hexane with the extraction and hydrolysis of matairesinol conjugates in an ethanol–bidistilled water–hydrochloric acid (2:2:1, v/v/v) solvent system. To optimize the extraction efficiency the hydrochloric acid content and the refluxing period was varied. Higher concentrations of hydrochloric acid shorten the hydrolysis time but increase the de-

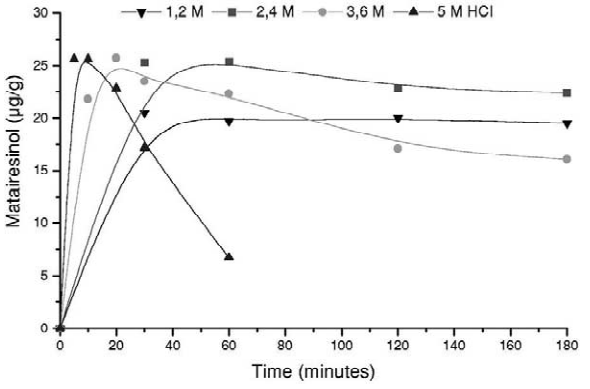

Fig. 4. Release of matairesinol from flax seed by acid hydrolysis.

composition of matairesinol (Fig. 4). At the same time an unknown compound (Fig. 2, X) showing current–voltage curve similar to that of matairesinol is formed. A hydrolysis time of 75 min at a hydrochloric acid content of 2.4 *M* leads to the highest extraction efficiency while matairesinol remains stable under these conditions.

Purification of the hydrolysis mixture before HPLC analysis was not required. The acid solution was adjusted to pH 3–4 with sodium hydroxide and then diluted with mobile phase. Each flax seed sample was analyzed two times and the mean values are summarized in Table 1. The relative standard deviation of seven double determinations of different flax seed samples is 1.2 μg/g. The limit of quantitation ($S/N=5$) was found with 5 μg/g. As described above matairesinol and bisphenol A show nearly identical recovery for a standard mixture. Therefore the percentage recovery for bisphenol A (80.3±5.0%) in spiked flax seed samples determined from seven double determinations was adopted as recovery for matairesinol.

Table 1
Matairesinol content in flax seed

Flax seed sample	Content of matairesinol[a] (μg/g)
1	28.5
2	24.4
3	19.0
4	14.6
5	12.9
6	8.6
7	7.0

[a] Mean value of two determinations.

4. Conclusion

It was shown that HPLC with coulometric array detection is a very suitable technique for analyzing phenolic compounds like lignans in complex matrices. Only minimal pretreatment of sample is required due to the selectivity and sensitivity of the coulometric electrode array detector. For qualitative analysis the retention time and the current–voltage curves can be used. Additionally the standard sigmoid *I–V* curves (hydrodynamic voltammograms) can be generated for all solutes in a single chromatographic run since each of the electrodes can be set at a different potential. This is accomplished by plotting the cumulative current as function of the potential within a given time slice [15]. Rapid qualitative information is the benefit of the coulometric array detection. It is not necessary to inject the sample several times and to change the potential of the working electrode from one to the next chromatographic run as it is necessary for a single cell detector.

Using this approach matairesinol can be determined as aglycone even in low concentrations. The quantitative results (Table 1) are in a good agreement with those published by Mazur et al. [5] and Liggins et al. [9]. From 15 compounds tested as internal standard bisphenol A fulfills the requirements needed. It is not present in the flax seed extract, it is stable during acid hydrolysis, it can be used to take account of the losses during all steps of the sample preparation and it is a compromise with respect to electrochemical behaviour and retention time.

Acknowledgements

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