Available online at www.sciencedirect.com
ScienceDirect
Journal of Trace Elements in Medicine and Biology 21 (2007) 43–51
Journal of Trace Elements in Medicine and Biology
www.elsevier.de/jtemb

NUTRITION

Relationship between trace metal concentration and antioxidative activity of ancient rice bran (red and black rice) and a present-day rice bran (Koshihikari)

Isao Kaneda[a,c,*], Fukuzo Kubo[b], Hiromu Sakurai[c]

[a]*Office Komenuka-club, Basic Research Department, 4-16-5 Higashishirakawadai, Suma-ku, Kobe 654-0102, Japan*
[b]*Real Company Limited, 1-3-8 Goshodori, Hyogo-ku, Kobe 652-0885, Japan*
[c]*Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan*

Received 22 March 2005; accepted 21 October 2006

Abstract

Antioxidative activity and polyphenol and trace metal content in bran from ancient rice varieties (red and black rice) and a present-day variety of rice (Koshihikari) were measured. The antioxidative properties of rice bran in terms of scavenging and quenching activity for reactive oxygen species (ROS), including superoxide anion radicals ($\cdot O_2^-$), hydroxyl radicals ($\cdot$OH), singlet oxygen ($^1O_2$) and lipid peroxide (LOO$\cdot$), correlated well with polyphenol and trace metal content. In particular, the possibly that Mn content greatly contributes to the antioxidative properties of rice bran was revealed.

Keywords: Ancient rice bran; Present-day rice bran; Trace metal; ICP-MS; Antioxidative activity

Introduction

The number of stress-induced diseases increases year by year worldwide [1,2]. Issues related to increasing stress levels include endogenous factors, such as age-associated deterioration of vital functions, nutritional imbalance and reduced immunologic competence, as well as exogenous events, such as increased UV exposure arising from a depletion of the ozone layer and exposure to environmental pollutants. It has been proposed that the intake of antioxidative compounds and the induction of antioxidative enzymes involving catechin, α-tocopherol (vitamin E), ascorbic acid (vitamin C), superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) that remove reactive oxygen species (ROS) [3,4] can prevent diseases caused by such stress. Accordingly, in recent years, several antioxidative components have been found in natural sources, which are both safe and effective. Some antioxidative compounds that effectively eliminate ROS, including singlet oxygen ($^1O_2$), superoxide anion radicals ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot$OH) and lipid peroxide (LOOH), have been used as components of cosmetics and health foods [5,6]. We recently found that ancient rice, which is regarded as a staple of the Japanese diet, is highly effective in scavenging ROS [7]. Furthermore, rice bran contains natural compounds

*Corresponding author. Office Komenuka-club, Basic Research Department, 4-16-5 Higashishirakawadai, Suma-ku, Kobe 654-0102, Japan. Tel.: +81 78 742 3157; fax: +81 78 742 3157.
E-mail address: isao-k-kirakira@coffee.ocn.ne.jp (I. Kaneda).

0946-672X/$ - see front matter
doi:10.1016/j.jtemb.2006.10.003

with antioxidative properties, such as γ-oryzanol and phytic acid [8,9]. Rice bran is also an important dietary source of several trace metals, including K, Ca, Mg and Mn [10].

Recently, we reported the determination of trace element concentrations in several varieties of ancient rice and in varieties of present-day rice [11]. We have also measured polyphenol levels in rice [7]. On the basis of these results [7,11], we examined the relationship of trace metal and polyphenol content with antioxidative properties of rice.

Materials and methods

Samples

Ancient varieties of rice and a present-day variety of rice grown in 2001 in Japan were used. Ancient black rice samples were obtained from three different districts (Toyama Prefecture and Nakashinkawa-gun and Hiraka-gun of Akita Prefecture). Ancient red rice samples were obtained from six districts, which were Toyama, Shiga, Kyoto, Shizuoka, Nara and Fukuoka Prefectures. For the present-day rice, a Koshihikari variety from Niigata Prefecture was used.

Hulled rice was crushed, and the rice bran was used for test samples. The rice bran was immersed in a mixture consisting of 50% CH_3CN and 50% EtOH or 50% MeOH, stirred for 10 min and left to stand for 1 h at room temperature. The mixture was then centrifuged for 10 min at 3000 rpm. The obtained supernatant is referred to as the 'centrifuge-treated extract'.

Reagents

The following reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan): hematoporphyrin dihydrochloride (HP), xanthine oxidase from buttermilk (XOD), trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), sodium tungstate (VI) dehydrate, and phosphomolybdic acid-*n*-hydrate. 2, 2, 6, 6-Tetramethyl-4-piperidone hydrochloride (TMPD) and 6-hydroxypurine (hypoxanthine, HPX) were obtained from Sigma Aldrich (St. Louis, MO, USA). 5, 5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from Labotec (Tokyo, Japan). Diethylenetriamine-*N,N′,N″,N‴,N⁗*-pentaacetic acid (DTPA) and Fe(III)-EDTA were obtained from Dojindo (Kumamoto, Japan). All other chemicals used were of commercially available analytical reagent grade.

The following standards were used. ICP-grade multi-element standard solution BM (Al, Cu, Fe, Mn, Pb and Zn) and gallic acid monohydrate were obtained from Wako Pure Chemicals, and ICP-grade accu trace reference standard solution (Al, Sb, As, Be, Cd, Cr, Co, Cu, Pb, Mn, Mo, Ni, Se, Tl, Th, U, V and Zn) was purchased from AccuStandard (New Haven, CT, USA).

Preparation of rat liver microsomes

Wistar strain rats (male, 8-weeks old) (Shimizu Experimental Material Co., Kyoto, Japan) were fasted for 24 h, anaesthetized with ether and then sacrificed. The liver was removed and homogenized in a 1.15% KCl solution using a Potter-Elvehjem type homogenizer (Iuchi, Tokyo, Japan). The homogenate was then centrifuged for 60 min at 100,000 rpm using a Beckman XL-90 Ultra-centrifuge (Beckman Coulter, Fullerton, CA, USA), re-homogenized and then stored at −80 °C until use [12,13].

Preparation of Folin–Dennis reagent

One hundred gram of sodium tungstate (VI) dihydrate, 20 g phosphomolybdic acid *n*-hydrate and 50 mL of H_3PO_4 were added to 700 mL of purified water and dissolved. After refluxing for 2 h, the solution was cooled and the volume adjusted to 1 L. To make a saturated Na_2CO_3 solution, 35 g Na_2CO_3 was added to 100 mL of purified water and dissolved at a temperature between 70 and 80 °C. The solution was allowed to stand overnight at room temperature and the precipitate was then removed.

Analytical methods

Determination of trace metals by ICP-MS

The ancient rice bran varieties (red and black rice) and the present-day rice bran (Koshihikari) were treated with 62% HNO_3, 30% H_2O_2 and 60% $HClO_4$ at 200 °C for 3 h on a hotplate, and then diluted with 6% HNO_3 (69%) for measurement. ICPM-8500 (Shimadzu, Kyoto, Japan) was used to determine values for eight different metals, including Al, Cr, Mn, Fe, Co, Cu, Zn and Mo [14–16]. The metals contents were used calibration curve method, and the detection limit was 10 μg/kg.

Reactive oxygen species (ROS) scavenging activities by ESR-spin trap method

The centrifuge-treated extracts of the different varieties of bran were used as samples for measurement. Using an ESR spectrometer FR-30 (JOEL, Tokyo, Japan) and a high-sensitivity quartz cell (Labotec), ROS scavenging activity was measured by the spin trap method using DMPO as the spin trap agent [17,18]. Signal intensity due to DMPO–ROS spin adduct was expressed as the relative intensity ratio against the signal intensity due to Mn (II). The ROS scavenging activity of the rice bran extract was expressed as an IC_{50} value, which is defined as the concentration at which a 50% inhibition rate is achieved compared to the control. Eq. (1) to determine the inhibition rate was used as