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Feruloyl esterase hydrolysis and recovery of ferulic acid from jojoba meal☆

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Abstract

There is growing interest in recovering ferulic acid from plant sources for use as feedstock for several high-value applications. Jojoba meal was examined as a potential source of ferulic acid. The feruloyl esterase domain of the *Clostridium thermocellum* cellulosomal xylanase was employed to hydrolyze ferulic acid from defatted jojoba meal. Esterase treatment produced 6.7 g of ferulic acid/kg of jojoba meal. The predominant source (86%) of the ferulate was found to originate from the meal's water-soluble simmondsin fraction. Seven feruloyl simmondsin species from jojoba meal were identified by liquid chromatography–mass spectroscopy. Only one species, a didemethylsimmondsin ferulate, displayed an enzymatic hydrolysis rate distinctly faster than the other feruloyl simmondsins. Complete hydrolysis of all feruloyl simmondsin species was achieved in 24–48 h at 60 °C with a 100:1 meal:enzyme weight ratio. Ferulic acid was efficiently recovered from the medium by ethyl acetate extraction. The recovered ferulic acid was readily converted to ethyl ferulate, demonstrating a facile procedure for producing a valuable product from defatted jojoba meal.
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Keywords: Simmondsin; Feruloyl esterase; Ferulic acid

1. Introduction

Seeds of the jojoba plant (*Simmondsia chinensis*) are a source of valuable waxes used in skin and hair care products. The defatted meal, while rich in protein cannot be fed to animals unless a water-soluble toxic fraction, called simmondsin, is first removed (Cokelaere et al., 1992). The simmondsin fraction has been characterized as a family of cyanocyclohexyl glycoside compounds (Elliger et al., 1973, 1974). The simmondsin

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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fraction represents 9–10% of the dry weight of the defatted meal (Holser and Abbott, 1999). Simmondsin extraction allows defatted meal to be utilized as animal feed. Finding high-value uses for the extracted simmondsin would significantly improve the economics of the meal extraction process. Medicinal uses for jojoba meal's simmondsin fraction have been suggested, but have yet to be realized (Cokelaere et al., 1992).

Ferulic acid is a phenolic compound distributed widely throughout the plant kingdom. It has potential uses as a precursor to natural vanillin and as an UV absorber and antioxidant in skin care formulations (Taniguchi et al., 1999; Compton and Laszlo, 2002; Vandamme and Soetaert, 2002; Rouhi, 2003). Many studies suggest ferulic acid may have beneficial health effects (Ou and Kwok, 2004). Typically, ferulic acid is associated with the complex polymers of the plant cell wall in relatively low quantity, thus, making its recovery from such sources economically unfavorable (Clifford, 1999). There are a few known instances where ferulic acid is found bound to small molecules in relatively high concentrations, such as the phytosterol/phytostanol fraction of rice, corn, and barley oils (Taniguchi et al., 1999). A substantial fraction (16–20%) of the jojoba simmondsin fraction is feruloylated (Van Boven et al., 1994, 1995; Holser and Abbott, 1999), suggesting this as a potential new source of ferulic acid. Thus, ferulic acid derived from the simmondsin fraction may provide additional value to the jojoba meal extraction process.

In the present study, a method to liberate enzymatically ferulic acid from jojoba meal is examined. The *Clostridium thermocellum* cellulosome is an extracellular multiprotein complex with endo- and exocellulase, xylanase, β-glucanase, and feruloyl esterase activities (Blum et al., 2000). A recombinant feruloyl esterase domain from the complex is readily expressed in *Escherichia coli* and the produced esterase is easily isolated (Blum et al., 2000). This thermostable feruloyl esterase (FAEZ) is active with a variety of substrates from soluble xylan fragments to intact cell walls, and thus, potentially reactive with simmondsin ferulates. Selective enzymatic release of ferulic acid should facilitate the recovery and isolation of this valuable product. A facile approach for recovery of ferulic acid and conversion to ethyl ferulate is also investigated.

2. Experimental

2.1. Materials

Defatted jojoba meal and crude simmondsin extract were obtained from MGP Ingredients, Inc. (Atchison, KS). The simmondsin fraction was prepared by solvent extraction (Holser and Abbott, 1999). Meal and simmondsin extract were used without further treatment. Exposure of meal and meal extracts to light was minimized, except where noted. Purified simmondsin 2′-*trans*-ferulate was a generous gift of Professor M. Van Boven (Katholic University, Leuven, Belgium). Ferulic acid, ethyl ferulate (ethyl-4-hydroxy-3-methoxy cinnamate), acetonitrile, and ethanol were purchased from Sigma–Aldrich. Hydrochloric acid (HCl) was purchased from Fisher Scientific. FAEZ was expressed in *Escherichia coli* and isolated as described previously (referred to as FAE_{XynZ} in Blum et al., 2000). The purified enzyme had an activity of 925 U/g with methyl ferulate as the substrate.

2.2. High performance liquid chromatography (HPLC)

Simmondsin ferulate species and ferulic acid were determined using a Thermo Separations Products (San Jose, CA) HPLC system consisting of a AS3000 autosampler, P4000 pump, SCM1000 solvent degasser, UV6000LP diode array detector, and a Prodigy C8 column (5 μm, 250 mm × 4.6 mm; Phenomenex, Torrance, CA). Samples were diluted into methanol and passed through Gelman 0.45 μm 13LC PVDF syringe filters prior to injection. The injection volume was 10 μl. Ferulic acid and ferulate-containing species were detected at 325 nm (7 nm bandpass). Calibration standards were prepared from pure ferulic acid, ethyl ferulate, and simmondsin 2′-*trans*-ferulate.

For determination of ferulic acid, a binary gradient system based on acetic acid, water, butanol, and methanol was used as detailed previously (Compton et al., 2000). Simmondsin ferulate species were eluted from the column with 0.25% aqueous acetic acid (solvent A) and methanol (solvent B) using a linear gradient of 70/30 A/B to 30/70 A/B over 20 min.

HPLC–mass spectroscopy was conducted with the aforementioned HPLC equipment interfaced with a Finnigan (San Jose, CA) MAT-LCQ instrument operated in the negative ion mode. The liquid feed exit-