Detection of 1,4-Dihydroxy-2-Naphthoic Acid from Commercial Makgeolli Products

Research Note

Ji-Eun Eom1, Sang-Chul Kwon2, and Gi-Seong Moon1*

1Department of Biotechnology, Chungju National University, Chungbuk 368-701, Korea
2Food Safety Support Organization, Korea Food Industry Association, Seoul 137-867, Korea

Abstract

To support beneficial effects of makgeolli for human health, we investigated for the presence of 1,4-dihydroxy-2-naphthoic acid (DHNA), a bifidogenic growth stimulator (BGS), from commercial makgeolli products. Among eleven makgeolli products (A~K), four showed positive peaks for DHNA in high performance liquid chromatography analysis. Makgeolli product A in particular contained the highest concentration of DHNA (0.44 ppm), as confirmed by liquid chromatography-mass spectrometry. Furthermore, BGS activity of the makgeolli product A was higher than those of products in which DHNA was not detected. These results indicate that makgeolli can be a good source for DHNA and that DHNA-enriched makgeolli could be developed by modifying manufacturing procedures and controlling its microbiota.

Key words: makgeolli, 1,4-dihydroxy-2-naphthoic acid, bifidogenic growth stimulator

INTRODUCTION

Makgeolli is a Korean traditional rice wine with a low content of alcohol (6~8%), which is relatively less toxic to the liver and stomach. Unlike other alcoholic beverages, makgeolli contains abundant nutrients such as sugars, proteins, vitamins, and amino acids including valine, leucine, serine, proline, and glycine (1). Yeasts are mainly involved in the fermentation that occurs in makgeolli production, as well as many species of lactic acid bacteria (LAB) (2). Makgeolli is recognized as functional alcoholic beverage and its consumption has markedly increased in Korea. Also, consumption in other countries such as Japan has increased, resulting in increased export of makgeolli (3); however, the shelf life of non-sterilized makgeolli containing yeast and LAB can be as short as 10 days, even under refrigeration conditions. Studies have sought to increase the shelf life of non-sterilized makgeolli (4,5). One study reported an extended shelf-life of up to 30 days using heat-inactivation of saccharolytic enzymes and re-inoculation of yeast (6). Studies aimed at developing functional makgeolli have assessed the effect of adding biologically active materials such as pears, Gugija-Liriope tuber, and black garlic extract, among other substances (7-9).

1,4-Dihydroxy-2-naphthoic acid (DHNA) was recently identified as a bifidogenic growth stimulator (BGS) (10). DHNA also displays inhibitory activity against clinical isolates of Helicobacter pylori that are resistant to clarithromycin (11) and suppresses bone resorption (12). DHNA is an intermediate in the biosynthetic pathway of vitamin K2 (menaquinone), which plays a role in respiratory chain of bacteria as an electron carrier (13). Therefore, most LAB produce DHNA as an intermediate compound, with a small amount exported from the bacteria. It would be helpful to isolate DHNA-producing LAB for commercial use as a starter strain in the manufacturing of functional makgeolli products.

Before this goal can be achieved, however, it is necessary to ascertain whether current commercial makgeolli products contain DHNA. Therefore, we undertook this effort in this study, using high performance liquid chromatography (HPLC) to determine if DHNA was present in each sample of makgeolli. Additionally, we compared the BGS activity of DHNA-containing makgeolli products with those of makgeolli products where DHNA was not detected.

MATERIALS AND METHODS

Makgeolli products

To analyze DHNA from commercial makgeolli products, 11 makgeolli products (A~K) from several companies were purchased from a local market (Table 1).

Bacterial strains and culture conditions

For BGS activity test, Bifidobacterium longum FI10564 and Bifidobacterium lactis BL 750 (Culture Systems, Mishawaka, IN, USA) were cultivated in reinforced clos-
Table 1. Makgeolli products analyzed in this study

| Products | Starch source                     | Additives (sweetener) | Sterilization |
|----------|-----------------------------------|-----------------------|---------------|
| A        | Rice (100%)                       | Aspartame             | No            |
| B        | Rice (100%)                       | Aspartame, Acesulfame K | Yes          |
| C        | Rice (100%)                       | Aspartame             | No            |
| D        | Rice (80%), flour (20%)           | Aspartame             | Yes          |
| E        | Rice (50%), flour (30%), corn starch (20%) | Aspartame, Acesulfame K | Yes |
| F        | Rice (100%)                       | Aspartame             | Yes          |
| G        | Rice (40%), flour (40%), starch sugar (20%) | Aspartame, Acesulfame K | Yes |
| H        | Rice (100%)                       | Aspartame             | No            |
| I        | Rice (100%)                       | Aspartame             | No            |
| J        | Rice (80%), flour (10%), starch sugar (10%) | Aspartame, Acesulfame K | No |
| K        | Rice (100%)                       | Aspartame, Acesulfame K | Yes          |

tridial medium (RCM ) broth (Difco, Detroit, MI, USA) at 37°C in an anaerobic jar (Oxoid, Cambridge, UK).

**HPLC analysis for DHNA**

For HPLC detection of DHNA from makgeolli samples, 10 mL of each makgeolli sample was freeze-dried. The freeze-dried samples were each diluted with 150 μL of water and mixed with 300 μL of methanol. The mixtures were centrifuged at 5,000 × g for 10 min and the supernatants were filtered with 0.45 μm pore size syringe filters (Millipore, Billerica, MA, USA). The filtrates were used as injection samples for HPLC using an ACE 5 C18 column (4.6 × 150 mm; Advanced Chromatography Technologies, Aberdeen, Scotland). Column temperature was maintained at 45°C during analysis. The flow rate was 1 mL/min, injection volume was 20 μL, and detection wavelength was 254 nm. For construction of standard curve, reagent grade DHNA (Sigma-Aldrich, St. Louis, MO, USA) was used. The mobile phase was composed of acetonitrile : methanol : water : acetic acid (15:25:225:0.1) and the pH was adjusted to 5.5 with 5% (w/v) ammonium hydroxide.

**Liquid chromatography-mass spectrometry (LC-MS) analysis**

For confirmation of DHNA from one of the makgeolli products (A), DHNA fractions evident on HPLC were collected and subjected to LC-MS. LC utilized a Prominance 20A apparatus (Shimadzu, Kyoto, Japan). MS utilized a LCMS-IT-TOF system (Shimadzu). An XR-ODS LC column (3 × 75 mm, Shimadzu) was used. The column temperature was equilibrated at 45°C. Sample injection volume was 20 μL and flow rate was 0.2 mL/min. Mobile phase composition was the same as the aforementioned HPLC condition.

**BGS activity**

For the BGS activity test of makgeolli product samples, B. longum FI10564 and B. lactis BL 750 were used as the target bifidobacteria. Briefly, makgeolli product samples were centrifuged at 6,000 × g for 10 min. The resulting supernatants were syringe-filtered as described above and the filtrates were used as the test samples. One hundred microliters of each filtrate was added to RCM broth (Difco) that had been previously inoculated with a bifidobacterial strain (2% of final concentration). These inocula were incubated at 37°C for 12 hr in an anaerobic jar supplemented with a GasPak EZ Anaerobe Container System (BD, Sparks, MD, USA) and the optical density at 600 nm (OD600) was measured with time.

**RESULTS AND DISCUSSION**

To investigate whether commercial makgeolli products contain DHNA, eleven commercial makgeolli products were purchased from a local market and analyzed by HPLC. Four of the product samples (A, E, F, and J) showed positive peaks for DHNA. Two products, A and F, could be quantified using a standard curve which ranged 0.0–1.0 μg/mL (ppm) (data not shown). Product A, which was produced from a local brewing company located in Chungbuk province, displayed the most content of DHNA (0.44 ppm) as shown in Fig. 1B. The makgeolli product F contained 0.089 ppm of DHNA. Although the study involved a relatively small sampling of makgeolli products, the fact that four of the eleven products contained detectable levels of DHNA is promising, and indicates the potential of commercial makgeolli products as reliable sources of DHNA.

To confirm the presence of DHNA in the HPLC peak from makgeolli product A, DHNA fractions were collected and subjected to LC-MS. The mass spectra of DHNA from a standard solution and the makgeolli product sample revealed an m/z 203.03 (Fig. 1C and D), which corresponded to a de-protonated DHNA ion [M-H]. The result confirmed the presence of DHNA in makgeolli product A. Furthermore, we investigated whether the DHNA-containing makgeolli product A displayed BGS activity, which is a biological function of DHNA. For the test, the bifidobacterial strains B. longum FI10564 and B. lactis BL 750 were used. B. longum FI10564 was
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Previously isolated from a stool sample of a healthy human volunteer (14) and *B. lactis* BL 750 is commercially available and used in the dairy industry as a probiotic. For *B. longum* FI10564, BGS activity of makgeolli products B and H, in which DHNA was not detected, was not evident. The OD of 0.76 and 0.75 were not significantly different with that of a control where *B. longum* FI10564 strain was inoculated alone (0.70). However, makgeolli product A, which had been confirmed to contain DHNA, did present BGS activity, evident as an OD of 0.94 (Fig. 2A). For *B. lactis* BL 750, the BGS activity of makgeolli product A was higher than makgeolli products B and H. After 12 hr culture, the OD of the control was 0.76 and those of test samples A, B, and H were 1.35, 0.94, and 0.79, respectively (Fig. 2B). These results indicate that DHNA-enriched makgeolli products can improve BGS activity, although other factors can also influence the activity. In case of makgeolli product B, other factors including fibers might have affected the activity; if so, their effect was not significant.

Initially, we had expected that most of the makgeolli products would contain DHNA as a functional ingredient. In reality, four of the eleven products contained detectable levels of the functional material. Nevertheless, mak-
makgeolli products may be good sources for DHNA, pending modification of the manufacturing procedure, including use of starter strains that produce DHNA. To achieve this goal, it will be necessary to monitor the microbiota and DHNA content of makgeolli during fermentation.

Most of the previous research and patents in this field have related to manufacturing procedures, quality characterization, and biological functions. More recently, makgeolli has been recognized as a functional food (1, 15, 16). In particular, makgeolli contains farnesol, an anti-cancer or anti-tumor agent (17), which has encouraged consumption of makgeolli products in Korea and exportation to other countries, including Japan (18); therefore, it is very important to enlarge the capacity of functional properties of makgeolli for commercial aspects. For that reason, DHNA could also be a functional material candidate linked to makgeolli products.

The present data indicate the potential of makgeolli as a good source for DHNA. DHNA-enriched makgeolli products may be developed by modifying the manufacturing procedures. Planned studies will focus on the development for DHNA-enriched makgeolli product.

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REFERENCES

1. Kwon RH, Chae GY, Ha BJ. 2011. The effects of the makgeolli on the antioxidative activity in the endotoxin LPS-treated rats. J Food Hyg Safety 26: 166-170.
2. Min JH, Baek SY, Lee JS, Kim HK. 2011. Changes of yeasts and bacterial flora during the storage of Korean traditional makgeolli. Kor J Mycol 39: 151-153.
3. Kim MJ. 2010. The effect of consumer ethnocentric tendency and foreign culture acceptance on preference of traditional alcoholic beverage: focus on makgeolli and sake. J Foodserv Manag Soc Kor 13: 7-31.
4. Lee JW, Park JW. 2010. Quality characteristics of makgeolli during separation storage methods. Food Eng Prog 14: 346-353.
5. Lee JW, Shim JY. 2010. Quality characteristics of makgeolli during freezing storage. Food Eng Prog 14: 328-334.
6. Bae JH, Kim GW, Shon SK, Park SY, Kim GW. 2010. Manufacturing method of Takju. Kor patent 10-0936994.
7. Lee DH, Kim JH, Lee JS. 2009. Effect of pears on the quality and physiological functionality of makgeolli. Kor J Food Nutr 22: 606-611.
8. Baek SY, Nam YG, Ju JI, Lee JS. 2011. Changes of quality characteristics during storage of Guggia-Liriope tuber makgeolli made by Saccharomyces cerevisiae C-2. Kor J Mycol 39: 122-125.
9. Jeong Y. 2011. Physicochemical and microbial properties of Korean traditional rice wine, makgeolli, supplemented with black garlic extracts during fermentation. J Food Sci Nutr 16: 142-149.
10. Isawa K, Hojo K, Yoda N, Kamiyama T, Makino S, Saito M, Sugano H, Mizoguchi C, Karama S, Shibasaki M, Endo N, Sato Y. 2002. Isolation and identification of a new bifidogenic growth stimulator produced by Propionibacterium Freudenreichii ET-3. Biosci Biotechnol Biochem 66: 679-681.
11. Nagata K, Inatsu S, Tanaka M, Sato H, Kouya T, Taniguchi M, Fukuda Y. 2010. The bifidogenic growth stimulator inhibits the growth and respiration of Helicobacter pylori. Helicobacter 15: 422-429.
12. Matsubara M, Yamachika E, Tsujigiwa H, Mizukawa N, Ueno T, Murakami J, Ishida N, Kaneda Y, Shirasu N, Takagi S. 2010.Suppressive effects of 1,4-dihydroxy-2-naphthoic acid administration on bone resorption. Osteopora Int 21: 1437-1447.
13. Furuchi K, Katakura Y, Ninomiya K, Shiyo S. 2007. Enhancement of 1,4-dihydroxy-2-naphthoic acid production by Propionibacterium freudenreichii ET-3 fed-batch culture. Appl Environ Microbiol 73: 3137-3143.
14. Moon GS, Wegmann U, Gunning AP, Gasson MJ, Narbad A. 2009. Isolation and characterization of a theta-type cryptic plasmid from Bifidobacterium longum FI10564. J Microbiol Biotechnol 19: 403-408.
15. Shin MO, Kang DY, Kim MH, Bae SJ. 2008. Effect of growth inhibition and quinone reductase activity stimulation of makgeolli fractions in various cancer cells. J Korean Soc Food Sci Nutr 37: 288-293.
16. Shin MO, Kim MH, Bae SJ. 2010. The effect of makgeolli on blood flow, serum lipid improvement and inhibition of ACE in vitro. J Life Sci 20: 710-716.
17. Joo JH, Jetten AM. 2010. Molecular mechanisms involved in farnesol-induced apoptosis. Cancer Lett 287: 123-135.
18. http://www.kfri.re.kr. Korea Food Research Institute.

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