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Separation and determination of secoisolariciresinol diglucoside oligomers and their hydrolysates in the flaxseed extract by high-performance liquid chromatography

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Abstract

Flaxseed contains the largest amount of lignan secoisolariciresinol diglucoside (SDG) oligomers and is the richest dietary source of SDG. SDG oligomers in the flaxseed extract are often hydrolyzed to break the ester linkages for the release of SDG and the glycosidic bonds for the release of secoisolariciresinol (SECO). The hydrolysates of SDG oligomers are complicated because of the production of esters in an alcohol-containing medium. In this study, a new gradient reversed-phase high-performance liquid chromatography (HPLC) method has been developed to be suitable for the separation and determination of: (1) SDG oligomers extracted from the defatted flaxseed powder by a 70% aqueous methanol solution; (2) SDG oligomers and their alkaline hydrolysates, including SDG, *p*-coumaric acid glucoside and its methyl ester, ferulic acid glucoside and its methyl ester in an alkaline hydrolytic solution; and (3) the succedent acid hydrolysates, including secoisolariciresinol monoglucoside (SMG), SECO, anhydrosecoisolariciresinol (anhydro-SECO), *p*-coumaric acid and its methyl ester, ferulic acid and its methyl ester, 5-hydroxymethyl-2-furfural (HMF) and its degradation product in an acid hydrolytic solution. The content of SDG oligomers in a defatted flaxseed powder was found to be 38.5 mg/g on a dry matter basis, corresponding to a SDG content of 15.4 mg/g, which was determined after alkaline hydrolysis. Furthermore, this study presented a major reaction pathway for the hydrolysis of SDG oligomers.

Keywords: Flaxseed; SDG oligomers; Secoisolariciresinol diglucoside; Secoisolariciresinol monoglucoside; Secoisolariciresinol; Anhydrosecoisolariciresinol; *p*-Coumaric acid; Ferulic acid

1. Introduction

Lignans are a large group of phenolic compounds widely distributed in edible plants [1], and belong to the group of phytoestrogens [2]. Flaxseed (*Linum usitatissimum*, Linn., Linaceae) contains the largest amount of lignan, secoisolariciresinol diglucoside (SDG) among all the grains, legumes, fruits, and vegetables [3,4], and is the richest dietary source of the plant-based SDG [3–18], which can be metabolized to the mammalian lignans, enterodiol and enterolactone by human intestinal microflora [2,18–21]. Lignans are becoming increasingly important for their possible application in the fields of pharmacy and nutrition, and have been found to possess a variety of biological properties [22]. Secoisolariciresinol (SECO) and SDG are known to have a number of potential health benefits, including reduction of serum cholesterol levels, delay in the onset of type II diabetes, and decreased formation of breast, prostate and colon cancers [15,16,21,23–26], which may be partially attributed to their antioxidant properties [16,27,28].

SECO and SDG cannot be directly determined in the flaxseed extract because SECO is present as a glycoside, SDG, which is further ester-linked with 3-hydroxy-3-methyl-glutaric acid

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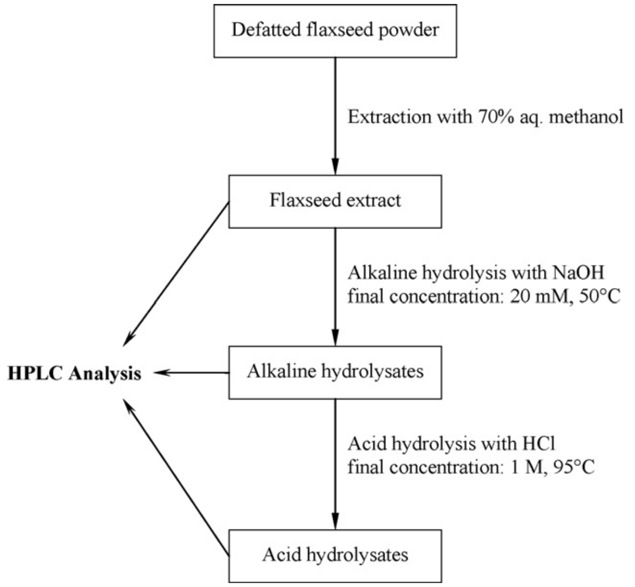

Fig. 1. Summary of the application of the developed HPLC method on the analysis of SDG oligomers and their hydrolysates.

(HMGA) to form SDG oligomers with a molecular weight of around 4000 [4]. Other phenolic compounds such as *p*-coumaric acid and ferulic acid are also present in glucosidic forms as a part of oligomers [4,6–8,11,15–17,22]. Hydrolysis, by which both ester linkages and glycosidic bonds are broken [29], is often done to simplify the subsequent chromatographic analysis of the extracts containing SDG oligomers [22]. Although strong acid hydrolysis breaks both ester linkages and glycosidic bonds whereas alkaline hydrolysis breaks ester linkages, strong acid treatment is destructive to some lignans or causes transformation reactions from one lignan to another lignan [30]. Therefore, it is necessary at first to transform the complex SDG oligomers by alkaline hydrolysis into free SDG, *p*-coumaric acid glucoside, and ferulic acid glucoside, and then an additional acid hydrolysis should be carried out to release free aglycone SECO from SDG [9,10,22]. The instability of SECO during the acid hydrolysis step results in the conversion of SECO to anhydrosecoisolariciresinol (anhydro-SECO) as an artifact by elimination of a water molecule from the diol structure of SECO [9,10,12,22,31]. In addition, secoisolariciresinol monoglucoside (SMG) has been found to be an intermediate product during the deglycosylation of SDG to form SECO by human intestinal bacteria [20]. Furthermore, Luyengi et al. [32] found a phenylpropanoid glucoside, which was a cinnamic acid methyl ester, from the defatted flaxseed. This indicates that the esters of *p*-coumaric acid and ferulic acid glucosides are formed when hydrolysis of flaxseed oligomers is performed in alcohols.

A number of analytical methods have been reported for the determination of the hydrolysates of SDG oligomers in the flaxseed extracts mainly by gas chromatography (GC) [22] and high-performance liquid chromatography (HPLC) [5–11,14,17,20,33]. The SDG oligomers in the flaxseed extracts [6–8] and their alkaline hydrolysates including SDG, *p*-coumaric acid glucoside, and ferulic acid glucoside [5–8,11,17] have been separated by the gradient reversed-phase HPLC. The acid hydrolysates of SDG oligomers including SECO, *p*-coumaric acid, and ferulic acid have been separated by the isocratic reversed-phase HPLC [10]. Charlet et al. [9] developed an isocratic reversed-phase HPLC procedure for the quantification of anhydro-SECO. Sicilia et al. [12] separated SECO, anhydro-SECO, and other lignans in the flaxseed extract after acid hydrolysis by a gradient reversed-phase HPLC.

To our knowledge, there is no previous information concerning the simultaneous determination of SDG oligomers, their alkaline hydrolysates, and the succedent acid hydrolysates. In this study, a new gradient reversed-phase HPLC method was developed to be suitable for the simultaneous separation and determination of SDG oligomers and their alkaline and acid hydrolysates, including SDG, SMG, SECO, anhydro-SECO, *p*-coumaric acid glucoside and its ester, ferulic acid glucoside and its ester, *p*-coumaric acid and its ester, ferulic acid and its ester, 5-hydroxymethyl-2-furfural (HMF) and its degradation product.

2. Experimental

2.1. Materials and reagents

The flaxseed sample, grown in Wulanchabu, Inner Mongolia, was purchased from Inner Mongolia Flaxseed Oil Health Promotion Center (Hohehot, Inner Mongolia, China). The commercial flaxseed lignan (40% of lignan) was obtained from Hunan Deray Biological Industry Group Co. Ltd. (Huaihua, Hunan, China). HPLC-grade methanol was purchased from Merck KGaA (Darmstadt, Germany). SECO (95% purity), *p*-coumaric acid (98% purity), ferulic acid (98% purity), and HMF (99% purity) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Water was purified using a Millipore Simplicity system (Billerica, MA, USA). Acetic acid, sodium hydroxide, and hydrochloric acid were of analytical grade. Preparative thin-layer chromatography (TLC) plates were prepared by depositing 10 g of silica gel (GF254, Qingdao Haiyang Chemical Co. Ltd.; Qingdao, China) onto a glass plate (20 cm × 20 cm, 2 mm). The thickness of the adsorbent (silica gel) layer is about 0.5 mm.

2.2. High-performance liquid chromatography

HPLC was conducted on a Waters liquid chromatograph equipped with a 1525 binary pump and a 2996 photodiode array detector from Waters Corporation (Milford, MA, USA). SDG oligomers and their alkaline and acid hydrolysates (Fig. 1) were separated and analyzed by using a Waters XTerra® RP18 column (4.6 mm × 250 mm, 5 μm) and a guard column (4.6 mm × 12.5 mm, 5 μm) at 30 °C. The mobile phase consisted of solvent A (0.5% of acetic acid in water) and solvent B (methanol). The following linear gradient procedure was used: 0–20 min, 15% of B; 20–30 min, 15–28% of B; 30–40 min, 28% of B; 40–60 min, 28–55% of B; 60–70 min, 55–85% of B; 70–80 min, 85% of B; and back to 15% of B within 1 min. The system was equilibrated with 15% of B for 10 min at the end of each run. The flow rate was 1.0 ml/min. The detecting wavelength was set between 210 and 400 nm, and the chromatographic peaks were measured at a wavelength of 280 nm to