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Hydroxyl radical scavenging activity of microparticles prepared from solid fermentation by edible-medicinal fungi

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Kelp waste is the main solid waste in the kelp processing industries. At the same time the hydroxyl •OH radical is one of the main chemical species which can damage all types of macromolecules in an organism. In this study, the possibility of incubation of popular edible-medicinal fungi producing hydroxyl radical scavenging activity polysaccharides on the kelp waste medium is discussed. Experiments were conducted to optimize the growth rate of the fungi solid fermentation. Each component of the fermentation mixture was investigated with hydroxyl radical scavenging assays respectively. Results showed that the edible-medicinal fungi could grow on this medium and produce abundant polysaccharides which have the capacity for hydroxyl radical scavenging. Assays of each component indicated the scavenging rate of the hot water extracts of these fermentation products and their polysaccharides were much higher than established by BHT control.

Keywords*:* Hydroxyl radical, Edible-medicinal fungi

INTRODUCTION

Kelp waste is a principle oceanic solid waste in large scale kelp cultivation and the related factories, such as the production of iodine, mannitol, sodium alginate and fucoidin [1] which contains minerals, crude fibers and proteins. With the development of these processing industries, kelp waste has been a potential source of environmental contamination that can cause water eutrophication and a potential source of red tide bloom by the draining of this industrial waste to the ocean together with the amounts of organic substance and nutritive salts [2]. Discarding this is also a great loss of natural resources. Edible-medicinal fungi fermented on kelp waste will have an excellent capacity for hydroxyl radical scavenging and polysaccharides producing with a low cost [3].

The hydroxyl •OH radical is one of the main chemical species controlling the oxidizing capacity of the global earth atmosphere. It can damage almost all types of macro molecules: nucleic acids, carbohydrates, amino acids and lipids. The hydroxyl radical has a very short in vivo half-life of approximately 10^{-9} seconds and a high reactivity [4]. This makes it a very dangerous compound for the organism [5].

It can be scavenged by antioxidants such as glutathione and melatonin and dietary antioxidants such as mannitol and vitamin E [6].

Hypsizygusmarmoreus is also called the Zhengjigu, jade mushroom, a spot jade mushroom, in China, belonging to *Hypsizygus, Tricholomataceae, Agaricales, hymenomycetidae, Basidiomycetes*. This Edible-medicinal fungi contains 8 kinds of amino acids necessary for us humans and several kinds of polysaccharide. Its extracts drawn by hot water from the fruit body have the role of clearing away the free radicals in the human body, which suggests that the solid kelp waste fermentation may have the effects of hydroxyl radical scavenging.

MATERIALS AND METHODS

Fungi strain and Solid fermentation

The kelp waste material was obtained from factories engaged in sodium alginate production in Weihai, China. The fungi *Hypsizygusmarmoreus* was preserved in our lab and initially incubated on a potato dextrose agar PDA medium (fresh potato 20%, glucose 2% and agar 1.5%) in a Petri dish at 25˚C for 10 days.

Agar plugs of 10 mm in diameter with young mycelia were punched out by a puncher and inoculated into 370 mL tissue culture bottles. It contained 50g kelp waste with addictive glucose and wheat bran.

The antioxidant BHT was used as positive control (C1). The medium without fermenting fungi was used as negative control (C2). Each treatment had three replications and each replication included three parallel tissue culture bottles.

Single factor experiments: water content, PH value, wheat bran content, temperature and glucose content were conducted to optimize the growth rate of *Hypsizygusmarmoreus.*

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Treatment of the experimental samples

The kelp waste product after 30 day's fermentation, with mature mycelia was dried and shattered into microparticles (160~200 mesh).

The fermented powder suspension was made by the microparticles suspended in distilled water. The prepared concentration of this powder suspension is 50 mg/mL.

Hot water extracts were taken by bathing (100˚C) of the micro particles for 2 hours, to extract the soluble part of the product which contained polysaccharides and other soluble substances. After precipitation and lyophilization, it was dissolved in distilled water with a hot water extraction of 50 mg/mL.

The polysaccharides solution was then made by four fold volumes of ethanol (95%) added to the hot water extraction. Centrifuged at 6,000 rpm for 30 min., left overnight at 4˚C, the polysaccharides were then obtained after precipitation and lyophilization. The solution was then re-dissolved (50mg/mL) in distilled water as experimental samples.

All the chemicals used in the study were of analytical grade.

Assays of hydroxyl radical scavenging activity and EC_{50}

Fenton's reagent is the most common reaction producing HO·, developed by Henry John Horstman Fenton. Ferrous Iron (II) is oxidized by hydrogen peroxide to ferric iron (III), a hydroxyl radical and a hydroxyl anion. Iron (III) is then reduced back to iron (II), a peroxide radical and a proton by the same hydrogen peroxide.

(a) $Fe_2+ + H_2O_2 \rightarrow Fe_3+ + OH\cdot + OH-$
(b) $Fe_3+ + H_2O_2 \rightarrow Fe_2+ + OOH\cdot + H+$

There is a maximum absorbance peak at 550 nm, adding a griess reagent to the fenton's reagent system, in a specified concentration range, the light absorption of the fenton's reagent system is positively related to the concentration of HO·. So the light absorption OD value will be reduced when the inhibitor of HO· exists in the system, which can test the hydroxyl radical scavenging activity of the substance. The formula is listed below as formula (1), where ODc is the absorbance without samples and ODu is the absorbance in the presence of the samples of the fermented products.

$$\text{Hydroxyl radical scavenging activity}(\%) = [(ODc-ODu)/ODc]\times 100 \quad (1)$$

The EC_{50} value is the effective concentration at which hydroxyl radicals were scavenged by 50% and was obtained by interpolation from regression analysis.

RESULTS AND DISCUSSION

Fermentation of Hypsizygusmarmoreuson kelp waste medium

a *b* *c*

Fig. 1. *Hypsizygusmarmoreus* fermented on a PDF medium (a) and a kelp waste medium (b,c)

Hypsizygusmarmoreus, as shown in Figure 1 can grow on the solid medium mainly composed of kelp waste. It produced white, moderately long and dense mycelia during the fermentation, which indicated kelp waste could be employed as an alternative medium for the fermentation of *Hypsizygusmarmoreus*. Experiments were conducted to optimize the growth rate of *Hypsizygusmarmoreus*: water content, PH value, wheat bran content, temperature and glucose content. The results of 5 single factor experoments are shown in figure 2 and the selected optimum solid fermentation conditions for growth and achiving a maximum growth rate were shown in table 1.

Table 1.Optimum Solid Fermentation

Optimum parameters	Results
Water content (%)	70
PH value	6.5
Bran content (%)	0.25
Glucose content (%)	0.16
Temperature (℃)	22
Maximum growth rate (mm/day)	2.16±0.35

According to the single factor optimization of the growth rate for *Hypsizygusmarmoreus*, the optical solid conditions were a water content of 70%, a PH value of 6.5, bran content of 25% and glucose content of 16%. The temperature of the fermentation system was 22˚C. The experiment to attain a maximum growth rate was conducted under

these conditions, which showed that the growth rate for *Hypsizygusmarmoreus* was 2.16 ± 0.35 mm/d.

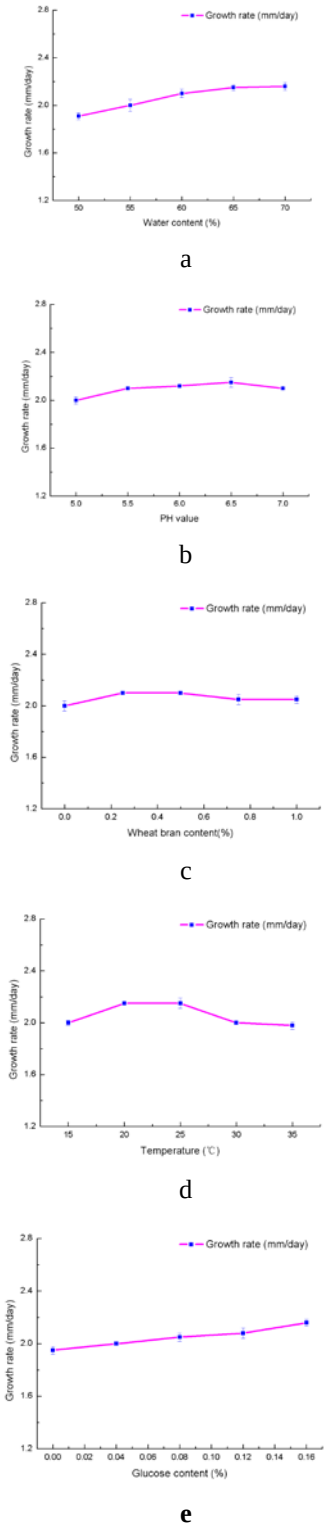

Fig. 2. Growth rate affected by the water content (a), PH value (b), wheat bran content (c), Temperature (d) and Glucose content (e)

The hot water extracts and the polysaccharides from Hypsizygusmarmoreus fermentation

The dried and shattered kelp waste product was suspended in distilled water. The soluble part of the product which contained polysaccharides and other soluble substances was in hot water extracts, then the polysaccharides were drawn from hot water extracts, with the yield following these steps weighed, calculated and listed in table 2.

Table 2. Yield of the Target Product in (%).

	Hot water extracts	Polysaccharides
Yield	18.35±0.62	10.58±0.42

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the microparticles suspension, hot water extracts and polysaccharides obtained from solid fermentation, the kelp waste medium and BHT solution are shown in Figures 3 to 5.

The Hydroxyl radical scavenging activity of the obtained samples from *Hypsizygus* incubation (T in figures 3 to 5), BHT(C1) and the seaweed waste alone (C2) as a control sample was tested. The data was presented as the mean of three independent experiments ± SD.

The formula is listed below as formula (2), where ODc is the absorbance without samples and ODu is the absorbance in the presence of the samples of the fermented products.

$$\text{Scavenging effect} = (ODc - ODu)/ ODc \qquad (2)$$

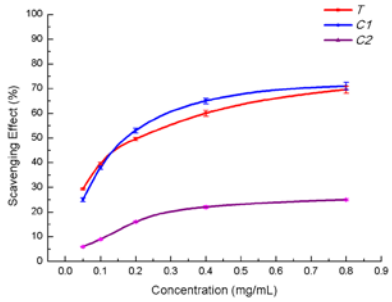

Fig. 3. Hydroxyl radical scavenging capacities of the powder suspension (T) obtained from Hypsizygus.

In accordance with figure 3, a dose-dependent increase of the hydroxyl radical scavenging activity of the microparticle is exhibited. The hydroxyl radical scavenging activity of the microparticles suspension was slightly less than BHT but more than the kelp waste medium without fermenting. But the hot water extracts (figure 4), especially the

polysaccharides (figure 5) drawn from the fermentation products had much more Hydroxyl radical scavenging activity than BHT and a kelp waste medium without fermenting.

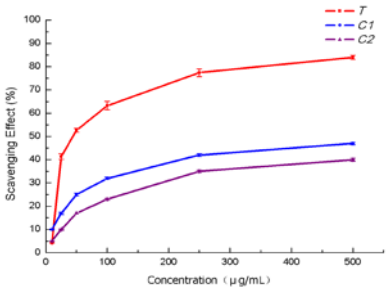

Fig. 4. Hydroxyl radical scavenging capacities of the hot water extracts (T) obtained from *Hypsizygus*.

Compared with the data for hydroxyl radical scavenging, among the fermented powder suspension, hot water extraction and polysacch-arides solution and BHT solutions (as positive control), *Hypsizygusmarmoreus* solid fermented products have a high hydroxyl radical scavenging activity, which also increases with the higher concentration of polysaccharides which exist in both the suspension and extracts solution at a diferent consentration.

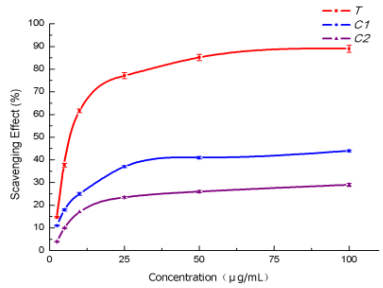

Fig. 5. Hydroxyl radical scavenging capacities of the polysaccharides (T) obtained from *Hypsizygus*

Results of EC_{50} for each sample

The effective concentrations at which the hydroxyl radicals were scavenged by 50% (EC_{50}) were calculated according to the scavenging rate of the microparticle suspension, hot water extracts and its polysaccharides each at this 50mg/mL concentration respectively. The EC_{50} for each sample was listed in table 3.

Table 3. EC50 Value (μg/mL)

Sample	Results
Powder suspension	204±10.3
Hot water extract	7.44±0.51
polysaccharides	1.73±0.14
BHT	151±13.0
Kelp waste medium	2910±32

As shown in table 3, the EC_{50} value of the fungi sample, microparticles suspension obtained from *Hypsizygusmarmoreus* fermentation was 204μg/mL, which was significantly lower than that of the kelp waste without *Hypsizygus* fermentation (2910μg/ml) as control 2, but higher than the BHT solution (151μg/ml) as control 1.The EC_{50} for the hot water extracts obtained from *Hypsizygusmarmoreus* fermentation was 7.44μg/mL, which was significantly lower than that of the kelp waste without *Hypsizygus* fermentation and the BHT solution. The EC_{50} value for the polysaccharides obtained from fermentation was 1.73μg/mL, significantly lower than that of the kelp waste without *Hypsizygus* fermentation and the BHT solution.

According to the EC_{50} values, the microparticle obtained from *Hypsizygusmarmoreus* fermentation exhibited significantly higher hydroxyl scavenging capacity than the kelp waste medium, the hot water extracts exhibited a significantly higher hydroxyl scavenging capacity than BHT and the kelp waste medium and the polysaccharides obtained from *Hypsizygusmarmoreus* fermentation exhibited a much higher hydroxyl scavenging capacity than BHT and the kelp waste medium.

CONCLUSIONS

In this study, *Hypsizygusmarmoreus* was shown to grow on the solid medium mainly composed of kelp waste, which indicates that kelp waste can be employed as an alternative medium for fermentation of *Hypsizygus* .

Compared with the hydroxyl radical scavenging activity of BHT and the kelp waste medium without fermentation, the activity of the hot water extracts and polysaccharides from *Hypsizygusmarmoreus* were higher than both of these, while the ferment microparticles suspension had a larger hydroxyl radical scavenging activity than the negative control but less than BHT**.**

Since a dose-dependent increase of the hydroxyl radical scavenging activities of the polysaccharides was exhibited and the EC_{50} of the polysaccharides was obviously much lower than the other components from the fermentation, the polysaccharides from the *Hypsizygusmarmoreus* kelp waste solid fermentation system is clearly the most effective hydroxyl radical scavenging substance in solid fermentation.

The hydroxyl radical scavenging results showed the product of the kelp waste fermentation of *Hypsizygus* have a high useful value. The efficient and high biological activity conditions of the biological conversion of kelp waste ferment of

edible-medicinal fungi has provided a potential pathway for the biological transformation of kelp waste into high value-added industrial products for application in medical and food stuffs products in natural hydroxyl radical scavenging. It is also a practical and environmentally friendly resource recycling method.

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СПОСОБНОСТ ЗА ОСТРАНЯВАНЕ НА ХИДРОКСИЛНИ РАДИКАЛИ ОТ МИКРОЧАСТИЦИ, ПОЛУЧЕНИ ПРИ ТВЪРДО-ФАЗНА ФЕРМЕНТАЦИЯ НА ЕДЛИВИ МЕДИЦИНСКИ ГЪБИ

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(Резюме)

Отпадъците от кафявите морски водорасли са основния твърд птпадък при тяхното производство. От друга страна хидроксилните радикали •OH са едно от главните химични вещества, които поразяват всички видове макромолекули в човешкия организъм. В настоящата работа се обсъжда възможността за ферментация на известни едливи медицински гъби с остатъци от кафяви водорасли, при което се получават полизахариди с противо-радикална активност. Проведени са експерименти за оптимизиране на растежа на гъбите при твърдо-фазна ферментация. Всеки компонент на ферментационната смес е изпитан за противо-радикалова акивност. Резултатите показват, че едливите медицински гъби могат да растат на тази среда и да произвеждат обилно полизахариди, които имат способността да отстраняват хидроксилни радикали. Анализът на всеки компонент показва, че противо-радикаловата способност в екстракти с гореща вода от ферментационните среди е много по-висока отколкото при BHT контрол.