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Quantitative Analysis of Allantoin in Various Rice Varieties

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ABSTRACT Identification and quantification of allantoin in rice grain of selected varieties were investigated. Allantoin was isolated from Jeokjinjubyeo, and its structure has been elucidated on the basis of spectral data. Allantoin was extracted with a 70% acetone and analyzed by high performance liquid chromatographic methods without a previous chemical derivatization. The concentration of allantoin in selected rice varieties was in a narrow range from 2 to 18 mg per 100 g of brown rice. The highest content was detected in Heugkwang with 18.6 mg per 100 g brown rice, while the lowest was 2.59 mg in Yeomyung.

Keywords : *Oryza sativa* L., rice, allantoin, HPLC

Allantoin is first detected in *Platanus orientalis* in 1881 (Schubert & Boland, 1990). Since this pioneer discovery, detailed investigations have been made of the biosynthetic route of allantoin, its catabolism with the transfer of N to amino acids, as well as the control of each of these metabolic steps, such that nowadays the whole process is well understood, including at the gene level (Crawford *et al.*, 2000). Allantoin is frequently present in hygiene products, various cosmetic lotions and creams, and other cosmetic and pharmaceutical products. It is also known as an antiphlogistic, antioxidant, and soothing keratolytic that has an antitrichomonal effect and induces cell proliferation (Ashcroft *et al.*, 2000). FDA list its safe and effective skin protectant at 0.1 to 2.0% (Thornfeldt, 2005). Allantoin is also used to treat wounds, ulcers, burns, dermatitis, psoriasis, impetigo, and acne. When formulated with surfactant and benzalkonium chloride, it is an effective hand sanitizer and onychomycosis therapy (Baumann, 2003).

Rice (*Oryza sativa* L., Poaceae) is the principle cereal food in Asia, and an important source of food, providing 20% of the total direct human caloric intake world-wide, as well as being the predominant staple food for many developing countries (FAO, 2004). Although several colored rice varieties are used, the most commonly consumed rice varieties have whitish kernels. Recently, as bioactive compounds from natural food resources are widely considered to be valuable for human health, framing and consumption of colored rice consuming is increased even in North East Asia. A bunch of experimental studies have reported that colored brown rice supplementation decreased oxidative stress *in vivo* and increased antioxidant capacity *in vivo* and *in vitro* (Toyokuni *et al.*, 2002; Xia *et al.*, 2003; Finocchiaro *et al.*, 2007). Such healthy properties have been related to three classes of phytochemicals present in rice: γ-oryzanol, tocols, and polyphenols (Reddy *et al.*, 1995; Xu & Godber, 1999; Han *et al.*, 2004; Tian *et al.*, 2004). However, no result reported has been able to access the allantoin contents in rice grain, as well as no method to quantify the allantoin.

Thus, the aim of the present work was to isolate the allantoin as the authentic standard and to develop a quantification method to evaluate the allantoin in various rice variety collections. In this study, we present the results of the identification, distribution and quantification of the allantoin in rice varieties.

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MATERIALS AND METHODS

Isolation and Identification of Allantoin

Red rice, Jeokjinjubyeo, was grown at the National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Gyeonggi-do, Korea, in 2003. The red rice (5 kg) was finely powdered, extracted with methanol (10 L × 3) at room temperature (24-26℃) for 1 day, and filtered. The combined filtrate was concentrated under vacuum at 30℃. The MeOH extract (300 g), defatted with n-hexane, was first partitioned between CH_2Cl_2 and H_2O. CH_2Cl_2 portion was fractionated by silica gel column chromatography using hexanes-EtOAc solvent gradient. The fractions were pooled into 17 major fractions on the basis of their TLC profiles. Allantoin (127 mg) was recrystalized from fraction 15. The melting points were determined using a B-chi B-540 (B-chi Lab. Postfach, Schweiz) apparatus and are uncorrected. The UV spectra were recorded on a HP 8453 UV-Vis spectrophotometer (Agilent Technologies, Inc., CA, USA) in MeOH solution. The IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer (Jasco Co. Ltd., Tokyo, Japan) as KBr disks. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker CXP-500 spectrometer (Bruker Biospin Co. Ltd., F-llanden, Switzerland). Chemical shifts are shown in δ values with tetramethylsilane (TMS) as an internal reference. EI-MS were obtained on a Hewlett Packard Model 5989B GC/MS spectrometer (Agilent Technologies, Inc., CA, USA) and HR-EI-MS were measured on a JMS700 spectrometer (Jeol Ltd., Japan).

Allantoin (1): white powder; mp 233℃; IR (KBr) ν_{max} 3439 (NH), 3344 (NH), 3190 (NH), 3061 (NH), 2764, 2361, 1782 (C=O), 1712 (C=O), 1658, 1602, 1531, 1184 cm^{-1}; EI-MS (70 eV) m/z (rel. int., %) 158 $[M]^+$ (1.2), 130 (32.0), 115 (15.8), 87 (45.8), 60 (100); HR-EI-MS m/z 158.0398 $[M]^+$ (calcd for $[C_4H_6O_3N_4]^+$: 158.0440); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 10.54 (1H, s, H-1), 8.06 (1H, s, H-3), 6.88 (1H, d, J = 8.1 Hz, H-6), 5.79 (2H, s, H-8), 5.23 (1H, d, J = 8.1 Hz, H-4); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 173.7 (C-5), 157.5 (C-7), 156.9 (C-2), 62.6 (C-4).

Sample Preparations for HPLC analysis

Forty four varieties of rice were harvested in the Experimental Station of National Institute of Crop Science, Suwon in 2005. Fully pulverized rice grains (2 g per sample) were extracted with 25 ml of an acetone-water mixture (7:3, v/v) under 30 min sonication in an ultrasonic bath at the ambient temperature. The extracts were rapidly filtered through a Acrodisc® PTFE syringe filter and kept refrigerated before assay.

Chromatographic conditions

Allantoin analysis of investigated extracts was performed on the HP 1100 series HPLC system, consisted of quarternary pump and diode array detector (Agilent Technologies, Inc., CA, USA). Chromatographic separations were performed on the Wakosil 5NH_2 column (Wako, 4.6×250 mm, 5 μm particle size). The samples were introduced through a Rheodyne injector, with a 20 μL sample loop. Data acquisition and analysis were performed using HP Chemstation.

Determination of allantoin was performed using the mobile phase acetonitrile/water (70/30, v/v).

Chromatography of standard solutions and investigated extracts was performed under isocratic conditions, at a flow rate of 1.0 ml min^{-1}. Separation was achieved at ambient condition, with a total run time of 15 min. Column effluent was monitored at 210 nm. Allantoin peaks in the investigated extracts were identified by comparing their retention times with that of standard isolated.

RESULTS AND DISCUSSION

Chromatography revealed well-resolved the allantoin. Fig. 2 shows a chromatogram of this allantoin in tested rice sample extract. The retention time of allantoin was 5.2±0.7 min. A calibration curve was constructed and the linearity of the method was estimated by regression analysis of allantoin peak area against the concentration (Fig. 1). Allantoin peak identification was performed by comparing its retention time with that of standard compound (Fig. 2). The regression equation of peak area (y) against compound concentration (x, μg mL^{-1}) is given in Fig. 1. Our results indicate that a very good linearity of the method was

achieved under the chromatographic conditions described.

Using this HPLC method, the allantoin concentrations in 44 varieties of rices, grown in Suwon, Korea in 2005, were measured (Table 1). The allantoin in 44 varieties of rices were on average 10.7±4.6 mg/100 g brown rice with a narrow range of 2 to 18 mg/100 g brown rice. The highest content was detected in Heugkwang with 18.6 mg/100 g brown rice. Meanwhile, Yeomyung showed the lowest level of allantoin with 2.6 mg/100 g brown rice. Although a greater variability of contents of allantoin was expected, the concentration of that in rice was in the range of 0.002 to 0.018% and its content was much lower than that of Dioscorea Rhizome which contains approximately 0.11 to 0.72% of allantoin (Sagara *et al.*, 1989).

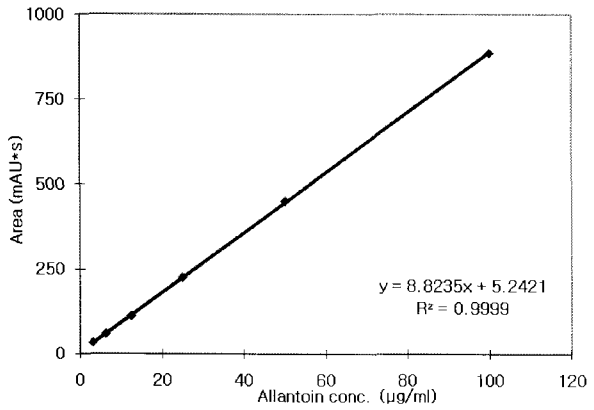

Fig. 1. The standard calibration curve of allantoin isolated from Jeokjinjubyeo.

The reasons of the lowest allantoin content in rice are unclear, so far. However, the low variability of allantoin contents supposed to be in accordance with the physiological role of this secondary metabolite in higher plant tissues. Among the other ureides, amino acids, nitrates or

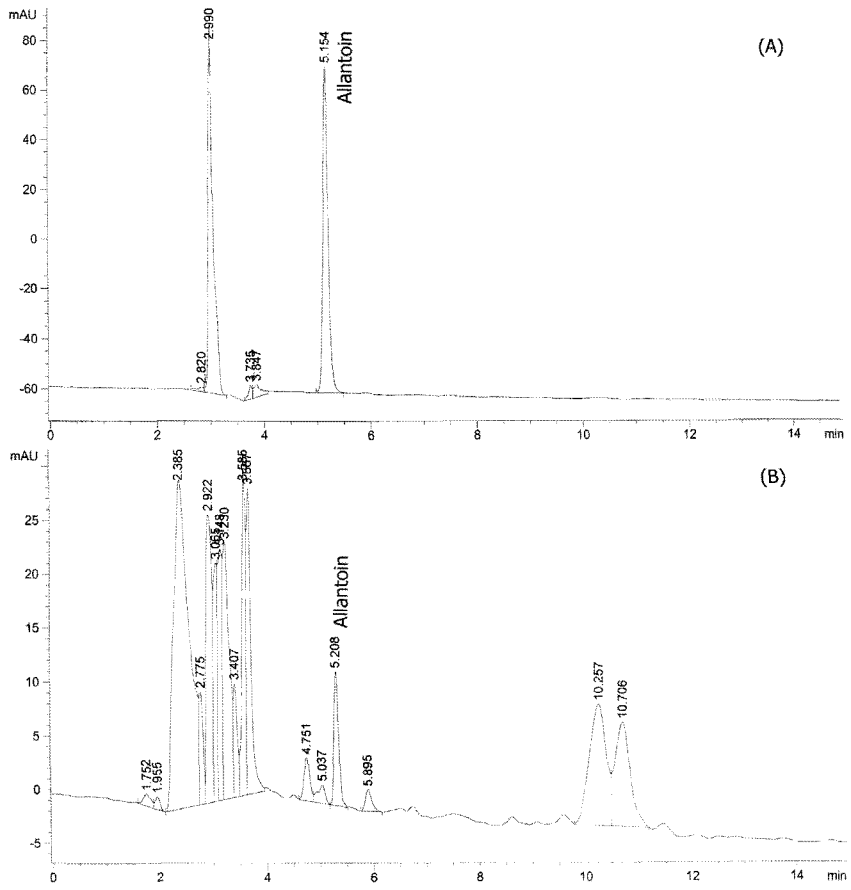

Fig. 2. HPLC chromatogram of allantoin standard (t_R = 5.154 min) (A) and the representative chromatogram of tested sample extract (B). UV detection at 210 nm. Allantoin peaks in the investigated extracts were identified by comparing their retention times with that of standard.

Table 1. Results of allantoin quantification in selected rice varietiesa[†].

Variety	Seed color	Allantoin conc (mg/100 g)
Arum	white	6.9
Baekjinju	white	17.6
Choochung	white	11.2
Chungchungchal	white	9.6
Daepyung	white	15.1
Dasan	white	5.6
Dongjin	white	4.5
Dongjin1	white	17.0
Goami	white	9.9
Goami2	white	17.0
Hanarum	white	15.7
Heugjinju	white	12.6
Hwachung	white	3.6
Ilpum	white	3.1
IR 24	white	5.2
IR 36	white	5.3
IR 56	white	7.3
IR 64	white	11.2
IR 72	white	6.8
IR 8	white	8.8
Manho	white	12.5
Milyang23	white	7.5
Nagdong	white	5.6
Nampoong	white	7.9
Niponbare	white	7.8
Nongan	white	6.0
Saechoochung	white	10.9
Saesangjoo	white	11.6
Samduk	white	15.1
Seogan	white	18.2
Seolgaeng	white	14.6
Seolhyangchal	white	13.2
Shindongjin	white	16.0
Shinseonchal	white	7.5
Taesung	white	15.4
Yeomyung	white	2.6
Youngan	white	10.6
Jeokjinju	red	13.3
Heughyang	black	9.0
Heungnam	black	7.4
Heugkwang	black	18.6
Chosengheugchal	black	15.8
Heugjinju	black	15.3
C3GHi	black	16.4
Mean±SD		10.7±4.6

[†]All of the rices were analyzed in duplicate.

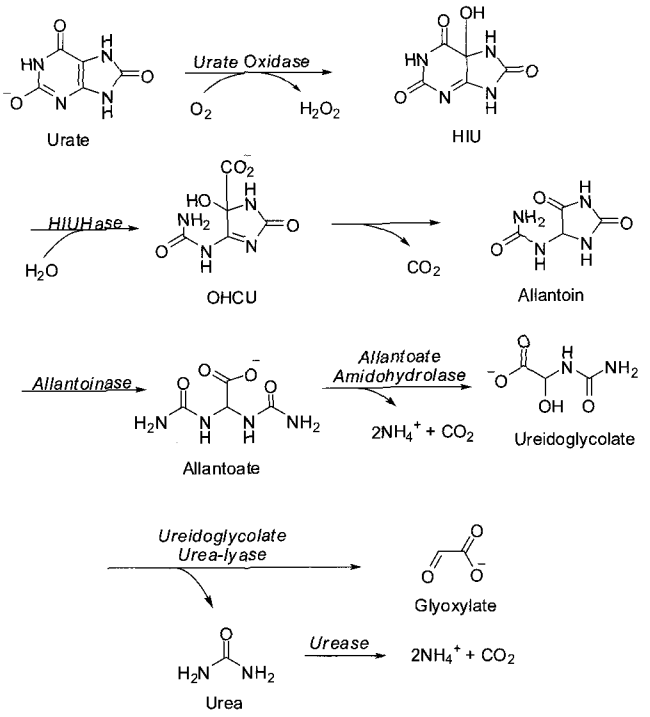

Fig. 3. Transformation of allantoin from urate to glyoxylate and ammonia (Todd, *et al.*, 2006).

amides, allantoin is considered one of the forms in which nitrogen is transported through the plant organism (Todd *et al.*, 2006) (Fig. 3). This type of nitrogen transport is frequent in the plant kingdom: up to now, allantoin is detected in various organs of plants belonging to 23 families; in some of them, allantoin is a typical constituent (Schliemann, 1984; Mazzafera & Goncalves, 1999; Maksimovic *et al.*, 2004).

Necessary quantities of allantoin are mainly produced synthetically by oxidation of uric acid with alkaline potassium permanganate, or by heating urea with dichloroacetic acid (Budavari, 1989). Because of economic advantages of synthesis over isolation from plant material, allantoin-containing herbal drugs have lost their importance as sources for the industrial production of allantoin. However, those drugs still have an importance in dermatology and cosmetology, because adequate extracts are frequently used.

A number of reports have discovered that rice has a wide range of phytonutritions. γ-Oryzanol and tocopherol are well known as an antioxidant in rice bran (Osawa *et al.*, 1992). Oryzafuran, polymeric procyanidins, and 4-carbo-

methoxy-6-hydroxy-2-quinolone have been also reported their antioxidant activities (Chung & Woo, 2001; Oki *et al.*, 2002; Han, *et al.*, 2004). Even its level in brown rice might not be considered, allantoin has a potential as a phytonutritions. Thus to breed the superior rice cultivars containing the high allantoin level for better human uses, this quantification method and results should be considered with investigation of genetic patterns between various germplasms.

In conclusion, we isolated allantoin from Jeokjinjubyeo and evaluated its level in 44 collected rice varieties. The contents of allantoin are between 0.002 to 0.018% of brown rice, and there was no significance between the allantoin level of colored rice and that of normal white rice varieties. These results might be an application for quality control purposes and process control in the industry, and also advantageous in breeding programs.

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