IJIE Int. J. Indust. Entomol. 33(1) 31-35 (2016)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2016.33.1.31

Comparison of optimal temperature and time conditions for highest α-glucosidase inhibitory activity from various of Korea mulberry teas

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Abstract

The influence of commonly used steeping times and temperatures about mulberry tea on α-glucosidase inhibitory activity were studied. The effects of product and preparation variables on the in-cup chemical composition of mulberry tea extracts is of interest because the appearance and taste characteristics and the possible health effects of a tea liquor arise from the chemical components extracted from the leaf during mulberry tea preparation. A comprehensive study was therefore undertaken to determine the contributions of product and provide a basic data for development of high quality mulberry tea products against diabetes. Specific eight mulberry tea were collected from each region of Korea (A-I) and when it was extracted on 85°C for 2min, α-glucosidase activity was best for 98-102%. According to various temperature and time extraction methods, this study was carried out to optimize teamaking conditions for maximal DNJ extraction from Korean mulberry tea and and it would be of particular interest for people who drink mulberry tea to control blood glucose levels.

Int. J. Indust. Entomol. 33(1), 31-35 (2016)

Received : 19 Sep 2016
Accepted : 23 Sep 2016

Keywords:
mulberry,
1-Deoxynojirimycin (DNJ),
α-glucosidase,
temperature,
steeping time

Introduction

Mulberry 1-deoxynojirimycin (DNJ), one of the poly-hydroxylated alkaloids, can be considered as analogues of glucose in which the ring oxygen has been replaced by nitrogen. DNJ with potential antidiabetic application is known to inhibit α-glucosidase, which hydrolyzes α-glucose residues within an oligosaccharide chain. The anti-diabetes mechanism of silkworm *Bombyx mori* L. powder and extracts was found to inhibit α-glucosidase in the human small intestine, effectively. Related to this factors, the major functional component of silkworm powder is DNJ, an intestinal α-glucosidase inhibitor present in mulberry leaves (Asano, 2003) and sericulture products such as silkworm powders (Asano *et al*., 2001), which exerts a blood glucose-lowering effects and thereby prevention of diabetes mellitus.

Diabetes mellitus has become a common disease not only in developed countries but also in developing countries due to the changes in people's lifestyle and dietary habits (Horton, 1995). α-glucosidase inhibitor is usually used to prevent or medically treat type II diabetes (Non insulin dependent diabetes mellitus, NIDDM) (Floris *et al*., 2005). These inhibitors combine with intestine α-glucosidase and block the uptake of postprandial blood glucose. In particular, 1-Deoxynojirimycin (DNJ) and its derivatives have been extensively investigated for their α-glucosidase inhibitory effects on postprandial hyperglycemia,

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and applied in nutraceuticals and medicine for preventing or delaying progression of type 2 diabetes (Asai *et al*., 2011; Kimura *et al*., 2007; Vichasilp *et al*., 2012).

The DNJ content is naturally synthesized in some higher plants, such as mulberry and dayflower (Yagi *et al*., 1976, Shibano *et al*., 2004). Mulberries are widespread and important crops for fruit, and silkworm feeding, as well as being excellent amenity trees. Mulberry leaf extracts contain rutin, isoquercetin, and various derivatives of kaempferol and quercetin glycosides that can scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical (Naowaratwattana *et al*., 2010). At present, mulberry dry tea products are commercially supplied as functional foods in many countries. In previous data, it was 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). In Thailand, although mulberry trees *(Morus* spp.) have been cultivated for a long time, the DNJ content and a-glucosidase inhibitory activity of most mulberry varieties have not been determined (Vichasilpa *et al*., 2012).

Consumers with anti-diabetes and cardiovascular disease are are take in mulberry tea against the dieases. According to the mentioned contents, 60 kg human is needed 6 mg DNJ to suppress high blood glucose levels. According to various temperature and time extraction methods, this study was carried out to optimize teamaking conditions for maximal DNJ extraction from Korean mulberry tea. Our findings provide a basis for development of high quality mulberry tea products, and it would be of particular interest for people who drink mulberry tea to control blood glucose levels.

Materials and methods

Sample preparation and extraction

Specific eight mulberry tea were collected from each region of Korea (A-I; Na-Ju, Ye-Cheon, Soon-Cheon, Hab-Cheon, San-Cheong, Young-Cheon, Je-Ju, Jin-An, Sang-Ju). All mulberry tea samples were cleaned, dried, ground into powder, and kept at 4°C until α-glucosidase inhibitory activity analysis. Mulberry tea (4 g) was extracted with 400 mL of water at 70°C for 2 min using shaking water bath. The extracted solution was concentrated on 14.5 mL by rotary evarporator, and then it was filtered using 0.45 μm syringe for analysis.

Effect of various temperature and time extraction on optimum condition

For determining the effect of the extraction temperature, 4 g of mulberry tea was extracted with 400 mL of water at various temperatures (55, 70, 85, 95°C) for 2 min using a temperature-controlled shaking water bath. The optimal temperature (85°C) was then used to determine the impact of the extraction time. For this, 4 g of mulberry tea was extracted with 400 mL of water at 85°C for various lengths of time (0.5, 1, 2, 5, 10 min).

Determination of α-glucosidase inhibitory activity

The inhibitory activity of the fermentation broth against α-glucosidase was determined by reaction between α-glucosidase and 4-nitrophenyl α-D-glucopyranoside (4-NPG) according to the protocol by Yamaki and Mori (2006). The fermentation broth was serially diluted with an equal volume of distilled water and dispensed into wells of the plates (20 μL per well) followed by the addition of 5 μL of suspension of rat intestine acetone powder (Sigma-Aldrich), 12mM 4-NPG 50 μL as substrate, and 75 μL of 0.1M potassium phosphate buffer (pH 6.8). The mixture was incubated at 37°C for 35 min to allow α-glucosidase to react with 4-NPG and produce 4-nitrophenol.The reaction was terminated with the addition of Na_2CO_3 (50 μL, 200 mM). Formation of 4-nitrophenol in each well was measured by the intensity of absorbance at 405 nm using a microplate reader (BioTek Instruments Korea Ltd. Model Synergy HT).

< Calculation >

Inhibition (%) = A_{405}(inhibition) – A_{405}(control) / A_{405}(enzyme) - A_{405}(blank) X 100

Statistical analysis

Each experiment was carried out in triplicate, all data were the average of three independent experiments and analyzed by SPSS (version 18.0), and expressed as mean ± standard deviation (SD). Results were considered significant at $p < 0.05$.

Results and Discussion

α-glucosidase inhibitory activity from optimum temperature extraction

It has been reported that rat and human α-glucosidase is strongly inhibited by mulberry leaf extract (Anno *et al*., 2004; Miyahara *et al*., 2004; Oku *et al*., 2006). For commercial products such as green tea, the target compound and its concentration in the product should be known in order to achieve the best therapeutic results. In the case of mulberry tea, DNJ is the key compound because it strongly inhibits α-glucosidase and mulberry leaves contain high concentrations of it (50% of total imino sugars) (Asano *et al*., 2001). Therefore, it is important to provide a basis information for development of high quality mulberry tea products, and the temperature of the water was considered as one of the important factors that could affect the yield of DNJ extracted from mulberry tea.

Generally, green tea active ingredients, caffeine and polyphenols, are usually isolated from raw material by extraction on 70°C (Castiglioni *et al*., 2015). So, optimal temperature condition of mulberry tea also based on 70°C for α-glucosidase inhibitory activity. The results obtained are presented in Fig. 1 and α-glucosidase inhibitory activity of most mulberry teas was 70-80%. For comparing with this result, it was investigate the impact of temperature (55, 70, 85, 90°C) for 2 min (Fig. 2.). As shown this result, when it was extracted on 85°C for 2min, α-glucosidase activity was best for 98-102%. In

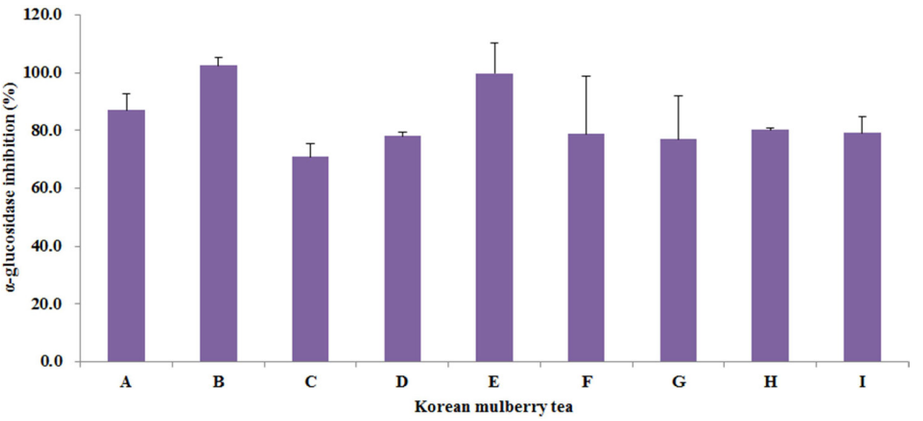

Fig. 1. α-glucosidase inhibitory activity of extracts on 70°C, 2 min from various of mulberry tea. Values are mean ± SD of triplicates. (A-I) Korean mulberry tea products (from Na-Ju, Ye-Cheon, Soon-Cheon, Hab-Cheon, San-Cheong, Young-Cheon, Je-Ju, Jin-An, Sang-Ju)

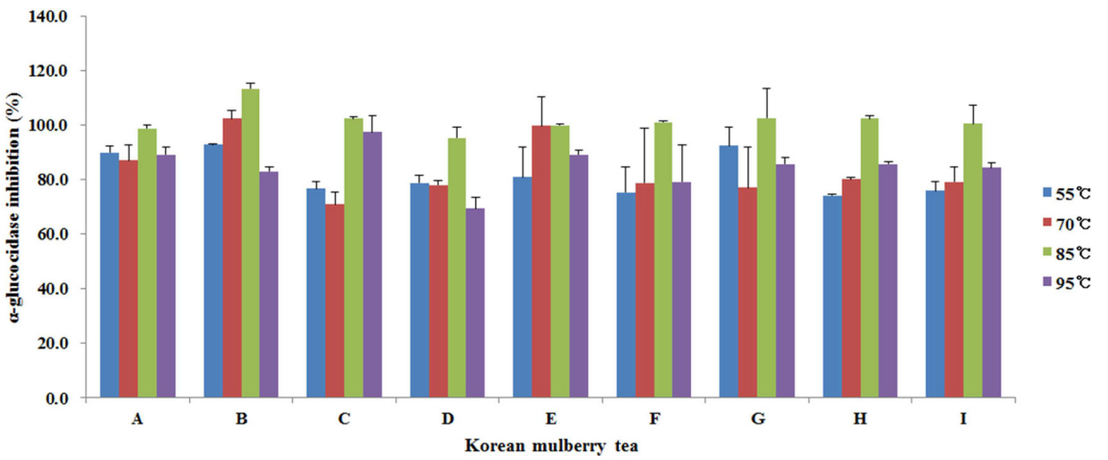

Fig. 2. α-glucosidase inhibitory activity of extracts on 55, 70, 85, 95°C, 2 min from various of mulberry tea. Values are mean ± SD of triplicates. (A-I) Korean mulberry tea products (from Na-Ju, Ye-Cheon, Soon-Cheon, Hab-Cheon, San-Cheong, Young-Cheon, Je-Ju, Jin-An, Sang-Ju)

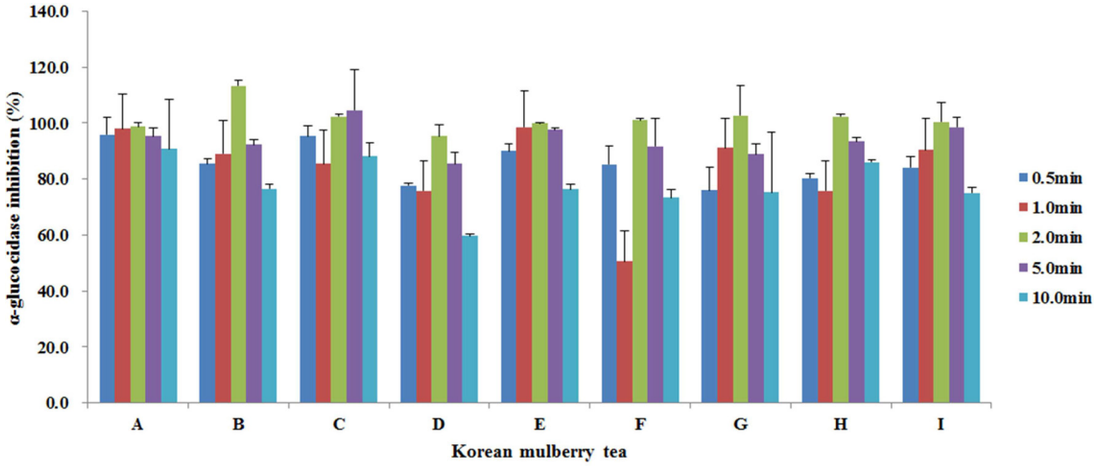

Fig. 3. α-glucosidase inhibitory activity of extracts on 0.5, 1, 2, 5, 10 min (85°C) from various of mulberry tea. Values are mean ± SD of triplicates. (A-I) Korean mulberry tea products (from Na-Ju, Ye-Cheon, Soon-Cheon, Hab-Cheon, San-Cheong, Young-Cheon, Je-Ju, Jin-An, Sang-Ju)

theory, high extraction temperatures can increase the yield of functional materials because the cell walls of the mulberry tea leaves become more permeable to the solvent and to the constituents and, thus, the solubility and diffusion coefficients of the tea functional materials are increased. However, the functional materials can also be subject to degradation when the extraction is conducted at too high temperatures due mainly to the epimerization of their structure. Therefore, extraction of functional materials from tea at the optimum temperature will help for people who drink mulberry tea to control blood glucose levels.

α-glucosidase inhibitory activity from optimum time extraction

The effect of extraction time on the yield of functional materials is good information for consumers. When preparing mulberry tea, the type of water used, water temperature, time the tea is left to infuse and the amount of tea leaves used are some of the main points in determining the flavor of the mulberry tea. Fig. 3 is a result graph of α-glucosidase inhibitory activity over extraction time. When it was extracted on 85°C for 2 min, α-glucosidase inhibitory activity was best for 98-113%.

Several factors regarding the extraction process have been shown to influence the functional materials in tea preparations, including: temperature, type of tea, concentration of tea, and steep time (Astill *et al.*, 2001; Castiglioni *et al.*, 2015). A general consumer is unlikely to monitor the exact amount of time that their tea is steeping but will keep it within the steep times recommended by the brand, usually 5 min or less. Much of the research in regards to steeping time and its influence on polyphenol content has been conducted in vitro and Others investigated the polyphenol content and antioxidant capacity after a greater range of steep times (0, 1, 2, 4, 6, 8, 10, 12, 14 and 20 min) but for only one specific type of black tea (Ceylon) (Fernando *et al.*, 2015) So, it need to set the guide line of mulberry tea's extraction time that consumers can easily utilize. Optimal extraction condition about 8 type products of Korean mulberry tea for α-glucosidase inhibitory activity was summarized (Table 1). This result provide a basic data for development of high quality mulberry tea products against diabetes.

Table 1. Recommended Brew times and temperature of Korean mulberry products for α-glucosidase inhibitory activity

Mulberry Tea	Optimum Minute, temperature	α-glucosidase inhibition(%)
A	2-5min, 85°C	95-98
B		92-113
C		102-104
D		85-95
E		97-99
F		91-101
G		88-102
H		93-102
I		98-100

Acknowledgments

This study was supported by the Department of Agricultural Biology, National Academy of Agricultural Science of the Rural Development Administration (project: PJ010817).

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