IJIE Int. J. Indust. Entomol. 35(2) 111-117 (2017)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2017.35.2.111

# Characterization of DNJ production for large-scale fermentation of mulberry leaf

O-Chul Kwon, Wan-Taek Ju, Hyun-Bok Kim, Gyoo-Byung Sung, and Yong-Soon Kim*

*Sericultural & Apicultural Materials Division, National Academy of Agricultural Science, RDA, Wanju-gun, 55365, Republic of Korea*

## Abstract

Mulberry leaves containing 1-deoxynojirimycin (DNJ) known to be a strong inhibitory effect for α-glucosidase. Thus, DNJ has been recognized as a potentially important source for prevent or treat hyperglycemia. More effective method for the DNJ high-production is needed because DNJ content of natural mulberry leaf are as low as 0.1%. Many researchers have studied for the DNJ high-production in mulberry leaves such as the harvest season, fermentation using microorganisms, optimal culture conditions, and optimal extraction conditions. In order to provide for useful data that is anticipated at the level of industrial scale, we investigated α-glucosidase inhibitory activity, pH value and DNJ content in large-scale based on the optimal culture conditions for mulberry leaf fermentation of small-scale in our previous study. The α-glucosidase inhibitory activity, pH value, and DNJ content in this study were measured from the mulberry leaf fermentation broth for 7 days. During mulberry leaf fermentation, the α-glucosidase inhibitory activity and DNJ content was increased until 2 to 4 days, but after 4 day was decreased. The pH value showed a decreasing trend up to 2 day, and little changes in 2 to 4 days. However, the pH was started to increase after 4 days.


Int. J. Indust. Entomol. 35(2), 111-117 (2017)

*Received* : 12 Dec 2017
*Revised* : 14 Dec 2017
*Accepted* : 15 Dec 2017

**Keywords**:
Mulberry leaf,
α-Glucosidase Inhibitory activity,
pH value,
1-Deoxynojirimycin (DNJ),
Large-scale fermentation

## Introduction

Mulberry family (Moraceae) comprises 37 genera with approximately 1,100 species distributed throughout tropical, sub-tropical, and temperate regions of the world (Adolkar *et al*., 2007; Clement and Weiblen, 2009; He *et al*., 2013). Mulberry (Morus alba L.) has long been used as a traditional medicine and food in Asian countries (Korea, China, and Japan) (Sastry, 1984; Jeong *et al*., 2014). In addition, mulberry has been used in East Asia (Korea, China, and Japan) as an herbal medicine for various pharmacological effects such as anti-hyperlipidemic (Kim *et al*., 1998; Dimo *et al*., 1998), anti-hypertensive (Fukai *et al*., 1985), anti-hyperglycemic (Singab *et al*., 2005), anti-allergic (Chai *et al*., 2005), hepatoprotective (Oh *et al*., 2002), and immuno-modulatory (Bharani *et al*., 2010). Mulberry leaves and fruits are commercially available in the form of various products, including teas, jam, marmalade, frozen desserts, pulp, juice, paste, ice cream, and wine in Korea and some other countries (Pawlowska *et al*., 2008; Kimura, 2011; Priya, 2012). The case of Korea, mulberry leaves have traditionally been used to treat diabetes, lower blood pressure, and to promote urination (Lee, 1981). Therefore, mulberry has been used as a potentially important source for prevent or treat.

***Corresponding author.**
Yong-Soon Kim
Department of Agricultural Biology, National Institute of Agricultural Science, RDA, Nongsaengmyeong-ro 166, Wanju-gun, 55365, Republic of Korea
Tel: +82632382845 / FAX: +82632383832
E-mail: kaiko0214@korea.kr

Mulberry has recognized as a potentially important functional food and source due to their biologically active compounds, such as 1-deoxynojirimycin (DNJ), flavonoids (anthocyanin, rutin, quercetin, and isoquercitrin), steroids, amino acids, polysaccharides, γ-aminobutyric acid (GABA), and vitamins (Yang and Tsai, 1994; Doi *et al*., 2001; Anno *et al*., 2004; Choi and Hwang, 2005; Wang *et al*., 2008; Quin *et al*., 2010; Zhang *et al*., 2016). Many Asian countries including Korea, China, and Japan practiced to take tea of mulberry leaves used as antidiabetic (Bajpai and Rao, 2014). Mulberry leaves have been known to be rich in iminosugars such as the glucose analogue 1-DNJ, N-methyl-DNJ, and 2-O-R-D-galactopyranosyl-DNJ (Asano *et al*., 2001). DNJ was isolated from natural product (Shibano *et al*., 2004), and it is firstly reported in 1976 in the root bark of Mulberry (Morus species) (Yagi *et al*., 1976). This compound was known to be the inhibition of maltase and sucrase in the human and rat small intestinal (Asano *et al*., 1994; Miyahara *et al*., 2004; Oku *et al*., 2006), and has a strong intestinal α-glucosidase inhibitor (Jeong *et al*., 2014).

Human biologically effective dose of DNJ is 6 mg per 60 kg body weight, while the DNJ content of mulberry leaf is as low as approximately 100 mg per 100 g (0.1%) of dry tea (Kimura *et al*., 2004; Gao *et al*., 2016). Thus, many researchers have studied for enhancement of DNJ production using mulberry leaves (Vichasilp *et al*., 2012; Jeong *et al*., 2014; Jiang *et al*., 2014; Ju *et al*., 2016). Jeong *et al*. (2014) and Jiang *et al*. (2014) reported that mulberry leaf fermentation by various microorganisms was increased in DNJ contents and α-glucosidase inhibitory activity in comparison to unfermented mulberry leaf.

In our previous study (Kwon *et al*., 2017), we confirmed the inhibitory activity of α-glucosidase was greatly increased average 20.12% in mulberry leaf fermentation by using B. subtilis KJ 21. In addition, we also confirmed the optimal culture conditions (about pH 7, inoculation amount 0.4%, and fermentation time of 2 to 4 days) increasing α-glucosidase inhibitory activity in mulberry leaf fermentation of small-scale. Therefore, the aim of this study was investigated α-glucosidase inhibitory activity, pH value and the DNJ content in large-scale of mulberry leaf fermentation based on a basic data of previous study.

## Materials and Methods

### Sample preparation

Mulberry leaves were collected from the Sericulture and Apiculture Division for Department of Agricultural Biology, RDA, Suwon, Republic of Korea. These samples were dried, ground to powder for fermentation, and stored at 4°C until use. Bacillus subtilis KJ 21 (KACC 19079) isolated from soil was cultured on mulberry leaf powder media.

### Mulberry leaf fermentation

The fermentation conditions were carried out based on results of our previous study (Kwon *et al*., 2017). The mulberry leaf fermentation medium contain the following compositions (g/L): $Fe_2(SO_4)_3$ 0.028g, $ZnCl_2$ 0.007g, $CaCl_2$ 0.15g, $K_2HPO_4$ 14g, $KH_2PO_4$ 6g, $(NH_4)_2SO_4$ 2g, $MgSO_4 \cdot 7H_2O$ 0.2g, $MnSO_4$ 0.0017g, and glucose 25g with 2.5% (w/v) mulberry leaf powder. All media were autoclaved at 121°C for 10 min. The initial pH value of the fermentation medium was approximately pH 7. Before fermentation, the seed cultures of B. subtilis KJ 21 was cultured on LB broth (Luria-Bertani) and incubated at 37°C with shaking 120 rpm for 1 day. The mulberry leaf fermentation was performed in a 2 L capacity in a 5 L fermentation tank of Marado-PDA (CNS Co., Ltd, Daejeon, Korea) and added 0.4% (v/v) of Bacillus inoculum (seed culture). Temperature and agitation speed of the fermentation were maintained at 37 °C and 180 rpm, respectively. The concentration of dissolved oxygen (DO) in the fermentation was maintained at about 0.2%. α-Glucosidase inhibitory activity, pH value, and DNJ content were measured from the mulberry leaf fermentation broth for 1 to 7 days.

### Measurement of α-glucosidase inhibitory activity

The inhibitory activity of α-glucosidase for the fermentation broth against was performed using the modified Yamaki and Mori method (2006). The inhibitory activity of α-glucosidase determined by reaction between α-glucosidase and 4-nitrophenyl α-D-glucopyranoside (4-NPG). The fermentation broth was centrifuged at 10,000 rpm for 10 min, and transferred the supernatant to a new Eppendorf tube. The supernatant 20 μL was mixed with 1M potassium phosphate buffer (pH 6.8) 75

μL, 12 mM 4-NPG 50 μL as substrate, and 20 μL of rat intestine acetone powder solution (Sigma-Aldrich, St. Louis, MO, USA). The mixture was incubated at 37°C for 35 min to produce 4-nitrophenol. The reaction was terminated by addition of 50 μL of 200 mM $Na_2CO_3$, and measured by the intensity of absorbance at 405 nm using a microplate reader (Model Synergy HT; BioTek Instruments Korea, Ltd., Seoul, Korea). The inhibitory activity of α-glucosidase was calculated using the following equation:

$$\text{Inhibition (\%)} = A_{405}(\text{inhibition}) - A_{405}(\text{control}) / A_{405}(\text{enzyme}) - A_{405}(\text{blank}) \times 100$$

The inhibitory activity of α-glucosidase is expressed as mean ± standard deviation (SD) of at three independent experiments. Significant differences relative to controls were analyzed using one-way ANOVA followed by Tukey's test ($p < 0.05$) using Prism (GraphPad Software Inc., La Jolla, CA, USA).

### DNJ determination

The DNJ content analysis was performed using the modified Jiang *et al*. (2014). The DNJ crude extract of mulberry fermentation broth was mixed with 0.4 M borate buffer (10 μL), 5mM FMOC-CI (9-fluorenylmethyl chloroformate) (20 μL), and then the mixture was reacted on 20°C for 20 min. Finally, 0.1 M glycine (10 μL) was added to the sample, which was adjusted to 950 μL with 10% acetic acid. The DNJ content was determined using SHISEIDO SP3203 HPLC system with fluorescence detection (Shiseido Co., Ltd., Japan; excitation 254 nm, emission 322 nm) and a Phenomenex C-18 (25 x 4.6 mm, 5 μm, USA) column. The chromatography conditions were as follows: column temperature 40°C; flow rate, 1mL/min; injection volume, 10 μL; mobile phase, acetonitrile (solvent A) - 0.1% of aqueous acetic acid (solvent B) 50:50 (v/v); and detection wavelength, 254 nm.

## Results and Discussion

### α-Glucosidase inhibitory activity and DNJ content according to scale-up of mulberry leaf fermentation

In previous study, we (Ju *et al*., 2015; Kwon *et al*., 2017) confirmed that α-glucosidase inhibitory activity and DNJ content for mulberry leaf powder fermented by B. subtilis KJ 21 were greatly increased average 20.12% and 1.5-fold respectively, than unfermented. Based on the optimal culture conditions (about pH 7, inoculation amount 0.4%, and fermentation time of 2 to 4 days) for mulberry leaf fermentation of small-scale in our previous study, when B. subtilis KJ 21 was cultured on mulberry leaf powder media for 1 to 7 days, the inhibitory activity of α-glucosidase according to scale-up are shown in Fig. 1. As a result, α-glucosidase inhibitory activity in large-scale of mulberry leaf fermentation for 7 days ranged from 66.59 ± 3.13 to 89.29 ± 3.28%. The highest α-glucosidase inhibitory activity was 89.29 ± 3.28% at 2 day of fermentation, while the lowest α-glucosidase inhibitory activity was 66.59 ± 3.13 at 7 day of fermentation. Overall, α-glucosidase inhibitory activity in large-scale of mulberry leaf fermentation was increased until 2 to 4 days, but after 4 day was decreased. Especially, in fermentation of 2 day and 3 day shown that α-glucosidase inhibitory activity were increased significantly by 11.98 and 11.02 % respectively, than 1 day of incubation. These results are similar to our previous study (Kwon *et al*., 2017) for mulberry leaf fermentation of small-scale. Therefore, mulberry leaf fermentation according to scale-up is not much difference to α-glucosidase inhibitory activity in small-scale.

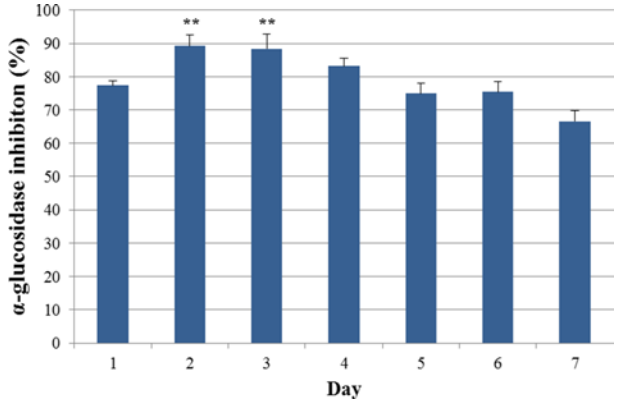


**Fig. 1.** α-Glucosidase inhibitory activity according to scale-up of mulberry leaf fermentation. *, Significant difference compared to 1 day of incubation. The values are expressed as mean ± SD (n=3), and the data were analyzed using one-way ANOVA. *P<0.05, **P<0.01, and ***P<0.001.

HPLC chromatograms of DNJ in mulberry leaf fermentation for 1 to 7 days are shown in Fig. 2. The DNJ was detected in retention time of 1.9 min. Overall, the DNJ content until 2 to 7 days showed high 1.11 to 1.19-fold, than 1 day of

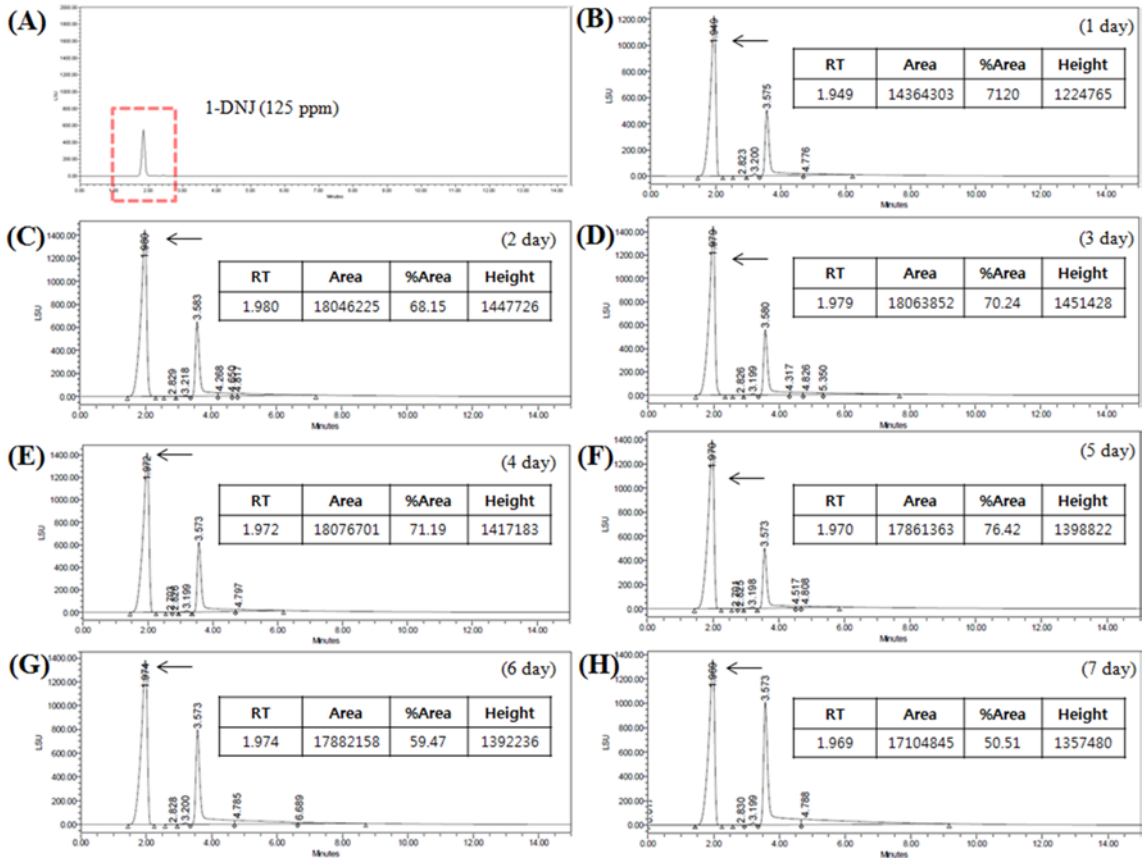


**Fig. 2.** HPLC chromatograms of DNJ in mulberry leaf fermentation. (A) DNJ reference standard; (B-H) HPLC chromatograms of DNJ produced in mulberry leaf fermentation for 1 to 7 days. Black arrows indicate DNJ peak.

fermentation. The DNJ content showed high in 2 day and 3 day of fermentation. The DNJ content in 2 day and 3 day were increased by 1.18 and 1.19-fold, respectively, then 1 day of fermentation. The DNJ content of mulberry leaf fermentation was increased after 1 day, and after 4 day was decreased. Jeong *et al*. (2014) reported that mulberry leaf powder extract fermented by microorganisms (Lactobacillus plantarum, Zygosaccharomyces rouxii, Wickerhamomyces anomalus, and B. subtilis) were increased DNJ contents (1.2 to 2-fold) and α-glucosidase inhibitory activity than unfermented mulberry leaf powder extract. In addition, Yatsunami *et al*. (2008) suggested that a strong correlation existed between DNJ content and α-glucosidase inhibitory activity in mulberry leaves. Vichasilp *et al*. (2012) suggested that α-glucosidase inhibitory activity of mulberry leaves were highly correlated with DNJ concentration, and α-glucosidase inhibitory activity is mainly due to DNJ. Increase or decrease of the DNJ content according to mulberry fermentation times in this study showed similar trend to the results of α-glucosidase inhibitory activity. Thus, these results showed that correlation existed between the DNJ content and α-glucosidase inhibitory activity in mulberry leaf fermentation.

## Changes in pH value of mulberry leaf fermentation by using B. subtilis KJ 21

The pH changes in fermentation scale-up of mulberry leaf for 1 to 7 days are shown in Fig. 3. The initial pH value of medium before fermentation was 6.8, and decreased rapidly to pH 5.8 after 2 days of mulberry leaf fermentation. In 2 to 4 days of fermentation were little changes of the pH values (pH 5.8, 5.82, and 5.83, respectively). However, the pH value was started to increase after 4 days and showed pH 6.79 in 7 days of fermentation. Sini *et al*. (2007) and Seo *et al*. (2017) reported the pH changes of fermentation by B. subtilis. Sini *et al*. (2007) reported that the pH value of shrimp shell fermentation by using B. subtilis showed a decreasing trend up to 8 day and thereafter started increasing. Seo *et al*. (2017) also reported that the pH value of mulberry leaf fermentation by B. subtilis

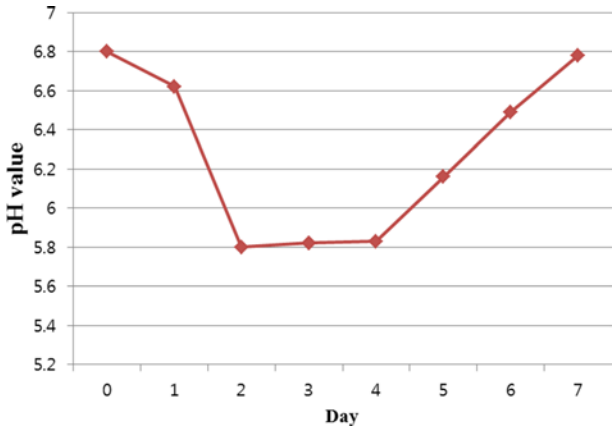


**Fig. 3.** Changes in the pH of mulberry leaf fermentation.

was decreased rapidly after 12 hours and was little changes until 48 hours, but started to increase after 72 hours. This is similar trend to the results of our pH changes of mulberry leaf fermentation by B. subtilis. An initial decrease in pH value might has caused due to the ability of the strain B.subtilis to initially use sugar as substrates for their growth (Sarkar *et al*., 1993) and simultaneously they produce acid via pyruvate (Leroy and De Vuyst, 2004). In addition, during fermentation, when the sugar substrate was depleted, proteolysis occurs and ammonia release due to utilization of amino acids for the bacteria growth might be caused the increase in pH (Sarkar *et al*., 1993; Kiers *et al*., 2000).

α-Glucosidase inhibitor combined with intestine α-glucosidase and blocking the absorption of postprandial blood glucose (Holman, 1998), and is usually used to prevent or medical care of type II diabetes (Floris *et al*., 2005). Mulberry leaves contain DNJ have been traditionally used as medicine for decreasing blood sugar, and DNJ was known to be a strong intestinal α-glucosidase inhibitor (Kimura *et al*., 1995; Asano *et al*., 2001; Jeong *et al*., 2014). This compound has been associated with the potential human health benefits due to biological actives such as anti-hyperglycemic and anti-obesity. Thus, many researchers have studied for the DNJ high-production in mulberry leaves.

In the present study, we confirm that α-glucosidase inhibitory activity in mulberry leaf fermentation by using B. subtilis KJ 21 is not much difference between small and large-scale. In addition, the α-glucosidase inhibitory activity and DNJ content were increased after 1 day, and after 4 day was decreased. When the pH value was rapidly decreased after 2 days of mulberry leaf fermentation, the α-glucosidase inhibitory activity and DNJ content were increased, while the α-glucosidase inhibitory activity and DNJ content were decreased with increasing pH after 4 days. During mulberry leaf fermentation, the pH changes in mulberry leaf fermentation might be affected the α-glucosidase inhibitory activity and DNJ content. Thus, these results will be able to provide additional useful data that is anticipated at the level of industrial scale. Furthermore, for mulberry leaf fermentation by using B. subtilis KJ 21 to be used as a level of industrial scale, further studies of the various operational parameters such as optimized oxygen supply and agitation are needed.

## Acknowledgments

This study was supported by the Department of Agricultural Biology, National Academy of Agricultural Science of the Rural Development Administration (project: PJ 01081704).

## References

Adolkar VV, Raina SK, Kimbu DM (2007) Evaluation of various mulberry Morus spp. (Moraceae) cultivars for the rearing of the bivoltine hybrid race Shaanshi BV-333 of the silkworm Bombyx mori (Lepidoptera: Bombycidae). Int J Trop Insect Sci 27(1), 6-14.

Anno T, Tamura K, Oono H, Tomi H (2004) Maltase, sucrase and α-amylase inhibitory activity of Morus leaves extract. Food Preserv Sci 30, 223-229.

Asano N, Tomioka E, Kizu H, Matsui K (1994) Sugars with nitrogen in the ring isolated from the leaves of Morus bombycis. Carbohydr Res 253, 235-245.

Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS, Ryu KS (2001) Polyhydroxylated alkaloids isolated from mulberry trees (Morus alba L.) and silkworms (Bombyx mori L.). J Agric Food Chem 49, 4208-4213.

Bajpai S, Rao AVB (2014) Quantitative determination of 1-deoxynojirimycin in different mulberry varieties of India. J Pharmacogn Phytochem 3(3), 17-22.

Bharani SE, Asad, M, Dhamanigi SS, Chandrakala GK (2010) Immunomodulatory activity of methanolic extract of Morus alba Linn. (mulberry) leaves. Pak J Pharm Sci 23(1), 63-68.

Chai OH, Lee MS, Han EH, Kim HT, Song CH (2005) Inhibitory effects of Morus alba on compound 48/80-induced anaphylactic reactions and anti-chicken gamma globulin IgE- mediated mast cell

activation. Biol Pharm Bull 28(10), 1852-1858.

Choi EM, Hwang JK (2005) Effects of Morus alba leaf extract on the production of nitric oxide, prostaglandin E2 and cytokines in RAW264.7 macrophages. Fitoterapia 76, 608-613.

Clement WL, Weiblen GD (2009) Morphological evolution in the mulberry family (Moraceae). Syst Bot 34, 530-552.

Dimo T, Rakotonirina S, Kamgang R, Tan PV, Kamanyi A, Bopelet M (1998) Effects of leaf aqueous extract of Bidens pilosa (Asteraceae) on KCl- and norepinephrine-induced contractions of rat aorta. J Ethnopharmacol 60(2), 179-182.

Doi K, Kojima T, Makino M, Kimura Y, Fujimoto Y (2001) Studies on the constituents of the leaves of Morus alba L. Chem Pharm Bull 49, 151-153.

Floris AV, Peter LL, Reinier PA, Eloy HV, Guy ER, Chris VW (2005) α-glucosidase inhibitors for patients with type 2 diabetes. Diabetes Care 28, 154-162.

Fukai T, Hano Y, Hirakura K, Nomura T, Uzawa J, Fukushima K (1985) Structures of two natural hypotensive diels-alder type adducts, mulberrofuran F and G, from the cultivated mulberry tree (Morus lhou Koidz). Chem Pharm Bull (Tokyo) 33, 3195-3204.

Gao K, Zheng C, Wang T, Zhao H, Wang J, Wang Z, Zhai X, Jia Z, Chen J, Zhou Y, Wang W (2016) 1-Deoxynojirimycin: Occurrence, extraction, chemistry, oral pharmacokinetics, biological activities and in silico target fishing. Molecules 21(11), 1-15.

He N, Zhang C, Qi X, Zhao S, Tao Y, Yang G, Lee TH, Wang X, Cai Q, Li D, Lu M, Liao S, Luo G, He R, Tan X, Xu Y, Li T, Zhao A, Jia L, Fu Q, Gao C, Ma B, Liang J, Wang X, Shang J, Song P, Wu H, Fan L, Wang Q, Shuai Q, Zhu J, Wei C, Zhu-Salzman K, Jin D, Wang J, Liu T, Yu M, Tang C, Wang Z, Dai F, Chen J, Liu Y, Zhao S, Lin T, Zhang S, Wang J, Wang J, Yang H, Yang G, Wang J, Paterson AH, Xia Q, Ji D, Xiang Z (2013) Draft genome sequence of the mulberry tree Morus notabilis. Nat Commun 4, 1-9.

Holman RR (1998) Assessing the potential for α-glucosidase inhibitors in prediabetic states. Diabetes Res Clin Pract 40, s21-s25.

Jeong JH, Lee NK, Cho SH, Jeong YS (2014) Enhancement of 1-deoxynojirimycin content and α-glucosidase inhibitory activity in mulberry leaf using various fermenting microorganisms isolated from Korean traditional fermented food. Biotechnol Bioprocess Eng 19, 1114-1118.

Jiang YG, Wang CY, Jin C, Jia, JQ, Guo X, Zhang GZ, Gui ZZ (2014) Improved 1-Deoxynojirimycin (DNJ) production in mulberry leaves fermented by microorganism. Braz J Microbiol 45, 721-729.

Ju WT, Kim HB, Sung GB, Kim YS (2015). Screening of 1-deoxynojirimycin (DNJ) producing bacteria using mulberry leaf. J Indust Entomol 31(2), 48-55.

Ju WT, Kim HB, Sung GB, Kim YS (2016) Comparison of optimal temperature and time conditions for highest a-glucosidase inhibitory activity from various of Korea mulberry teas. J Indust Entomol 33(1), 31-35.

Kiers JL, Van laeken AEA, Rombouts FM, Nout MJR (2000) In vitro digestibility of Bacillus fermented soya bean. Int J Food Microbiol 60, 163-169.

Kim SY, Lee WC, Kim HB, Kim AJ, Kim SK (1998) Antihyperlipidemic effects of methanol extracts from mulberry leaves in cholesterol-induced hyperlipidemia rats. J Korean Soc Food Sci Nutr 27, 1217-1222.

Kimura M, Chen F, Nakashima N, Kimura I, Asano N, Koya S (1995) Antihyperglycemic effects of N-containing sugars derived from mulberry leaves in streptozocin-induced diabetic mice. J Trad Med 12, 214-219.

Kimura T (2011) Development of Mulberry Leaf Extract for Suppressing Postparandial Blood Glucose Elevation [Internet] Available from: http://cdn.intechopen.com/pdfs/21464/InTech-Development of mulberry leaf extract for suppressing postprandial blood glucose elevation.pdf [published on 10 October 2011].

Kimura T, Nakagawa K, Saito Y, Yamagishi K, Suzuki M, Yamaki K, Shinmoto H, Miyazawa T (2004) Determination of 1□ deoxynojirimycin in mulberry leaves using hydrophilic interaction chromatography with evaporative light scattering detection. J Agric Food Chem 52, 1415-1418.

Kwon OC, Ju WT, Kim HB, Sung GB, Kim YS (2017) Effect of pH values and inoculation amounts for α-glucosidase inhibitory activity in mulberry leaf fermentation. J Indust Entomol 34(2), 38-44.

Lee SI (1981) Bonchohak, Mori Fructus. Suseowon, Seoul, Korea. 136-137.

Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15, 67-78.

Miyahara C, Miyazawa M, Satoh S, Sakai A, Mizusaki S (2004) Inhibitory effect mulberry leaf extract on postprandial hyperglycemic in normal rats. J Nutritional Sci Vitaminol 50, 161-164.

Oh H, Ko EK, Jun JY, Oh MH, Park SU, Kang KH, Lee HS, Kim YC (2002) Hepatoprotective and free radical scavenging activities of prenylflavonoids, coumarin, and stilbene from Morus alba. Planta Med 68(10), 932-934.

Oku T, Yamada M, Nakamura M, Sadamori N, Nakamura S (2006) Inhibitory effects of extractives from leaves of Morus alba on human and rat small intestinal disaccharidase activity. Br J Nutr 95, 933-938.

Pawlowska AM, Oleszek W, Braca A (2008) Quali-quantitative analyses of flavonoids of Morus nigra L. and Morus alba L. (Moraceae) fruits. J Agric Food Chem 56, 3377-3380.

Priya S (2012) Medicinal values of mulberry - An overview. J Pharm Res 5 (7), 3588-3596.

Quin CG, Li Y, Niu WN, Ding Y, Zhang RJ, Shang XY (2010) Analysis and characterisation of anthocyanins in mulberry fruit. Czech J Food Sci 28, 117-126.

Sarkar PK, Cook PE, Owens JD (1993) Bacillus fermentation of soybeans. World J Microbiol Biotechnol 9, 295-299.

Sastry CR (1984) Mulberry varieties, exploitation and pathology. Sericologia 24, 333-359.

Seo SH, Park SE, Kim EJ, Oh D, Son HS (2017) Characterization of Fermented Mulberry Leaf Using Bacillus subtilis. Korean Soc Food Sci Nutr 46(1), 108-114.

Shibano M, Fujimoto Y, Kushino K, Kusano G, Baba K (2004) Biosynthesis of 1-deoxynojirimycin in Commelina communis: A difference between the microorganisms and plants. Phytochem 65, 2661-2665.

Singab AN, El-Beshbishy HA, Yonekawa M, Nomura T, Fukai T (2005) Hypoglycemic effect of Egyptian Morus alba root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. J Ethnopharmacol 100(3), 333-338.

Sini TK, Santhosh S, Mathew PT (2007) Study on the production of chitin and chitosan from shrimp shell by using Bacillus subtilis fermentation. Carbohydr Res 342, 2423-2429.

Vichasilp C, Nakagawa K, Sookwong P, Higuchi O, Luemunkong S, Miyazawa T (2012) Development of high 1-deoxynojirimycin (DNJ) content mulberry tea and use of response surface methodology to optimize tea-making conditions for highest DNJ extraction. LWT-Food Sci Technol 45, 226-232.

Wang J, Wu FA, Zhao H, Liu L, Wu QS, (2008) Isolation of flavonoids from mulberry (Morus alba L.) leaves with macroporous resins. Afr J Biotechnol 7, 2147-2155.

Yagi M, Kouno T, Aoyagi Y, Murai H (1976) The structure of moranoline, a piperidine alkaloid from Morus species. Nippon Nogeikagaku Kaishi 50(11), 571-572.

Yamaki K, Mori Y (2006) Evaluation of alpha-glucosidase inhibitory activity in colored foods: A trial using slope factors of regression curves. Nippon Shokuhin Kagaku Kaishi 53, 229-231.

Yang CH, Tsai TC (1994) Anthocyanins in mulberry fruit. Food Science 21, 319-330.

Yatsunami K, Ichida M, Onodera S (2008) The relationship between 1 and deoxynojirimycin content and α-glucosidase inhibitory activity in leaves of 276 mulberry cultivars (Morus spp.) in Kyoto, Japan. J Nat Med 62, 63-66.

Zhang D, Wan Y, Xu J (2016) Ultrasound extraction of polysaccharides from mulberry leaves and their effect on enhancing antioxidant activity. Carbohydr Polym 137, 473-479.