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Short Communication

Nuruk, a Traditional Korean Fermentation Starter, Contains the Bioactive Compound 2,6-dimethoxy-1,4-benzoquinone (2,6-DMBQ)

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***Nuruk*, a traditional Korean fermentation starter, contains 2,6-dimethoxy-ρ-benzoquinoe (2,6-DMBQ), also found in fermented wheat germ extract, and has anti-cancer and immune supporting effects. The presence of 2,6-DMBQ was confirmed by high performance liquid chromatography and mass spectrometry. Among the five traditional *nuruks* tested, the highest 2,6-DMBQ content of 1.16±0.07 mg/50 g was obtained from *nuruk* purchased in Hwaseong. The results of this study may explain the health-promoting functions of traditional Korean alcoholic beverages that employ *nuruk* as a fermentation starter.**

Key words: 2,6-dimethoxy-ρ-benzoquinoe, fermentation, high performance liquid chromatography, *nuruk*

Quinone is a class of bioactive compound that may be components of anti-cancer chemotherapy drugs [Dandawate *et al*., 2010]. 2,6-Dimethoxy-1,4-benzoquinone (2,6-DMBQ) exerts significant *in vitro* cytotoxicity against human tumor cell lines and also exhibits antibacterial activities (Fig. 1) [Tömösközi-Farkas and Daood, 2004; Lana *et al*, 2006; Kim *et al*., 2010]. 2,6-DMBQ has been identified in a variety of plant families, including wheat (*Triticum vulgaris*) and other food crops [Chen *et al*., 1994; Harasawa and Tagashira, 1994; Kwon *et al*., 2001]. 2,6-DMBQ and 2-methoxy-benzoquinone (2-MBQ) are especially abundant in wheat germ as the glycone type [Tömösközi-Farkas and Daood, 2004]. Lana *et al*. [2006] suggested that *Eucalyptus* tar could be a prominent substrate for 2,6-DMBQ production, because wood tar is inexpensive and contains abundant 2,6-dimethoxyphenol, which can be oxidized into 2,6-DMBQ.

Avemar® is the trade-name of a standardized, natural nutrient compound that has been extensively studied for the treatment of cancer and autoimmune diseases [Boros *et al*., 2005; Illmer *et al*., 2005; Telekes *et al*., 2009].

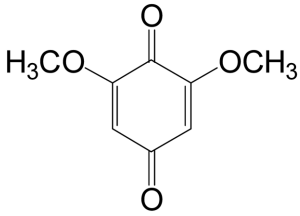

Fig. 1. Chemical structure of 2,6-DMBQ.

Avemar® is made by fermenting wheat germ with baker's yeast (*Saccharomyces cerevisiae*), which harbors β-glucosidase activity, and of which aglycone-type 2,6-DMBQ and 2-MBQ are important components [Tömösközi-Farkas and Daood, 2004; Telekes *et al*., 2009].

Nuruk, a traditional Korean fermentation starter, is produced by the natural proliferation of fungi and other microorganisms on crushed or whole wheat kernels [Yu *et al*., 1998]. Recent studies have demonstrated the biological activities of *nuruk* [Kim *et al*., 2008; Kwak *et al*., 2008; Lee *et al*., 2009]. Extracts of *nuruk* were associated with inhibitory effects on hypertension, platelet aggregation, migration, and angiogenesis of cancer cells [Lee *et al*., 2009]. *Nuruk* extract is also associated with decreases in lipopolysaccharide (LPS)-induced nitrite and interleukin (IL)-6 in RAW 264.7 cells by inhibiting the activation of p38 mitogen-activated

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protein kinase [Kim *et al*., 2008]. Four sterol compounds, ergosterol peroxide, stigmast-5-en-3β,7β-diol, ergosta-7,22-dien-3β,5α,6β,9α-tetraol, and daucosterol, have been isolated from *nuruk* [Lee *et al*., 2009]. Accordingly, renewed evaluation of *nuruk* is warranted to explore its apparent health-enhancing biological activities. In the present study, the presence of 2,6-DMBQ in *nuruk* and the 2,6-DMBQ contents of five different *nuruks* are reported.

Materials and Methods

***Nuruk* and chemicals.** *Nuruk* was purchased from five local markets (located in Andong, Busan, Hwaseong, Jinju, and Sangju) in Korea. Commercially available 2,6-DMBQ (TCI Chemicals, Tokyo, Japan) was used as the standard. Formic acid and methanol were purchased from Wako Pure Chemicals (Osaka, Japan).

Extraction of 2,6-DMBQ. *Nuruk* (20 g) was added to a 2-L Erlenmeyer flask containing 300 mL sterile water. The reaction mixture was incubated at 30°C for 24 h with vigorous shaking and centrifuged at 6,000 g for 10 min. After measurement of β-glucosidase enzyme activity as described by Hong *et al*. [2009], the supernatant was sterilized at 121°C for 15 min, filtered through a 0.45-μm filter (Whatman, Maidstone, England), and dried using a freeze-dryer (Ilshin Engineering Co., Seoul, Korea). The lyophilizate was harvested and stored at −80°C until further analysis.

High-performance liquid chromatography (HPLC) analysis. The operation conditions for HPLC analysis described previously were implemented with slight modification [Tömösközi-Farkas and Daood, 2004]. One gram of the lyophilizate was dissolved in 50 mL sterile water and extracted three times with 100 mL chloroform. The chloroform layers were pooled and evaporated to dryness using a vacuum evaporator (EYELA, Tokyo, Japan). The resulting dry pellet was dissolved in 20 mL mobile phase [methanol (24%, v/v) in water containing 1 mM formic acid, pH 4.2]. Analysis of 2,6-DMBQ was performed on a Shimadzu Prominence LC-20A HPLC system equipped with an UV/VIS SPD-20A detector (Shimadzu Co., Kyoto, Japan) and an inertsil ODS-3 C18 column (GL Sciences, Tokyo, Japan). The wavelength of the UV detector was set at 290 nm, and the flow rate of the mobile phase was 0.7 mL/min. Column temperature was maintained at 40°C.

LC-MS and LC-MS/MS analyses. Liquid chromatography-mass spectrophotometry (LC-MS) and liquid chromatography-tandem mass spectrophotometry (LC-MS/MS) experiments were performed using the Quantum Ultra Triple-Stage Quadrupole system (Thermo Fisher Scientific, Pittsburgh, PA). A C18 Hydrosphere column (GL Sciences, Tokyo, Japan) was used for separation. The mobile phase flowed at a rate of 200 μL/min. The ionization was achieved using an electrospray ionization source in the positive selected reaction monitoring (SRM) mode. The spray voltage, capillary temperature, sheath gas pressure, auxiliary gas pressure, and capillary temperature were 4.2 kV, 320°C, 50 psi, and 20 psi, and 320°C, respectively.

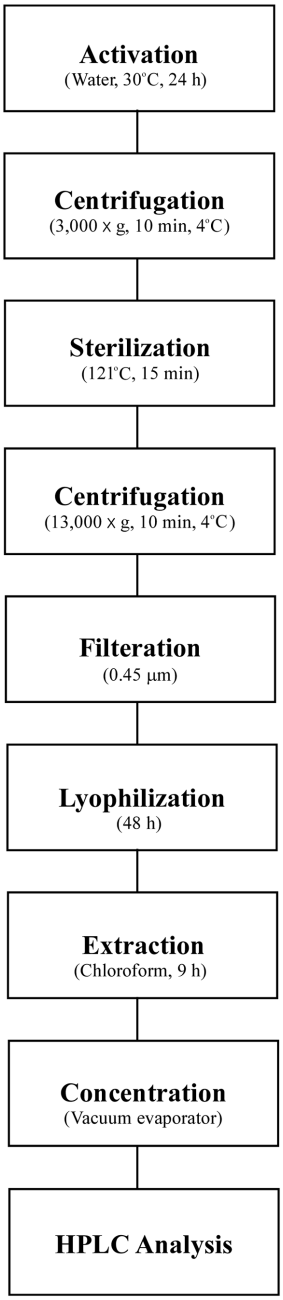

Fig. 2. Schematic representation of 2,6-DMBQ analysis.

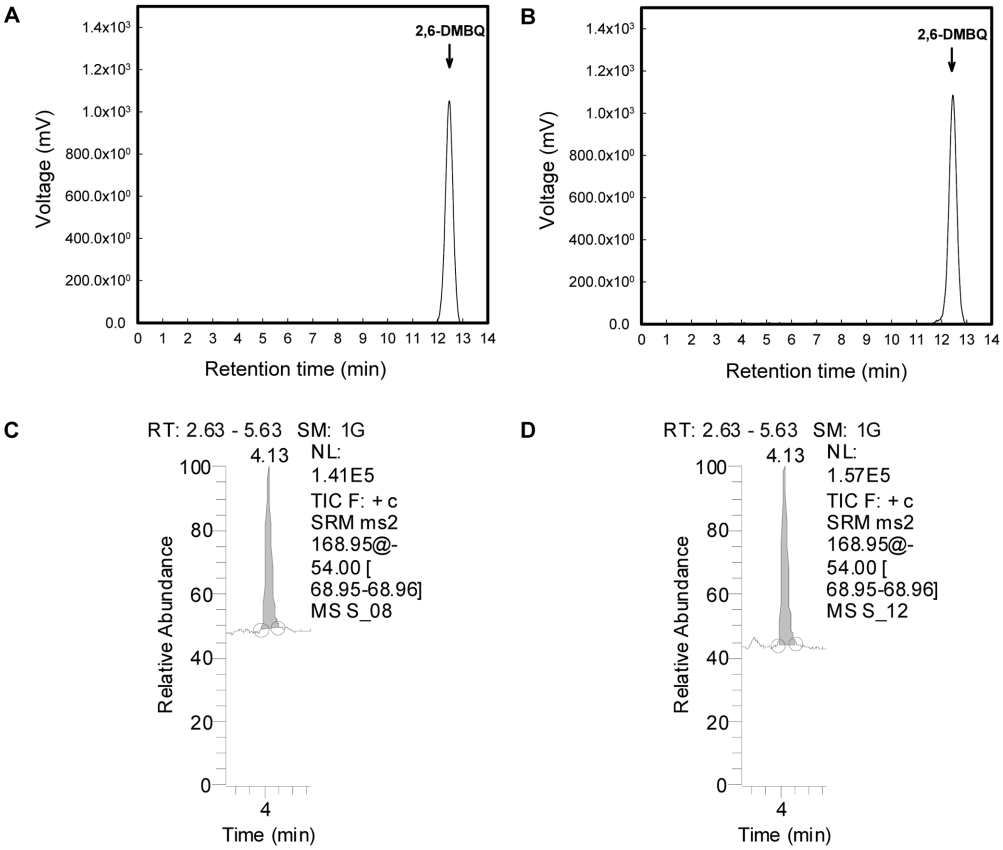

Fig. 3. Chromatograms of HPLC (upper panel) and LC-MS/MS (lower panel) for 2,6-DMBQ standard (A,C) and *nuruk* extract from Hwaseong (B,D). Commercially available 2,6-DMBQ (0.3 μg/mL) was used as standard.

Results and Discussion

The HPLC chromatograms of the samples prepared from *nuruk* were similar to that of the commercially available 2,6-DMBQ (standard) (Fig. 3). In addition, the *m/z* (mass-to-charge ratio) values of both the standard 2,6-DMBQ and *nuruk* extract were 168.91, and MS^2 fragmentation induced by a collision energy of 54 eV were the same for the samples and standard. The results of HPLC and LC-MS/MS analyses showed that *nuruk* contains 2,6-DMBQ. Among the five samples, the highest 2,6-DMBQ content was found in *nuruk* purchased in Hwaseong, which contains 2,6-DMBQ at 1.16±0.07 mg/50 g. *Nuruk* purchased in Jinju showed the lowest content (0.38±0.01 mg/50 g). However, 2-MBQ, which is found in fermented wheat germ, was not detected in *nuruk*. Contents of 2,6-DMBQ in *nuruk* from the five different local markets are shown in Table 1.

In the present study, 2,6-DMBQ was hypothesized to be produced by the actions of microbial β-glucosidases in *nuruk*. *Nuruk* contained much lower 2,6-DMBQ content than that of the commercial product Avemar®, which may be explained by the fact that *nuruk* is usually not made of wheat germ, but with wheat bran or wheat kernel [Lee *et al*., 1994] in which the intrinsic content of the glycone type of 2,6-DMBQ is low compared to that of wheat germ [Tömösközi-Farkas and Daood, 2004].

The specific microorganisms present in different *nuruk* and their enzymes may play important roles in converting the glycone form of 2,6-DMBQ to its aglycone form. *Nuruk* is naturally inoculated by airborne microorganisms, and thus contains a variety of fungi, yeast, and bacteria [Yu *et al*., 1998]. Accordingly, *nuruk* from different local areas may vary in microbial flora that produce β-glucosidase. *Nuruk* purchased in Hwaseong exhibited the highest β-glucosidase enzyme activity among the five *nuruk* samples (Table 1), which suggests that the enzyme activity of b-glucosidase is critical to the production of the aglycone form of 2,6-DMBQ in *nuruk*. Because the pivotal microorganisms involved in production of 2,6-DMBQ in *nuruk* are unknown, further study on enumeration and characterization of the microorganisms harboring β-glucosidase activity could be necessary.

In conclusion, to the best of our knowledge, this is the first study to demonstrate the presence of the bioactive compound 2,6-DMBQ in *nuruk*, which may help explain the health-promoting functions of traditional Korean fermented alcoholic beverages that employ *nuruk* as a

Table 1. Comparison of 2,6-DMBQ contents and β-glucosidase activities of *nuruk* samples

Nuruk	District of purchase	2,6-DMBQ (mg/50 g)*	β-Glucosidase activity (U/mg protein)
A	Busan	0.55±0.02	0.04±0.01
B	Sangju	0.49±0.12	0.04±0.01
C	Hwaseong	1.16±0.07	0.08±0.01
D	Andong	0.17±0.01	0.02±0.005
E	Jinju	0.38±0.01	0.04±0.01

*Each value is mean ± standard error of three independent experiments.

fermentation starter [Shin *et al*., 2008; Lee *et al*., 2010].

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