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Development of high 1-deoxynojirimycin (DNJ) content mulberry tea and use of response surface methodology to optimize tea-making conditions for highest DNJ extraction

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ABSTRACT

Mulberry 1-deoxynojirimycin (DNJ), a potent α-glucosidase inhibitor, suppresses postprandial blood glucose, thereby possibly preventing diabetes mellitus. At present, mulberry dry teas are commercially supplied as functional foods in many countries, but these products may not provide an effective dose (6 mg DNJ/60 kg human wt) due to their low DNJ content (about 100 mg/100 g of dry wt). Therefore, development of tea with higher DNJ content is desirable. To do this, we investigated distribution of DNJ content and α-glucosidase inhibitory activity in 35 Thai mulberry varieties. DNJ content in young leaves varied among mulberry varieties from 30 to 170 mg/100 g of dry leaves. Varieties having highest DNJ content were Kam, Burirum 60 and Burirum 51. Leaf position affected DNJ content: shoots > young leaves > mature leaves. DNJ concentration and α-glucosidase inhibitory activity were highly correlated ($r = 0.84$), suggesting that α-glucosidase inhibitory activity of mulberry leaves is mainly due to DNJ. Consequently, high DNJ content mulberry tea was produced from shoots of varieties such as Burirum 60, which contains 300 mg/100 g of dry wt. Tea-making conditions were optimized for highest DNJ extraction using response surface methodology. Approximate 95% of total DNJ in high DNJ content dry tea was extracted when temperature was maintained at 98 °C for 400 s; these conditions could be applicable for preparation of commercial products with high DNJ content. One cup (230 ml, a normal serving) of DNJ-enriched mulberry tea contained enough DNJ (6.5 mg) to effectively suppress postprandial blood glucose.

1. Introduction

Mulberry 1-deoxynojirimycin (DNJ) is a glucose analogue with an NH group substituting for the oxygen atom of the pyranose ring (Fig. 1A). Due to its potent α-glucosidase inhibitory activity (Watson, Fleet, Asano, Molyneux, & Nash, 2001), mulberry DNJ has gained much attention for use as a functional or medical food to control postprandial blood glucose, thereby potentially preventing diabetes mellitus (Kimura et al., 2007; Kong et al., 2008; Lee et al., 1994; Mingrone et al., 1999).

At present, mulberry dry tea products are commercially supplied as functional foods in many countries, *e.g.*, Japan, Korea, China and Thailand. Previously, we estimated that 6 mg DNJ/60 kg human is needed to suppress high blood glucose levels (Kimura et al., 2007); currently available commercial products (~ 100 mg/100 g of dry wt) do not provide an effective dose (Kimura et al., 2004; Nuengchamnong, Ingkaninan, Kaewruang, Wongareonwanakij, & Hongthongdaeng, 2007). Therefore, higher quality products enriched with DNJ would be desirable, and mulberry varieties with leaves rich in DNJ would be good sources of high quality dry tea. In Thailand, although mulberry trees (*Morus* spp.) have been cultivated for a long time, the DNJ content and α-glucosidase inhibitory activity of most mulberry varieties have not been determined. This is due in part to difficulty accessing various Thai mulberry cultivars and to lack of reliable quantitative methods. Recently, hydrophilic interaction chromatography has been coupled with tandem mass spectrometry (HILIC-MS/MS), and the method shows promise for selective and

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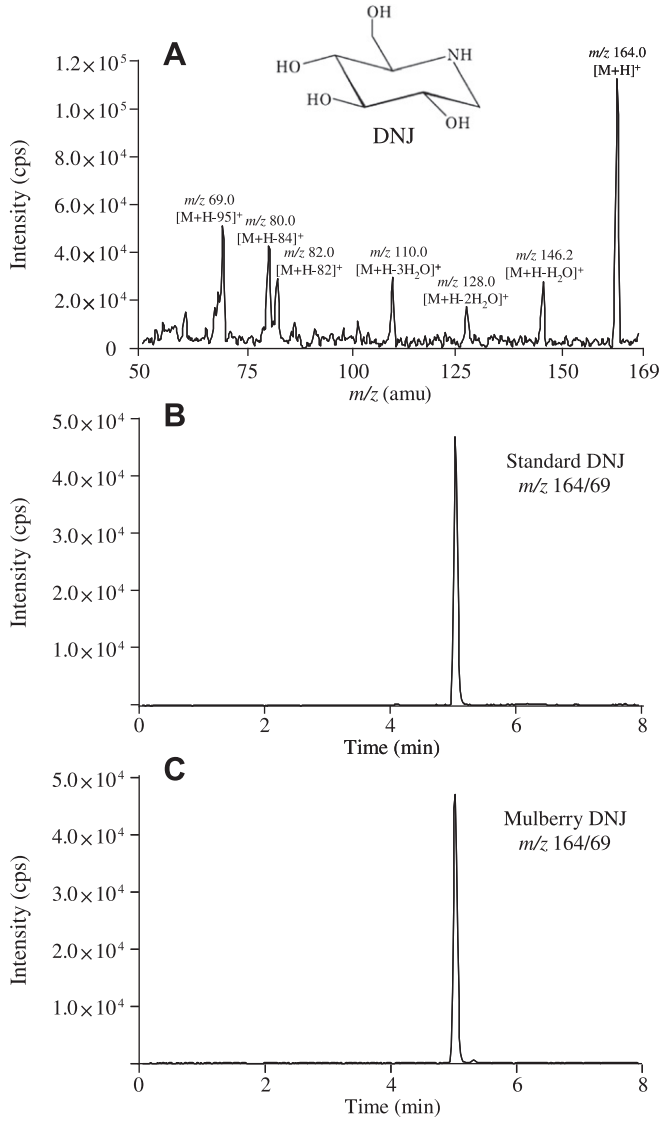

Fig. 1. Production scanning and multiple reaction monitoring (MRM) chromatograms of 1-deoxynojirimycin (DNJ). Samples were infused directly into the MS/MS with a syringe pump at a flow rate of 0.01 ml/min, and product ion scanning for *m/z* 164 $[M + H]^+$ was performed. (A) DNJ chemical structure; mulberry leaf extract (5 μl) was analyzed by HILIC-MS/MS with MRM for transition of the parent ion (*m/z* 164) to the product ion (*m/z* 69). (B) DNJ standard (500 ng/ml). (C) DNJ (500 ng/ml) extracted from mulberry leaves.

sensitive determination of mulberry DNJ (Nakagawa et al., 2008, 2010). Therefore, in this study (experiment 1), we used HILIC-MS/MS to quantify DNJ content in 35 Thai mulberry varieties and then evaluated the correlation between DNJ content and α-glucosidase inhibitory activity in order to improve the quality of mulberry dry tea.

Besides production of high quality dry mulberry tea products, tea-making conditions (hot water extraction of DNJ from dry mulberry tea leaves) should also be considered. Response surface methodology (RSM) is a statistical technique for determining the optimal (maximal or minimal) settings for experimental factors (Box & Wilson, 1951). RSM has been used to improve extraction efficiency from plant materials of chemical compounds such as lycopene, phenolic compounds, antioxidants, anthocyanins and folate (Cho, Choi, Lee, & Eitenmiller, 2010; Ghafoor, Choi, Jeon, & Jo, 2009; Lu, Engelmann, Lila & Erdman, 2008; Wijngaard & Brunton, 2009). Therefore, in experiment 2 we used RSM to optimize tea-making conditions for maximal DNJ extraction from mulberry dry tea. Our findings provide a basis for development of high quality mulberry tea products, and document optimal conditions for tea-making; the latter would be of particular interest for people who drink mulberry tea to control blood glucose levels.

2. Materials and methods

2.1. Chemicals

Standard DNJ was purchased from Wako (Osaka, Japan). Ethanol, acetonitrile, and distilled water were obtained from Kanto (Tokyo, Japan). All other reagents used were of analytical grade. Commercial mulberry dry tea products were bought from a market in Bangkok, Thailand.

2.2. Plant materials

Thirty-five Thai mulberry varieties (300 g) were collected from the conservatory fields of the Queen Sirikit Department of Sericulture (Sisaket, Thailand) in July 2008: these were Bai Mon, Bai Pou, Burirum 51, Burirum 60, Chiang Kam, Chiang Mai, Jark, Hang Plalod, Kaew, Kaew Chonabot, Kaew Kasang, Kaew Satuke, Kam, Kee Kai, Khun Phai, Krua, Pou, Prong, Mae Lokong, Meei, Nakhonratchasima 60, Noi, Phai, Phai Ubon, Sakonakhon 72, Se Da, Som, Som Yai, Sroi, Srisaket 33, Ta Dum, Ta Deang, Yai Burirum, Yai Srisaket, and Yuak. Leaves were classified into three groups based on branch positions, *e.g.,* shoots (leaf bud and the two leaves immediately below it), young leaves (the third to tenth leaves), and mature leaves (leaves below the tenth leaf). All leaf samples were cleaned, dried, ground into powder, and kept at 4 °C until DNJ analysis.

2.3. DNJ determination

Fifty mg of mulberry leaf powder was suspended in 5 ml of a solvent (ethanol and distilled water, 50:50) with sonication for 10 min. After removing insoluble substances by centrifugation at 3000 rpm for 5 min and subsequent filtration with a PTFE filter (0.45 μm pore size; Advantec, Tokyo, Japan), the solution (5 μl) was subjected to hydrophilic interaction chromatography has been coupled with tandem mass spectrometry (HILIC-MS/MS) for DNJ determination and to an α-glucosidase inhibition assay.

HILIC-MS/MS consisted of liquid chromatography (Shimadzu, Kyoto, Japan) coupled to an API 3200 MS/MS (Applied Biosystems, CA, USA). Under positive ion electrospray ionization condition, MS/MS parameters (*e.g.,* collision energy) were optimized with standard DNJ. The standard and samples prepared from mulberry leaves were separated in a TSK gel Amide-80 column (2.0 mm × 150 mm; Tosoh, Tokyo, Japan). The HILIC column was eluted using a binary gradient consisting of the following solvents: A, acetonitrile (containing 0.1% formic acid) and B, water (containing 0.1% formic acid). The gradient profile was as follows: 0–2 min, 20–60% B linear; 2–5.5 min, 60% B; 5.5–5.6 min, 60-20% B linear; 5.6–8 min, 20% B. The flow rate was 0.2 ml/min, and the column temperature was maintained at 40 °C. DNJ was detected in the post column effluent by MS/MS with multiple reaction monitoring (MRM) for transition of the parent ion of DNJ to the productions. The concentration of mulberry DNJ was calculated from the calibration curve for standard DNJ.

2.4. α-Glucosidase inhibition assay

α-Glucosidase inhibitory activity was measured by a modification of the procedure described by Ma, Hattori, Daneshtalab, and