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Sinapis alba seed meal as a feedstock for extracting the natural tyrosinase inhibitor 4-hydroxybenzyl alcohol

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ABSTRACT

Although mustards are ideal rotational crops in moisture-limited environments, biofuels production from mustard seed isn't economically feasible unless the de-oiled seed meal is used to generate co-products. 4-Hydroxybenzyl alcohol (HBA) derived from *Sinapis alba* (yellow mustard) seed meal is a potentially high value co-product that acts as a skin lightening agent. With increasing demand for skin lightening agents, there is need for the development of alternative products preferably derived from what are perceived as renewable, safer natural materials. Although HBA is known to have inhibitory activity on the tyrosinase reaction by reducing melanin production, the use of HBA from *S. alba*, a widely grown agricultural crop, has not been described. Our objective was to evaluate the potential of an HBA-containing *S. alba* extract to act as an inhibitor for the tyrosinase reaction by 1) optimizing the conditions for production and recovery of HBA from *S. alba* seed meal and 2) quantifying the inhibitory effect of *S. alba* seed meal extracts on the tyrosinase-activated reaction of L-3,4-dihydroxyphenylalanine (L-DOPA). The optimized procedure to produce an *S. alba* mustard seed meal extract with an HBA concentration of 2 mM included using ground mustard meal, 100 mM citrate-phosphate buffer (pH 3.8), and a 24-h incubation. The calculated IC_{50} value for the *S. alba* extract is equivalent to 6.0 µM demonstrating that potency of the extract is comparable to that achieved using standard skin lightening agents. The procedure for HBA extraction from seed meal is relatively rapid and does not require sophisticated equipment or solvent systems, making it easily adapted to large scale industrial processing facilities. The potential thus exists to use a feedstock that is readily available from an agriculturally important oilseed crop to produce HBA as a natural tyrosinase inhibitor.

1. Introduction

Production of biodiesel has increased more than 34-fold (Yeboah et al., 2013) because it is a renewable source of energy that has the potential to reduce net greenhouse gas emissions (Schneider and McCarl, 2003). Mustards are promising biodiesel crops that produce 1.5 times more oil with comparable or better characteristics than soybean oil (Blackshaw et al., 2011). Mustards also have desirable agronomic characteristics in that they are drought tolerant and well adapted to grow on marginal lands in the Mediterranean region, North America, and Asia (Saez-Bastante et al., 2016; Shim et al., 2017; Sarker et al., 2015). However, the value of mustard seed meal resulting from oil extraction must be increased to improve sustainability and on-farm profitability (Embaye et al., 2018). One approach is to use the meal as a feedstock to produce high value industrial coproducts, such as is the current focus, a skin lightening agent.

Skin lightening agents are required to supply a $20 billion industry with primary markets in Asia and India (Global Industries Analytics, 2017). Current lightening agents such as arbutin, azeic and ascorbic acid, and hydroquinone are commercially synthesized (Arndt and Fitzpatrick, 1965; Kumar et al., 2013; Breathnach et al., 1989; Shivhare et al., 2013). Many currently available lightening products have potentially toxic side effects such as transienterythema and skin irritation (Rendon and Gaviria, 2005). Also, there are concerns that other components present in the formulations can negatively impact human health (Gbetoh and Amyot, 2016). For example, mercury, hydroquinone, and clobetasol propionate are among the toxic compounds often found in high concentrations in commercial lightening products available in Canada and Africa (Gbetoh and Amyot, 2016). Recently, extracts from plant materials were screened for their potential use as skin lightening agents (Ya et al., 2015; Smit et al., 2009; Hu et al., 2017); however, many of these proposed extracts are moderately efficient. Also, the plants that are used as sources for skin lightening agents are not agricultural crops, thus creating a feedstock supply problem.

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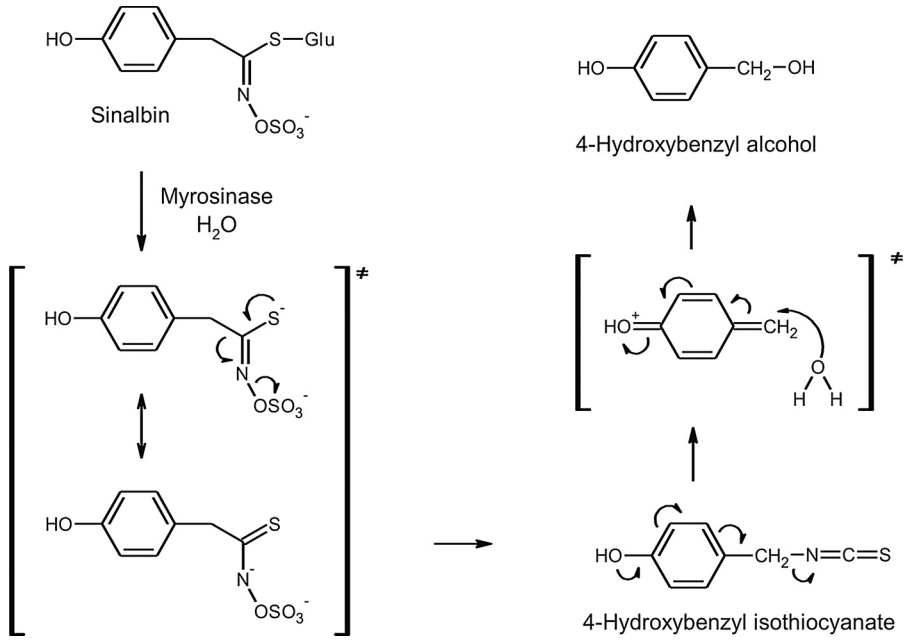

Fig. 1. Production of 4-hydroxybenzyl alcohol from sinalbin in aqueous solutions in the presence of myrosinase.

Recently, 4-hydroxybenzyl alcohol (HBA) was shown to have inhibitory activity on the tyrosinase reaction (Liu et al., 2007; Ortiz-Ruiz et al., 2015). While HBA is most often commercially synthesized, it can also be derived from several plants such as *Bletilla ochracea*, *Gastrodia elata*, and *Sinapis alba* (Cai et al., 2007; Hayashi et al., 2002; Borek and Morra, 2005). Unlike other plant sources, *S. alba* (yellow mustard) is a widely grown commercial crop that contains relatively high concentrations of the HBA precursor, sinalbin (Fig. 1). After oil is removed from *S. alba* seed for use in biodiesel production, the remaining meal by-product may contain up to 200 μmol sinalbin g^{-1} (Popova and Morra, 2014). In aqueous solutions, sinalbin is enzymatically converted to a series of products that includes an equimolar concentration of the unstable intermediate 4-hydroxybenzyl isothiocyanate that is further transformed to an equimolar amount of HBA (Fig. 1) (Borek and Morra, 2005). While this reaction has been characterized, no optimization of HBA production from *S. alba* meal has been reported. Thus, our objective was to evaluate the potential of an HBA-containing *S. alba* extract to act as an inhibitor for the tyrosinase reaction. To accomplish this objective, we 1) optimized the conditions for production and recovery of HBA from *S. alba* seed meal and 2) quantified the inhibitory effect of *S. alba* seed meal extracts on the tyrosinase-activated reaction of L-DOPA.

2. Materials and methods

2.1. Chemicals

Sinigrin (used as internal standard), HBA, *p*-phenyl acetonitrile, 3,4-dihydroxyphenylalanine (L-DOPA), and mushroom tyrosinase (monophenol monooxygenase, E.C. 1.14.18.1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sinalbin was isolated and purified from defatted *S. alba* seed meal in our laboratory. Acetonitrile, water, methanol, and other solvents were of HPLC or LC/MS grade. Solvents and all other chemicals (at least of analytical grade) were purchased from Sigma–Aldrich or Fisher Scientific (Pittsburgh, PA, USA).

2.2. HBA extraction from mustard meal

Mustard seed of *S. alba* (IdaGold variety) was obtained locally during the summer of 2016 (Latah County, ID, USA). Seed was cold crushed to produce seed meal that contained approximately 15% residual oil as described previously (Brown et al., 1991). For optimization of HBA extraction, ground mustard seed meal (0.1 g) was mixed with 5 mL of solvent in a 15-mL test tube and agitated using a Glas-Col Rugged Rotator (Glas-Col, Terre Haute, IN, USA) at 60 rpm for various times, after which meal debris was separated by centrifugation at 9600*g* and the supernatant pipetted into a clean test tube for analysis of HBA concentration. All extractions were carried out at room temperature (25 °C).

Solvent composition was optimized in two steps. In the first step, 0.1 g of autoclaved *S. alba* meal was spiked with HBA (2 mM final concentration) and 5 mL of aqueous methanol (from 0 to 100% methanol in 10% increments). Solutions were agitated using a Glas-Col Rugged Rotator for 24 h. In the second step, HBA was extracted from non-autoclaved *S. alba* meal (0.1 g) with 5 mL of aqueous methanol (0–100% methanol in 10% increments) by agitating for 24 h. For optimization of pH, 100 mM citrate-phosphate buffer (pH range 3.0–7.4) was used instead of water in the aqueous methanol solution. The time for extraction was varied from 1 to 48 h, and the meal to solvent ratio was varied from 1:5 to 1:50.

2.3. HPLC/DAD/TOF-MS analysis

HPLC analysis was performed using an Agilent 1200 Series HPLC with a diode array detector (DAD) coupled to an Agilent G1969A TOF-MS equipped with an ESI source (Agilent, Santa Clara, CA, USA). The chromatographic separation of intact glucosinolates was performed using a Zorbax SB-Aq, 50 mm × 4.6 mm, 3.5 μm rapid resolution column equipped with a Zorbax SB-Aq, 12.5 mm × 4.6 mm, 5 μm guard column (Agilent, Santa Clara, CA, USA) maintained at 30 °C. The injection volume was 5 μL. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient program started with isocratic elution using 5% B for 3 min, followed by a linear gradient to 70% B from 3 to 10 min. For the first 2 min of the analysis, the flow was diverted from the MS to prevent MS contamination and ion suppression with salts and other polar species. The flow rate was 0.4 mL min^{-1} and spectra were recorded from 190 to 400 nm.

Electrospray ionization was operated in the negative mode. The absolute values for electrospray ionization potential and collision-