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Modeling the recovery patterns from solid phase extraction purification of secoisolariciresinol diglucoside, *p*-coumaric acid glucoside, and ferulic acid glucoside from microwave-assisted flaxseed extracts

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A B S T R A C T

Flaxseed extracts obtained by microwave-assisted extraction (MAE) were cleaned of leftover salts from hydrolysis by solid phase extraction (SPE). The SPE set up was affordable, non-automated and vacuum-driven. The recovery patterns of secoisolariciresinol diglucoside (SDG), *p*-coumaric acid glucoside (PCouAG), and ferulic acid glucoside (FerAG) were modeled in two stages using regression procedures ($p < 0.05$). At stage one, the recovery patterns were predicted as a function of SPE eluent concentration in ethanol (10–50%). At stage two, the accuracy of the predictions was increased by enlarging the SPE eluent regressor's range (0–100% ethanol in water) and arranging the solvent system into three practical elution groups. The groups 1, 2 and 3 reflected the major loss, the major recovery and the minor loss of SDG, respectively. Second degree polynomial regression models were fitted for accurately predicting the recoveries of compounds. Microwave-assisted extracts obtained from 0.6 and 1.5 g defatted flaxseed meal were purified; the total SDG recoveries from the SPE funnel were 97.8 and 99.8%; and the SDG amounts obtained were 8.54 and 20 mg, respectively. The HPLC analysis of eluates pooled into practical groups allows for significant reductions in HPLC analysis time and solvent consumption which could have a positive impact on future purification studies. The results of this study allow for designing simplified, efficient and economical pilot scale studies for the purification of SDG from flaxseed extracts.

Keywords: Prediction accuracy; SDG; SPE; Purification losses; Lignan; MAE

1. Introduction

Lignans are phytoestrogens found in great amounts in flaxseed or flaxseed derivatives. Flaxseed lignans are bioconverted in the human digestive tract to the more potent mammalian lignans, enterodiol and enterolactone, which have beneficial effects for the human health such as reduction of the risk of developing cardiovascular diseases, the metabolic syndrome or certain types of cancer (Adolphe et al., 2010). The flaxseed lignan is secoisolariciresinol diglucoside (SDG); its concentration varies with the cultivated variety. SDG concentrations ranging from 1 to 1.9% on a fresh weight basis (wb) were reported by Nemes and Orsat (2011a). Flaxseed derivatives such as defatted flaxseed meal or flax hulls have higher SDG concentrations; they were found to contain about 2.3 and 4% SDG (wb) (Nemes and Orsat, 2011a). Flax hulls can be obtained from dry flaxseed by abrasive dehulling and separation of hulls from embryos by sieving or aspiration. Cui and Han (2006) found that the SDG content of flax hulls free of oil and mucilage was 2–10 times greater than that of the seed material used for dehulling. Wiesenborn et al. (2003) found an inverse linear relationship between the SDG and the oil contents of the hull, the intact seeds and the embryos fractions resulted from a dehulling process. The authors reported that the hulls contained 46 times more SDG than the embryos. Therefore, defatted flaxseed meal and flax hulls are preferred

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to whole flaxseed meal as raw materials for SDG extraction, concentration and purification.

In order to improve the yield of producing purified SDG from flaxseed, efficient extraction methods have to be used in addition to selecting raw materials concentrated in SDG. A recent review of patented technologies for the extraction and the purification of SDG from flaxseed revealed that SDG is obtained mostly from defatted and whole flaxseed meal by alcoholic extraction followed by alkaline hydrolysis and sequential purification by liquid/liquid extraction, and/or anion exchange chromatography followed by reversed phase C18 chromatography (Hosseinian and Beta, 2009). In the literature, a variety of methods have been reported for the extraction of SDG for the purpose of purification by SPE. Two categories of extraction methods can be distinguished: one that involves extraction with aqueous alcohols or mixtures of alcohols followed by alkaline hydrolysis (Johnsson et al., 2000; Muir and Westcott, 2000; Struijs et al., 2007; Thompson et al., 2006), and another that involves direct alkaline hydrolysis in aqueous or alcoholic solutions (Frank et al., 2004; Hyvarinen et al., 2006; Strandås et al., 2008). It has been demonstrated that the alcoholic extraction does not recover the lignan from the plant matrix completely thus limiting the extraction yield from the beginning. A direct alkaline hydrolysis approach is recommended as it is more efficient than using initial alcoholic extraction (Eliasson et al., 2003; Nemes and Orsat, 2011a). A conventional direct alkaline hydrolysis method was developed by Eliasson et al. (2003), and it requires 1 h of hydrolysis at room temperature with 1 M NaOH. A more efficient microwave-assisted extraction (MAE) method using a direct alkaline hydrolysis approach was recently developed by Nemes and Orsat (2010, 2011b); the MAE requires 3 min of extraction at 135 W (applied intermittently 30 s on/off) with 0.5 M NaOH. The MAE method was shown to extract the SDG from the flaxseed matrix completely; the coefficients of variation for three users over time were 2.3–4% indicating excellent repeatability; and the total losses of SDG throughout the extraction and the HPLC analysis were only 2.5% (Nemes and Orsat, 2011a). Therefore, it appears that MAE is the most efficient alternative to the methods presented above for the extraction of SDG from flaxseed.

Initial purification of SDG crude extracts was usually done by vacuum-driven SPE on C18 resins; the purpose was the fractionation of extracts in: (1) wash-fractions containing low molecular weight polar compounds, acids and leftover salts from hydrolysis, and (2) SDG-rich fractions that also contained *p*-coumaric acid glucoside (PCouAG) and ferulic acid glucoside (FerAG). The extracts were prepared for SPE by adjusting the pH to 3 (Johnsson et al., 2000; Strandås et al., 2008) or to 6.5–7 (Muir and Westcott, 2000; Struijs et al., 2007), then by concentration or drying. The C18 SPE columns were usually activated with alcohol, generally methanol, and then equilibrated with water (Frank et al., 2004; Johnsson et al., 2000; Struijs et al., 2007). The extracts, reconstituted in water (Frank et al., 2004; Johnsson et al., 2000; Muir and Westcott, 2000; Struijs et al., 2007), or in 10% methanol in water (Strandås et al., 2008), were applied to the activated and equilibrated C18 SPE columns. Then, the unwanted compounds were washed out with water (Frank et al., 2004; Johnsson et al., 2000; Struijs et al., 2007), or in some cases with water followed by 30% methanol in water (Strandås et al., 2008), or with acidified water followed by acidified 20% ethanol in water (Muir and Westcott, 2000). The wash fractions were discarded; and the SDG was eluted from the C18 SPE columns with pure methanol (Frank et al., 2004; Johnsson et al., 2000; Struijs et al., 2007; Thompson et al., 2006), 30% ethanol in water (Muir and Westcott, 2000), 40% methanol in water (Strandås et al., 2008), or 50% methanol in water (Johnsson et al., 2000). The purified extracts were usually analysed by HPLC (Frank et al., 2004; Johnsson et al., 2000; Muir and Westcott, 2000; Struijs et al., 2007), or by GC–MS (Thompson et al., 2006). Nuclear magnetic resonance and HPLC–MS were also used by Struijs et al. (2007).

The applications of initial purification of SDG extracts by C18 SPE are varied. Muir and Westcott (2000) used SPE for obtaining 80–90% pure SDG for incorporation in bakery products; and for cleaning of extracts before further purification by preparative HPLC in order to obtain >98% SDG. Johnsson et al. (2000) used SPE for removing the hydrolysis salts from extracts before HPLC analysis and further purification on silica gel 60 in view of obtaining >99% pure SDG. Frank et al. (2004) obtained >95% pure SDG for dietary supplementation experiments on rats. Strandås et al. (2008) applied SPE for obtaining high purity SDG for antioxidant capacity assessment; and for cleaning of extracts before further purification by semi-preparative HPLC in view of elucidating the composition and the structure of the flaxseed lignan macromolecule. Thompson et al. (2006) used SPE to clean lignan extracts before and after enzymatic hydrolysis.

With few exceptions, the details pertaining to the efficiency of published SPE procedures for initial purification, and the characteristics of the extracts obtained with them are not known. The recoveries of SDG from the SPE columns, relative to the SDG content of the applied extract, are not reported in the literature. Therefore, it is not possible to appreciate the extent of SDG losses as affected by the various published purification procedures. However, Johnsson et al. (2000) reported >99.5% recovery of SDG standard for the SPE procedure they used for desalting flax extracts before HPLC analysis; and SDG concentrations of >60% and 80–90% were reported by Westcott and Muir (1998) and Muir and Westcott (2000), respectively, for the initial purification of flaxseed extracts by C18 SPE.

The purpose of this work was to develop a SPE purification technique with a high SDG recovery, and to establish the recovery patterns of SDG, PCouAG, and FerAG as a function of eluent composition, practical eluent groups, and extract concentration of target compounds. The SPE technique was developed using flaxseed extracts obtained with our optimized (Nemes and Orsat, 2010, 2011b) and validated MAE method (Nemes and Orsat, 2011a) in order to maximize the recovery of pure SDG relative to the SDG content of the flaxseed material used for extraction.

2. Materials and methods

2.1. *Chemicals*

The SDG reference standard of HPLC grade (molecular weight 686.71, purity 97.6%) was purchased from Chromadex (Santa Ana, CA, USA). The solvents: hexane, methanol, acetonitrile and ethyl acetate were of HPLC grade and were purchased from Fisher Scientific (Ottawa, ON, Canada). Anhydrous ethanol was purchased from Commercial Alcohols (Brampton, ON, Canada). The reagents: sodium hydroxide (purity ≥98%), sulphuric acid (purity 95–98%), phosphoric acid (purity ≥85%) and di-potassium hydrogen phosphate (purity 98%) were purchased from Sigma–Aldrich (Oakville, ON, Canada).