Active oxygen radical scavenging activity of the plant polysaccharide processed foodstuff BIO BRAN

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Purpose

MGN-3 (BIO BRAN) is mainly composed of denatured arabinoxylan derived from rice bran, and obtained by partial degradation of soluble hemicellulose fraction using the carbohydrate-degrading enzyme complex, which is obtained from the cultured filtrate of Cortinellus shiitake mycelia (a species of Basidiomycota). MGN-3 is reported to increase NK cell activity in immunocompromised patients (Int. J. Immunotherapy; 14(1), 1998).
In this study, we determined the active oxygen radical scavenging activity of BIO BRAN by electron spin resonance (ESR).

Materials and Methods

The concentration of the crude material was adjusted to 20 mg/ml with water, the solution was shaken for 10 minutes, and the extract was centrifuged for 5 minutes at 3,000 rpm to obtain the supernatant as the sample. The sample was diluted with water to bring the total concentration to 0.2 mg/ml and 2.0 mg/ml for measurement. To determine the active oxygen radical scavenging activity of BIO BRAN, the scavenging effects of this foodstuff on superoxide anion radicals (superoxide), hydroxyl radicals in the Fenton reaction system, and hydroxyl radicals irradiated with ultraviolet light were examined.

Superoxide was measured in the hypoxanthine (HPX)/xanthine oxidase (XOD) reaction system by the spin-trapping method. Specifically, 50 μl of the sample was mixed with 50 μl of 2 mM HPX, 35 μl of 5.5 mM DETAPAC, and 15 μl of 9.2M DMPO. The measurement of the mixture was started immediately after the addition of 0.4 U/ml (50 μl) of XOD. DMPO- superoxide anion (O_2^-)adduct spectra, which were generated within the quartz cell at 120 minutes after agitation, were measured. The superoxide scavenging activity was represented as the signal intensity of DMPO-O_2^-

adduct relative to the signal intensity of the internal standard MnO.
The concentration of superoxide dismutase corresponding to the scavenging activity of the sample was determined based on the working curve of various superoxide dismutase concentrations.[table1]

table1

Scavenging Activity on O_2^-
−Hypoxanthine−xanthine oxidase reaction system−

9.2M DMPO	15 μL
2mM HPX	50 μL
5.5mM DETAPAC	35 μL
Samples	50 μL
0.4U/mL XOD	50 μL

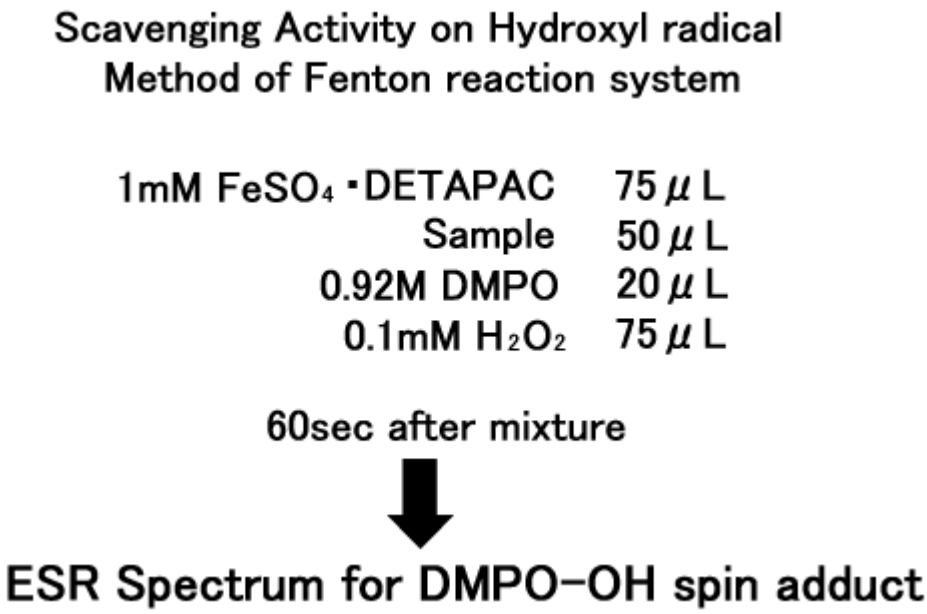
80sec after mixture

↓

ESR Spectrum for DMPO−O_2^- spin adduct

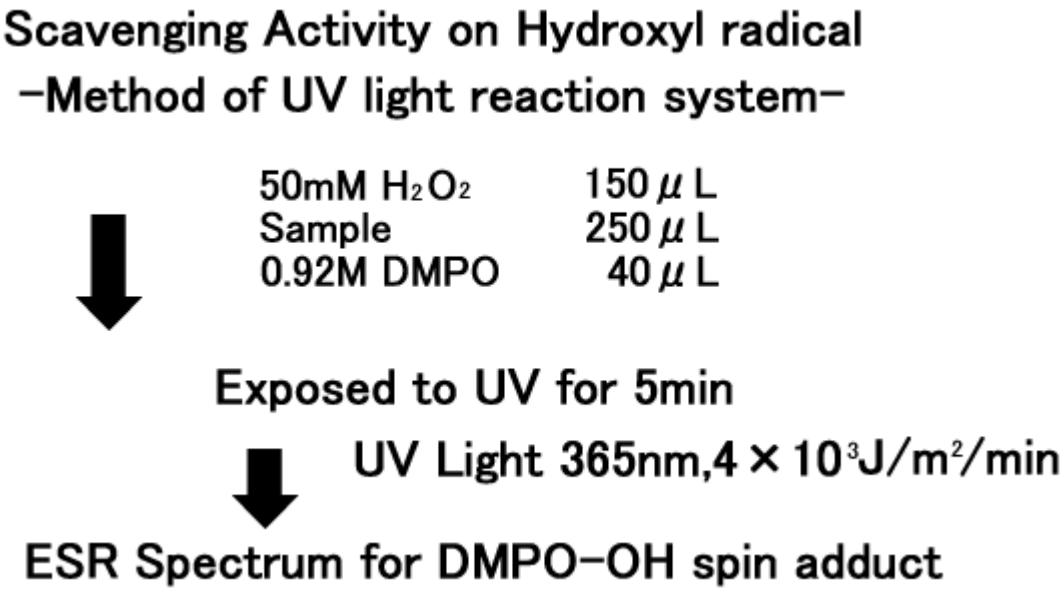
The hydroxyl radical scavenging activity of the sample was determined in the Fenton reaction system. 50 μl of the sample (20 mg/ml, 2.0 mg/ml and 0.2 mg/ml, respectively) was added to 1 mM $FeSO_4$ (75 μl) and mixed with 10-fold diluted DMPO (20 μl), to which 75 μl of 0.1 mM H_2O_2 was then added. The mixture was placed on the cell after 2-second agitation. Sweeping was started 60 seconds after the addition of H_2O_2.[table2]

table2



The hydroxyl radical scavenging activity of the sample was also determined in the reaction system with radiation from ultraviolet light. The sample (250μl) was mixed with 10-fold diluted DMPO (40μl), to which 150 μl of 100 mM H_2O_2 was added. After agitation, the sample was placed in a plastic container, and exposed to ultraviolet light (365nm, 4×10^3 J/㎡/min) for 5 minutes before the measurement.[table3]

table3



Spectrum analysis was conducted using an ESR apparatus (Nippon Denshi JES-FR30) under the following conditions: sweeping width of the magnetic field, 335.6 mT; modulation of the magnetic field, 0.1 mT; gain, 125; sweeping time, 2 min; reaction time, 0.1 sec; measurement temperature, room temperature.

[Results]

Superoxide scavenging activity of the sample increased in a dose-dependent manner. The scavenging rates were 4.4%, 23.0%, and 64.6% at the concentrations of 0.2 mg/ml, 2.0 mg/ml, and 20 mg/ml, respectively. [fig1] The working curve showed that the superoxide scavenging activities at the corresponding concentrations were -0.28 U/ml, 0.85 U/ml and 7.56 U/ml, as SOD-like activity respectively.[fig2] The hydroxyl radical scavenging activity of the sample in the Fenton reaction system also increased in a dose-dependent manner, with the scavenging rates being 3.3% for 0.2 mg/ml, 78.9% for 2.0 mg/ml, and 94.9% for 20 mg/ml.[fig3] The corresponding figures for ultraviolet-induced hydroxyl radical scavenging activity were 11.5%, 35.9%, and 72.6%.[fig4]

fig1

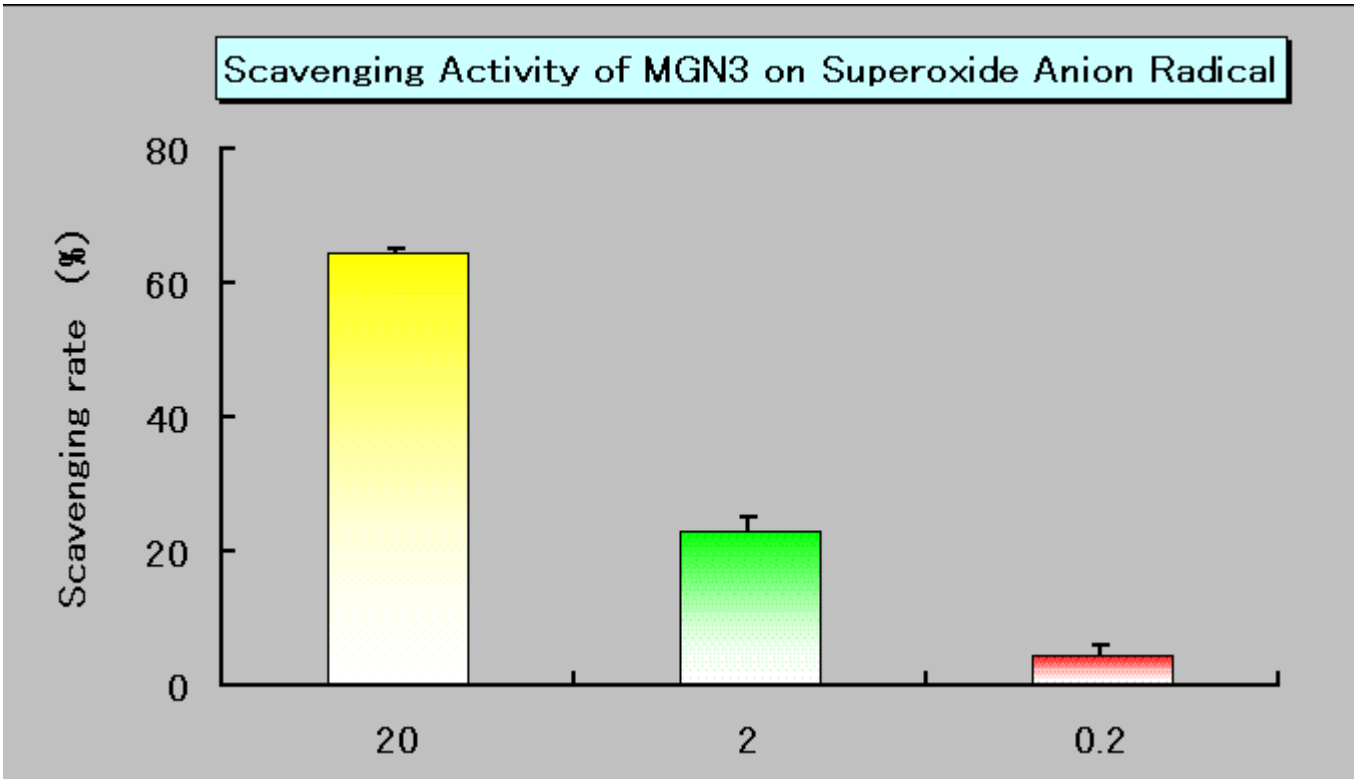


fig2

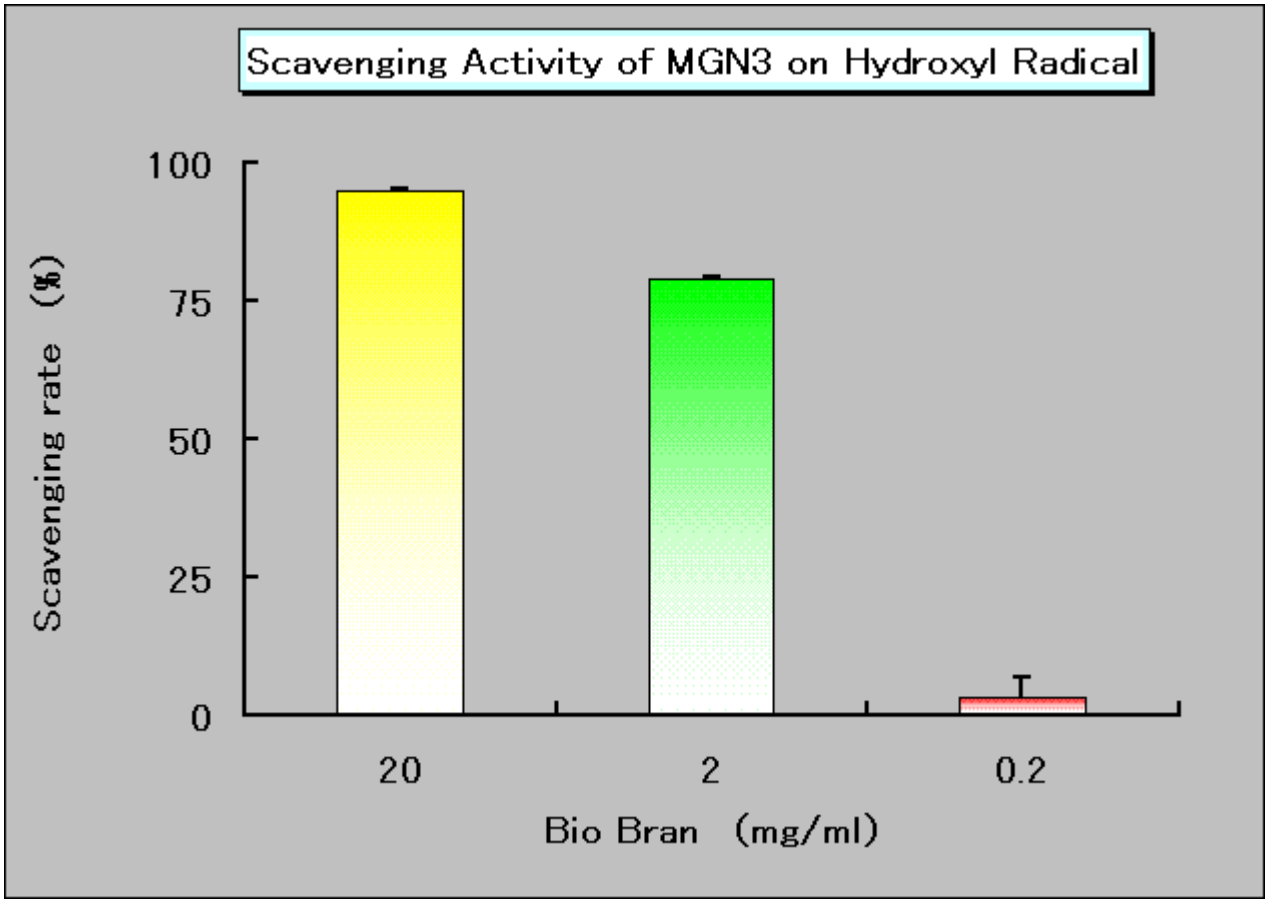

Scavenging Activity of MGN3 on Hydroxyl Radical
Scavenging rate (%)
100
75
50
25
0
20
2
0.2
Bio Bran (mg/ml)

fig3

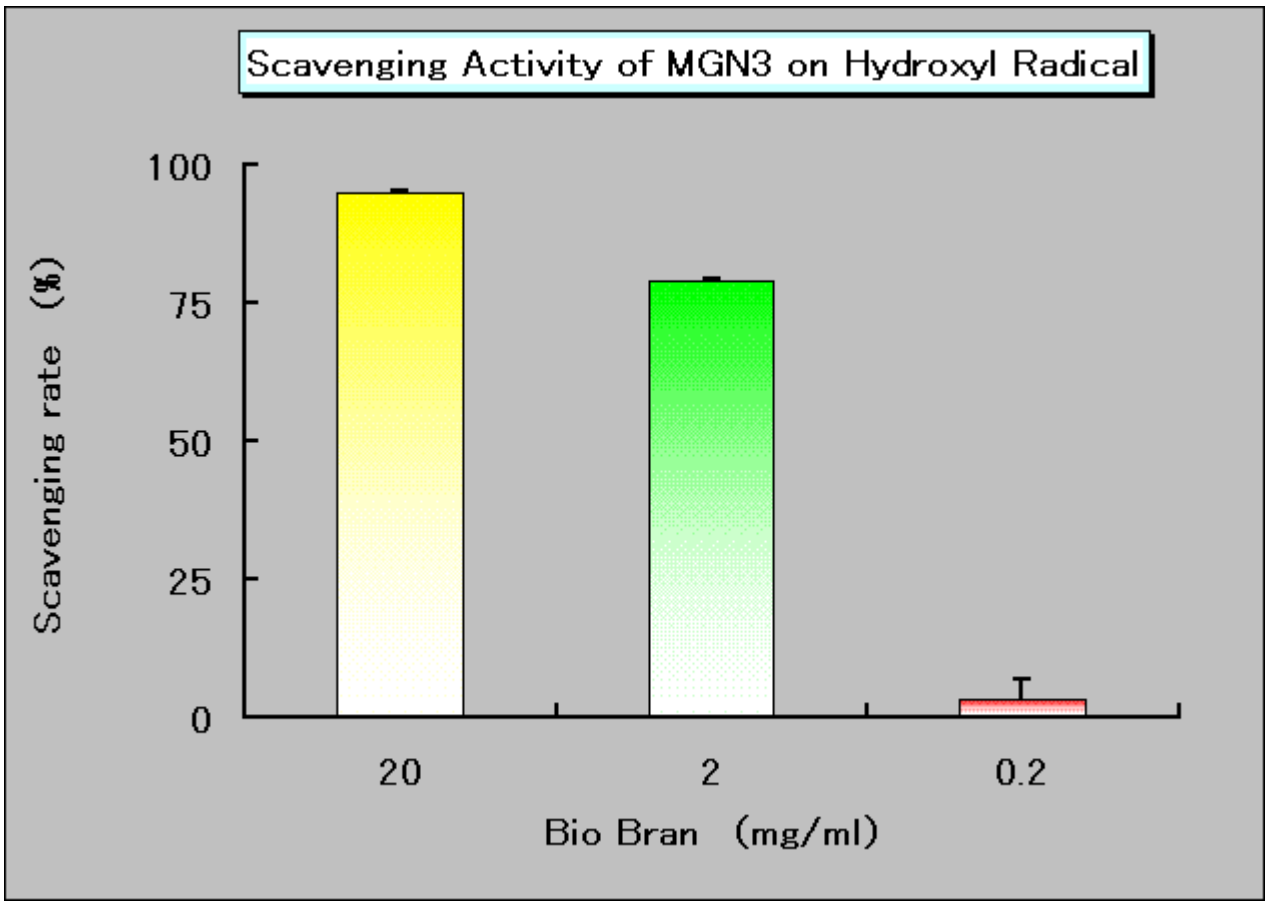
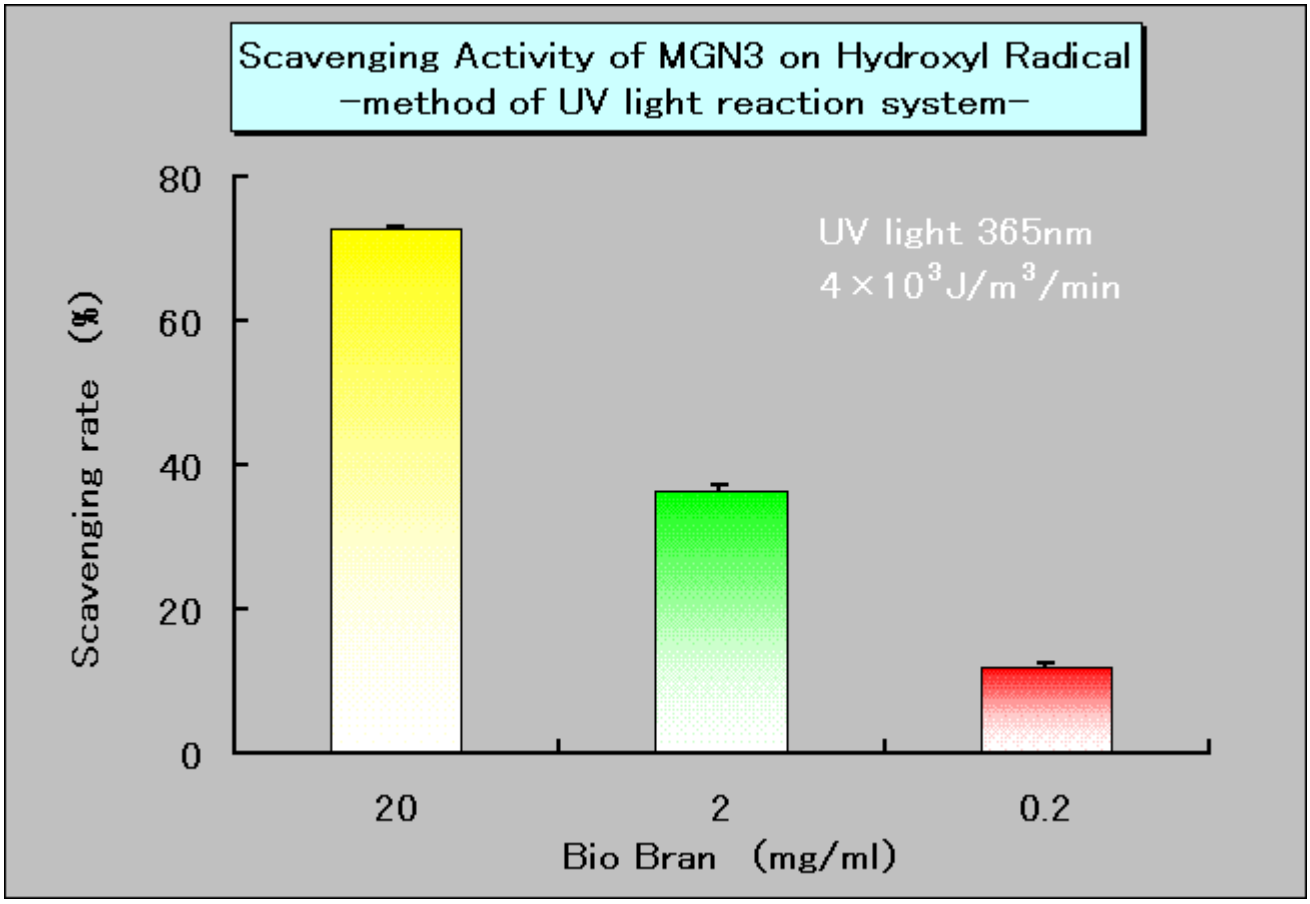

Scavenging Activity of MGN3 on Hydroxyl Radical
Scavenging rate (%)
100
75
50
25
0
20
2
0.2
Bio Bran (mg/ml)

fig4



Conclusion

In this study, we investigated the biological function of the plant polysaccharide processed foodstuff BIO BRAN in terms of active oxygen radical scavenging activity.The results indicated that the scavenging activities of BIO BRAN against superoxide anion radicals and hydroxyl radicals, which are thought to be involved in aging and the occurrence of disease, are high.In particular, hydroxyl radical scavenging activity in the Fenton reaction system was high. We are planning to perform an in vivo study on the active oxygen radical scavenging activity of BIO BRAN.