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O_2^- Scavenging Characteristics of Quercetin-Zn Complex

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Abstract O_2^- scavenging activity of quercetin-Zn complex was 1.2 times higher than that of quercetin and was the highest at pH 7.2, around 15℃. And also O_2^- scavenging ratio increased linearly while the concentration of quercetin-Zn complex was 5.5μmol/L and the temperature was 15℃ during 6minutes.

Key words quercetin-Zn complex, O_2^- scavenging characteristics

Introduction

The great leader Comrade **Kim Jong Il** said as follows.

"We must also deal properly with the work of adopting foreign successes in scientific and technological research applicable to our situation."("**KIM JONG IL** SELECTED WORKS" Vol. 10 P. 23)

Quercetin has various medical activities including anti-inflammation, antioxidation, antihypersensitivity, antibacterial, antivirus, antitumor and so on in human bodies, and Zn is trace amount of essential element as component in 200～300 species of enzymes[3－12].

Recently, it was reported that quercetin-Zn complex had significant antitumor activity and anti-inflammation activity[1].

We proceeded basic studies to elucidate molecular mechanism of medical activity by investigating its O_2^- scavenging characteristics. So far, it was been reported that quercetin-Zn complex had O_2^- scavenging activity[1], but data elucidating its O_2^- scavenging characteristics in detail have rarely been described.

1. Method and Materials

Material All reagents and solvents were analytical reagent grade.

Vit B_2(riboflavin), EDTA(ethylene diamin tetra acetic acid), NBT(nitro blue tetrazolium) were purchased from "Sigma".

Method Purity of quercetin was (96±2)%.

The quercetin-Zn complex was prepared by modifying previous method[1].

In a 150cm^3 three-necked, round-bottomed flask provided with an electromagnetic stirrer, reflux condenser and $CaCl_2$-guard tube were placed quercetin·$2H_2O$(0.3mmol) and absolute EtOH(25cm^3). Then the flask was heated to 60℃ on a water-bath. When the solid quercetin·$2H_2O$ dissolved, sodium carbonate(0.6mmol) was added quickly, and the solution was stirred and heated to reflux. After 1h, the zinc acetate(0.6mmol) was added and the solution

was stirred and heated to reflux for 6h. The mixture was cooled to room temperature and poured into H_2O(250cm^3). The pale yellow precipitate which was formed immediately, was set aside for 48h, then centrifuged by using a high speed cooling centrifugal separator, washed thrice with 1∶3 EtOH-H_2O, then several times with H_2O to free the precipitant from metal ion. The solid product was dried in vacuum for 48h. The yield was 72～85%.

Water contents of quercetin and quercetin-Zn complex are 10.72 and 6.43%, coinciding with two and three water molecules, respectively.

We used high speed cooling centrifugal separator "KUBOTA KR-20000S" and photoelectric photometer "HIRAMA PHOTOMETER MODEL 4C".

Quercetin-Zn complex has been characterized by IR, UV and atomic extinction analysis.

O_2^- scavenging activity was measured according to the modified NBT method[2]. The super oxide radicals(O_2^-) were produced by the oxidation of Vit B_2 itself exposed to fluorescent lighting and measured by the amount of NBT reduced by O_2^- . The reaction mixture of 3mL contained 8μmol/L Vit B_2, 667 μmol/L EDTA, 50 μmol/L NBT, 0.035mol/L NaH_2PO_4–NaOH buffer(pH 7.5) and 6 ～ 120 μmol/L tested compound. The reaction was carried out under the fluorescent lighting for 5min at room temperature. The amount of reduced NBT was detected by the absorbance at 560nm, since the reduced product, blue formazan, absorbs at this wavelength. The scavenging ratio(%) for O_2^- was calculated from the following expression.

$$\text{Scavenging ratio}=(A_0-A)/A_0\cdot 100$$

where A is the absorbance in the presence of quercetin or its Zn-complex, A_0 is the absorbance in the absence of quercetin or its Zn- complex.

2. Results and Discussion

O_2^- scavenging activity of quercetin-Zn complex Table shows O_2^- scavenging activities of quercetin and quercetin-Zn complex.

Table. O_2^- scavenging activity of quercetin-Zn complex

Material	Scavenging ratio/%
Quercetin·2H_2O	50.1±0.1
Quercetin-Zn·3H_2O	59.5±0.2

Values correspond to 0.1 μmol of materials.

As shown in table, O_2^- scavenging activity of quercetin-Zn complex is 1.2 times higher than that of quercetin.

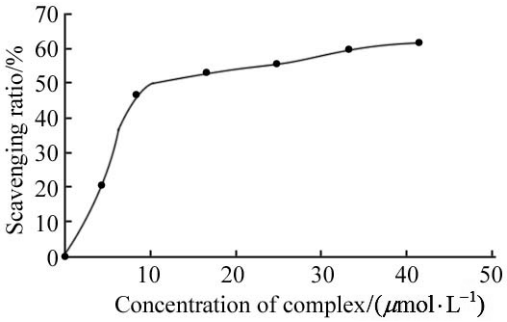

Fig. 1. The changes of O_2^- scavenging ratios with increasing of complex concentrations
Reaction condition: Vit B_2 8 μmol/L, EDTA 667 μmol/L , NBT 50 μmol/L, reaction temperature 15℃, reaction time 5min, pH 7.5

The changes of O_2^- scavenging ratios with increasing of quercetin-Zn complex concentrations We observed the changes of O_2^- scavenging ratios with increasing of complex concentrations(Fig. 1).

As shown in Fig. 1, O_2^- scavenging ratio increases linearly until complex concentration reaches 8.3 μ mol/L. This shows that the solution seems to form a homogeneous dispersion system and O_2^- ·scavenging ratio depends only on the concentration of quercetin-Zn complex. When the concentration of the complex is more than 8.3 μmol/ L because of association of quercetin-Zn complexes and of lack of Vit B_2, O_2^- scavenging ratio doesn't increase largely.

The changes of O_2^- scavenging ratios with increasing of quercetin-Zn complex reaction times Fig. 2 shows the varieties of O_2^- scavenging ratios with increasing of reaction times while the complex concentration in the solution is 5.5 μmol/L.

As shown in Fig. 2, O_2^- scavenging ratio increases linearly for 6min when the temperature was 15℃ or lower than 15℃.

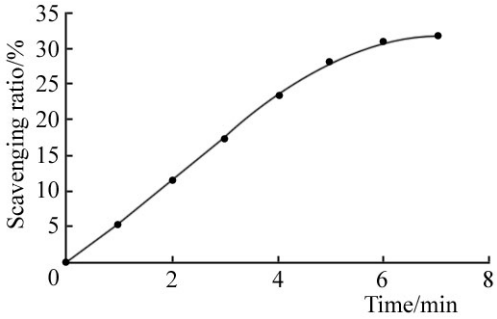

Fig. 2. The changes of O_2^- scavenging ratios with increasing of complex reaction times
Reaction condition: Vit B_2 8 μmol/L , EDTA 667 μmol/L , NBT 50 μmol/L, reaction temperature 15℃, pH 7.5, complex concentration 5.5 μmol/L

The effects of pH on O_2^- scavenging activities of quercetin-Zn complex We observed the effects of pH on O_2^- scavenging activities of quercetin-Zn complex, changing the pH of reaction solution (Fig. 3).

As shown in Fig. 3, O_2^- scavenging activity of quercetin-Zn complex is the highest at pH 7.2.

The effects of temperature on O_2^- scavenging activities of quercetin-Zn complex We observed the effects of temperature on O_2^- scavenging activities of quercetin-Zn complex, changing the temperatures of solution (Fig. 4).

As shown in Fig. 4, O_2^- scavenging activity of quercetin-Zn complex is the highest at about 15℃.

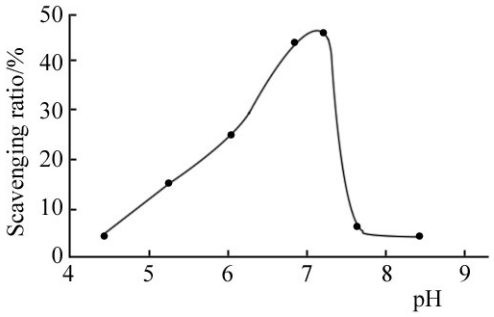

Fig. 3. The effects of pH on O_2^- scavenging activities of quercetin-Zn complex
reaction condition: Vit B_2 8 μmol/L , EDTA 667μmol/L, NBT 50μmol/L, reaction temperature 15℃, reaction time 5min, complex concentration 5.5 μmol/L

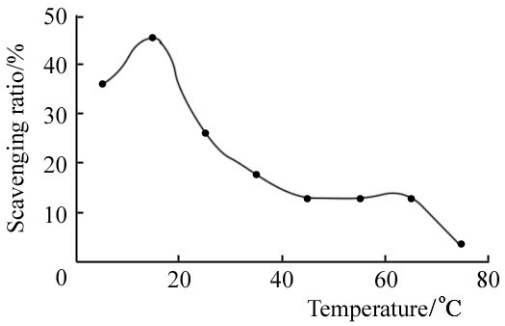

Fig. 4. The effects of temperatures on O_2^- scavenging activities of quercetin-Zn complex
reaction condition: Vit B2 8μmol/L, EDTA 667 μmol/L, NBT 50 μmol/L, reaction time 5min, pH 7.5, complex concentration 5.5 μmol/L

Conclusion

From the above mentioned experiment results, we found O_2^- scavenging activity of quercetin-Zn complex is 1.2 times higher than that of quercetin and is the highest at pH 7.2, about 15℃.

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