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Recovery of solanesol from tobacco as a value-added byproduct for alternative applications

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ABSTRACT

Solanesol in the waste streams of a bioprocess designed for alternative applications of low-alkaloid tobacco was recovered using three different extraction methods. Compared to the conventional heat-reflux extraction (HRE) and ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) using 1:3 hexane:ethanol (v/v) as the solvent after saponification treatment of tobacco biomass was found the most effective in terms of solanesol yield, processing time, and volume of solvent consumed. Quantification of solanesol was achieved by optimizing the mobile phase at 60/40 acetonitrile–isopropanol and lowering the oven temperature to 22 °C using a standard reverse-phase high performance liquid chromatography (RP-HPLC). The total solanesol recovered from tobacco biomass and chloroplast accounted for 30% (w/w) of the total solanesol in the fresh leaves. Since solanesol is the precursor of metabolically active quinones such as coenzyme Q10 and vitamin K analogues, extraction of solanesol from tobacco bioprocess waste is a feasible operation and could leverage the overall profitability of biorefining tobacco for alternative, value-added uses.

1. Introduction

Solanesol, an unsaturated polyisoprenoid alcohol containing all *trans* isoprene units (Erickson et al., 1959), is known to possess anti-bacterial, anti-inflammation, and anti-ulcer activities (Serebryakov and Nigmatov, 1990; Khidyrova and Shakhidoyatov, 2002). Industrially, it is employed by the pharmaceutical industry as an intermediate compound for the synthesis (both chemically and biotechnologically) of metabolically active quinones such as coenzyme Q10 and vitamin K analogues (Naruta, 1980; Hamamura et al., 2002; Choi et al., 2005). The demand of solanesol continues to escalate since coenzyme Q10 entered the market as a dietary supplement (Lipshutz et al., 2005) largely due to its perceived benefits in providing relief for migraine headache sufferers (Sándor et al., 2005), protecting people from Parkinson's disease and other neurodegenerative diseases (Matthews et al., 1998; Shults et al., 2002, 2004; Müller et al., 2003), and improving blood pressure and long-term glycemic control for patients with type 2 diabetes (Hodgson et al., 2002).

Biosynthesized via the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway (Fukusaki et al., 2004), solanesol is reportedly present in the middle lamina of tobacco leaves while almost absent from the stem, stalk and other parts of the plant (Rowland et al., 1956; Severson et al., 1977; Zhao et al., 1997, 2007b; Rao et al., 2008). Extensive efforts have been attempted to extract solanesol from fresh or waste tobacco leaves, including conventional heat-reflux extraction (Keca et al., 1997), solid phase extraction (Tang et al., 2007), ultrasound- (Keca et al., 1997; Chen et al., 2007) and microwave-assisted extraction (Zhou and Liu, 2006a), and supercritical fluid extraction (Ruiz-Rodriguez et al., 2008). Known factors affecting solanesol content in tobacco include the type and variety of tobacco, stage of growth, and method of curing (Stevenson et al., 1963; Sheen et al., 1978; Burton et al., 1989; Zhao et al., 2007a). The curing process has been shown to enhance the release of bound solanesol in the form of esters in flue-cured tobacco products (Severson et al., 1977; Chamberlain et al., 1988; Liu et al., 1998) and wastes (Zhao et al., 2005; Qiu et al., 2007).

Several of the aforementioned approaches have enabled commercial production of solanesol in China (Wang et al., 2005), the sole supplier of solanesol in today's market, because of its largest population of smoker in the world: over 350 million of its 1.2 billion people (Yang et al., 1999; Stillman et al., 2007). However, there remains a void in the literature on solanesol recovery from tobacco

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grown for alternative (nonsmoking) applications. Biorefining is a new manufacturing concept for converting renewable biomass to valuable products for industrial production (Andersen and Kiel, 2000; Kamm and Kamm, 2004; Gandini et al., 2006), has emerged as the most promising approach to ensure sustainable economic growth (Ragauskas et al., 2006; van Beilen and Poirier, 2008).

When grown for nonsmoking applications, tobacco plants could be managed under different agronomic schemes to attain high yield of leaf biomass with increased plant density, which might be suitable for mechanical harvesting (Anten et al., 2005). Moreover, different bioprocesses could be integrated and/or engineered to effectively separate and recover components from biological resources (Wildman, 2002; Whitfield, 2004). In the present study, quantification of solanesol using a reverse-phase high performance liquid chromatography (HPLC) was characterized and optimized. A low-alkaloid (low-nicotine) variety of tobacco grown under controlled environmental conditions was employed to assess the feasibility of recovering solanesol as a step of tobacco biorefinery for alternative applications. Leaf samples after a series of bioprocesses were subjected to different extraction procedures to compare their yield and efficacy in solanesol recovery.

2. Methods

2.1. Production of tobacco plants

Low-alkaloid tobacco (*Nicotiana tabacum* L. cv. MD-609LA) containing an average nicotine level of 0.6–0.8 mg/g dry weight, significantly lower than that of the common varieties containing nicotine up to 2.9 mg/g dry weight (Hoffmann and Hoffmann, 1998), was cultivated over a 4-month period by transplanting and hydroseeding techniques in the University of Maryland Greenhouse (College Park, MD).

For the production of the seedlings 288-cell Styrofoam trays were used. Each of the square-shaped cells has a volume of 9.7 cm^3 and the cells are distributed in a 12×24 arrangement. The tobacco seeds were scattered across the soil-filled Styrofoam cells floating on a basin of water. At the seedling stage, the plants were carefully transferred so that each soil cell was occupied by only one plant.

A couple weeks later, the overgrown tobacco plants were transplanted into individual pots (~1 gallon each) of sterilized soil. The temperature inside the greenhouse ranged between 14.8 and 32.1 °C (averaging 20.9 °C) from the 18th to 8th hour of the day and 17.2 and 32.7 °C (averaging 23.5) from the 8th to 18th hour. The higher temperatures typically occurred between 14 and 18 o'clock. The relative humidity averaged 51.0% from the 18th to the 8th hour; and 54.9% from the 8th to the 18th hour. No supplemental lighting was supplies so all growth occurred using natural day-length conditions. The plants were fertilized and watered as needed by hand. The plants were harvested when the plants reached the adult stage, as indicated by the formation of flower buds, which was approximately 6 weeks after the transplant date. The tobacco plants were labeled and delivered in bulk within two hrs on the same day to the laboratory for further processing.

2.2. Preparation of tobacco samples

Samples from different steps of the bioprocesses developed for tobacco protein extraction (Lo and Fu, 2008; Machado, 2008) were collected (Fig. 1). Fresh tobacco leaves were manually separated from the stalks and macerated upon arrival using a Meadows #35 hammer mill (Meadows Mills, Inc., North Wilkesboro, NC) with simultaneous addition of Na_2HPO_4–KH_2PO_4 buffer at a 2:1 (v/w) buffer-to-leaf ratio (Lo and Fu, 2008). The mixture of leaf-

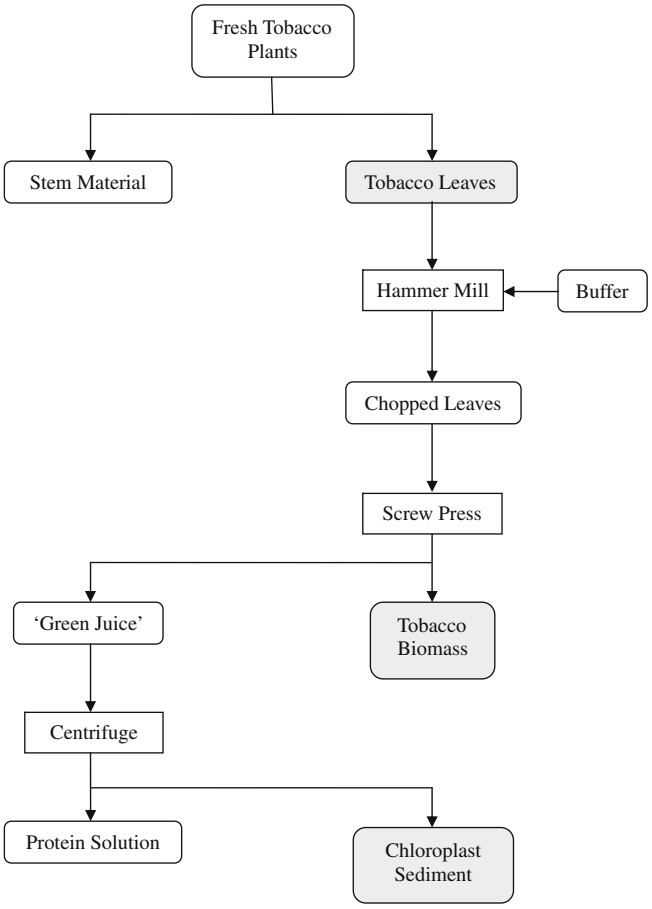

Fig. 1. Schematic diagram of processes involved in tobacco biorefinery. Samples collected from the shaded steps were subjected to the extraction treatments for solanesol recovery.

buffer solution was then screw-pressed to separate the liquid ("green juice") from tobacco biomass. The "green juice" was centrifuged under 30,000×*g* for 20 min at 4 °C using a L7-65 ultracentrifuge (Beckman-Coulter, Palo Alto, CA). The chloroplasts were precipitated at the bottom of the centrifugal tubes while the protein remains in solution.

The fresh leaves, leached biomass, and chloroplast sediment were dried in a Toastmaster Model 7091B convection oven (Toastmaster Corp., Menominee, MI) with temperature controlled at 50–55 °C to prevent denaturation of solanesol. The dried leaves were ground with a laboratory disc mill (Model 3600, Perten Instruments, Huddinge, Sweden). A No. 40 sieve (0.425 mm) was used to ensure uniform particle size.

2.3. Optimization of solanesol detection method

The solanesol concentration in the samples was determined using a high performance liquid chromatography (HPLC) system (Shimadzu LC-2010A, Shimadzu Scientific, Columbia, MD) equipped with serial dual plunger pumps, a column oven, an autosampler, and an ultraviolet–visual (UV–VIS) detector at 215 nm. A Waters reversed-phase μBondapak C18 column (3.9×300 mm, 10 μm particle size) with a guard column (Waters, Milford, MA) was used. Three oven temperatures, namely 25, 35, and 45 °C, were investigated. A solanesol standard (⩾90%, Product No. S8754) obtained from Sigma–Aldrich (St. Louis, MO) was first analyzed at various concentrations (with and without the solvents employed) to establish a calibration curve between solanesol concentrations