

Study on Hydroxyl Radical Scavenging Ability of Fermented Kelp Waste

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In order to develop a new pathway to scavenge hydroxyl radical and to utilize kelp waste, edible-medicinal fungi was fermented in medium mainly composed of kelp waste, and the products from fermentation were investigated with hydroxyl radical scavenging assays. Results showed that fungi could grow in this medium and produce abundant polysaccharides which have the capacity for hydroxyl radical scavenging. Assays indicated the capacity of fermented kelp waste for hydroxyl radical scavenging as the EC_{50} values were 0.77 ± 0.012 mg/mL while scavenging rate of its polysaccharides were much higher.

1. Introduction

The hydroxyl $\cdot OH$ radical is one of the main chemical species controlling the oxidizing capacity of the global earth atmosphere. It can damage virtually all types of macromolecules: carbohydrates, nucleic acids, lipids, and amino acids (Reiter, et al., 1995). The hydroxyl radical has a very short in vivo half-life of approximately 10^{-9} seconds and a high reactivity (Sies and Helmut, 1993). This makes it a very dangerous compound to the organism (Reiter, et al., 1997).

Unlike superoxide, which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction. Mechanisms for scavenging peroxy radicals for the protection of cellular structures includes endogenous antioxidants such as melatonin and glutathione, and dietary antioxidants such as mannitol and vitamin E (Reiter, et al., 1995).

Polysaccharides such as *Ganoderma lucidum* polysaccharides (Sanodiya, et al., 2009), lentinan (Shen, et al., 2010) and *Pleurotus eryngii* polysaccharides (Che, et al., 2014), from edible-medicinal fungus have been reported as antioxidants in recent years, which also have remarkable hydroxyl radical scavenging ability.

Kelp waste is a main oceanic solid waste in large scale of kelp cultivating and related factories, such as the production of sodium alginate, mannitol, iodine and fucoidin (Hurd, et al., 2014) which contains crude fibers, proteins and minerals. With the development of these processing industries, kelp waste has been a potential source of environmental contaminant (Zhang, et al., 2010) which can cause water eutrophication and potential element of red tide bloom by the draining of these industrial waste to the ocean with the amount of organic substance and nutritive salts. Discarding it is also a great loss of natural resources. Edible-medicinal fungus of polysaccharides production fermented on kelp waste will gain excellent capacity for hydroxyl radical scavenging with low cost.

2. Materials and methods

2.1 Fungi strains and solid fermentation

The kelp waste material was obtained from factories engaged in sodium alginate production in Weihai, China. The fungus *Cantharellus sp.* was screened and preserved in our Lab, and initially incubated on 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, 10 mm in diameter with young mycelia were punched out by a puncher and inoculated into 370 ml tissue culture bottles containing 50 g kelp waste with additive glucose and wheat bran.

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

Single factor experiments and RSM were employed to optimize the growth rate of *Cantharellus sp.*, and the second-order regression model with five-factor four-level design was established.

2.2 Preparation for experimental samples

Preparation for the fermented powder suspension, hot water extraction, the polysaccharides solution and BHT solution as positive control.

After 20 day's fermentation, the kelp waste product was dried and shattered into microparticles (160~200 mesh), then suspended in distilled water. The prepared concentration of this powder suspension is 50mg/mL.

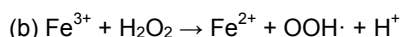
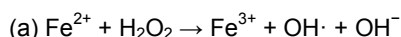
A water bath (100 °C) for 2 hours was proceeded to extract the soluble part of the product which contained polysaccharides and other soluble substances. After precipitation and lyophilization, it was dissolved in distilled water into hot water extraction of 50mg/mL.

Fourfold volumes of ethanol (95%) were added to the hot water extraction, centrifuged at 6,000 rpm for 30 min, stayed at 4°C overnight, and the polysaccharides were obtained after precipitation and lyophilization. The polysaccharides were also re-dissolved (50mg/mL) in distilled water for further study.

Finally, the control experiments of BHT which is known as an antioxidant were carried out under the same conditions. All chemicals used in the study were of analytical grade.

2.3 Assays for hydroxyl radical scavenging activity and EC₅₀

Fenton's reagent is the most common reaction producing HO·, which was developed in the 1890s 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.



Adding griess reagent to the fenton's reagent system, there is a maximum absorbance peak at 550 nm. In a definite concentration range, the light absorption of the fenton's reagent system is positively related with the concentration of HO·. So the light absorption OD value will reduce when the inhibitor of HO· exists in the system, which can test hydroxyl radical scavenging activity of substance. The formula is list below as formula (1), where OD_c is the absorbance without samples and OD_u is the absorbance in the presence of the samples of the fermented products. EC₅₀ value is the effective concentration at which hydroxyl radicals were scavenged by 50% and was obtained by interpolation from regression analysis.

$$\text{Hydroxyl radical scavenging activity (\%)} = \left[\frac{(\text{OD}_c - \text{OD}_u)}{\text{OD}_c} \right] \times 100 \quad (1)$$

3. Results and discussion

3.1 Fermentation of *Cantharellus sp.* on kelp waste medium

As is shown in Figure 1, *Cantharellus sp.* could grow on the solid medium mainly composed of kelp waste. It produced white, moderate long and dense mycelia during the fermentation, which indicated kelp waste could be employed as an alternative component for fermentation of *Cantharellus sp.*

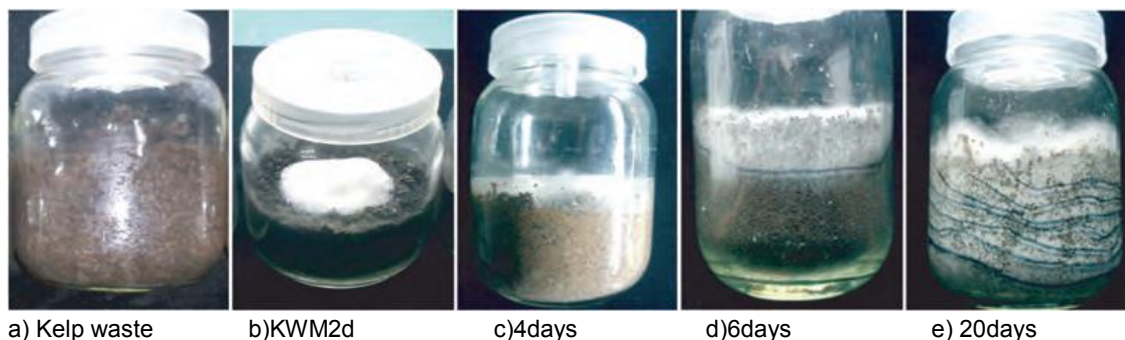


Figure 1: *Cantharellus sp.* fermented on kelp waste medium (KWM)

Single factor experiments and RSM was employed to optimize the growth rate of *Cantharellus sp.* then. The second-order regression model with five-factor four-level design was established.

By making a mathematical treatment, the maximum growth rate of *Cantharellus sp.* could be calculated. The selected optimal solid fermentation conditions for growth rate were shown in table 1.

Table 1: Parameters optimization of fermenting conditions

Optimal parameters		Results	
Water content (W_C , %)	64.9	Predictive value (mm/day)	2.88
PH value (P_H , 1)	6.5	Actual value (mm/day)	2.86
Bran content (B_C , %)	0.31	Fitting rate (%)	99.3
Glucose content (G_C , %)	0.12	Traditional growth rate(mm/day)	1.91
Temperature (T_F , °C)	21	Multiple of the increase(%)	51

According to the optimization of the growth rate for *Cantharellus sp.*, corresponding values of process variables were water content 64.9%, the PH value 6.5, bran content 0.31%, and glucose content 0.12%, temperature of solid fermentation condition was 21 °C. The maximum predicted growth rate for *Cantharellus sp.* was 2.88mm/day. The verification experiments were conducted completed under the optimal conditions, which gave the growth rate of 2.86 ± 0.223 mm/d. Fitting degree of 99.3% indicate close agreement of values predicted by the models and values in verification experiments.

3.2 The hot water extraction and the polysaccharides from *Cantharellus sp.* fermentation

After 20 day's fermentation, the kelp waste product was dried and shattered into microparticles (160~200 mesh), then suspended in distilled water. The final concentration of this powder suspension is 50mg/mL.

A water bath (100 °C) for 2 hours was then proceeded to extract the soluble part of the product which contained polysaccharides and other soluble substances. After precipitation and lyophilization, it was weighed and calculated the yield (as in Table 2), dissolved in distilled water into hot water extraction of 50mg/mL. Fourfold volumes of ethanol (95%) were added to the hot water extraction, centrifuged at 6,000 rpm for 30 min, stayed at 4 °C overnight, and the polysaccharides were obtained after precipitation and lyophilization. Weighed and calculated the yield of the polysaccharides (as in Table 2), the polysaccharides were also re-dissolved (50mg/mL) in distilled water for hydroxyl radical scavenging study.

Table 2: Yield of target product (%)

	Hot water extracts	Polysaccharides
<i>C.sp.</i>	16.5±0.32	8.0±0.23

3.3 Hydroxyl radical scavenging capacity

The hydroxyl radical scavenging activity of the microparticles suspension obtained from *Cantharellus sp.* fermentation, kelp waste medium and BHT solution were shown in Figure 2. A dose-dependent increase of the hydroxyl radical scavenging activities of the microparticle was exhibited apparently.

The formula is list below as formula (2), where OD_c is the absorbance without samples and OD_u is the absorbance in the presence of the samples of the fermented products. EC_{50} value is the effective concentration at which hydroxyl radicals were scavenged by 50% and was obtained by interpolation from regression analysis.

$$\text{Scavenging effect} = (OD_c - OD_u) / OD_c \quad (2)$$

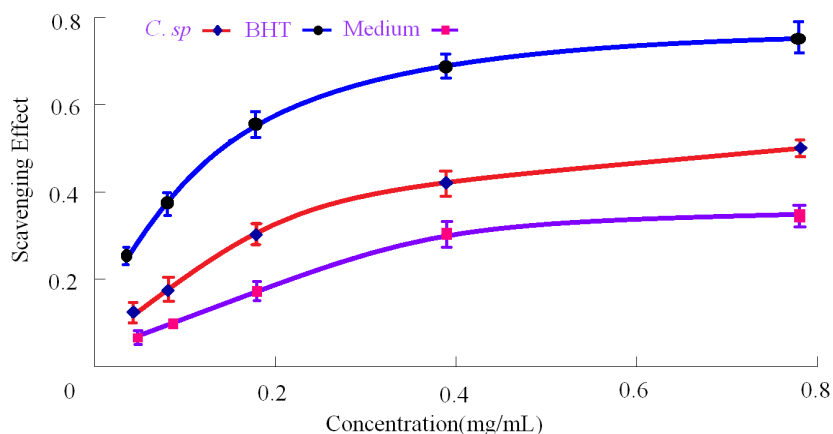


Figure 2: Hydroxyl radical scavenging capacities of the microparticles suspension obtained from *Cantharellus sp.*

Fermentation, kelp waste medium without fermented and the BHT solution. Data are presented as the mean of three independent experiments \pm SD.

The effective concentrations at which the hydroxyl radicals were scavenged by 50% (EC_{50}) were calculated according to the scavenging rate of the microparticles at different concentration.

Table 3: EC_{50} Value ($\mu\text{g/mL}$)

C.sp. sample	Control1	Control2
770 \pm 11.2	2910 \pm 32	151 \pm 13

As showed in table 3, the EC_{50} value of the *C.sp.* sample, microparticles suspension obtained from *Cantharellus sp.* fermentation was 770 $\mu\text{g/mL}$, which was significantly lower than that of the kelp waste without *Cantharellus* fermentation (2910 $\mu\text{g/mL}$) as control1, but higher than the BHT solution (151 $\mu\text{g/mL}$) as control2. According to the EC_{50} values, the microparticle obtained from *Cantharellus sp.* fermentation exhibited significantly higher hydroxyl scavenging capacity than the kelp waste medium.

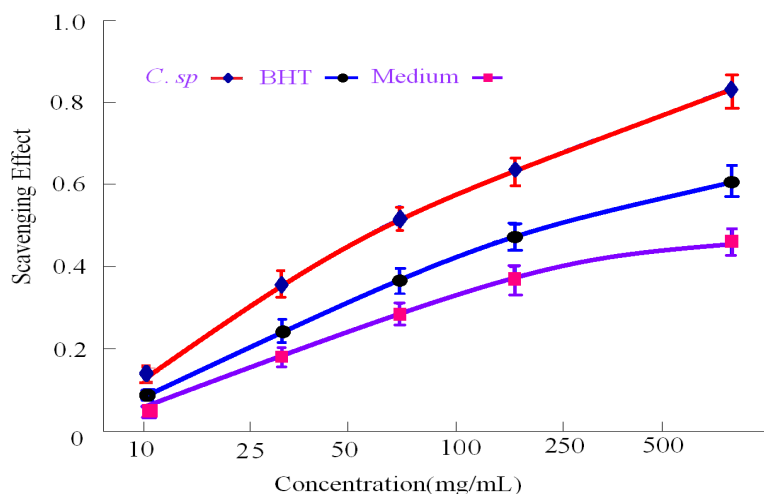


Figure 3: Hydroxyl radical scavenging capacities of the hot water extracts obtained from *Cantharellus sp.*

Fermentation, kelp waste medium without fermented and the BHT solution. Data are presented as the mean of three independent experiments \pm SD.

The effective concentrations at which the hydroxyl radicals were scavenged by 50% (EC_{50}) were calculated according to the scavenging rate of the solutions at different concentration.

Table 4: EC_{50} Value ($\mu\text{g/mL}$)

<i>C.sp.</i> sample	Control1	Control2
58.36 \pm 3.1	2910 \pm 32	151 \pm 13

As showed in table 4, the EC_{50} value of the *C.sp.* sample, the hot water extracts obtained from *Cantharellus sp.* fermentation was 58.36 $\mu\text{g/mL}$, which was significantly lower than that of the kelp waste without *Cantharellus* fermentation (2910 $\mu\text{g/mL}$) as control1, and the BHT solution(151 $\mu\text{g/ml}$) as control2. According to the EC_{50} values, the extracts obtained from *Cantharellus sp.* fermentation exhibited significantly higher hydroxyl scavenging capacity than BHT and the kelp waste medium.

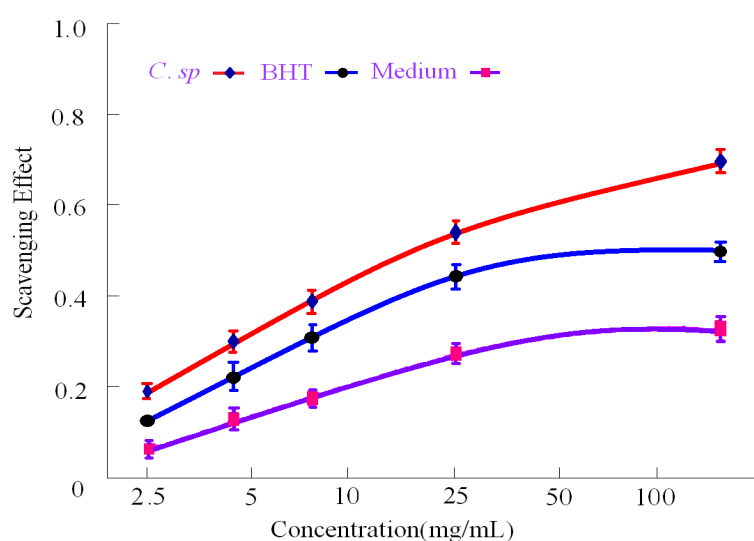


Figure 4: Hydroxyl radical scavenging capacities of the polysaccharides obtained from *Cantharellus sp.*

Fermentation, kelp waste medium without fermented and the BHT solution. Data are presented as the mean of three independent experiments \pm SD.

The effective concentrations at which the hydroxyl radicals were scavenged by 50% (EC_{50}) were calculated according to the scavenging rate of the solutions at different concentration.

Table 5: EC_{50} Value ($\mu\text{g/mL}$)

<i>C.sp.</i> sample	Control1	Control2
21.75 \pm 2.44	2910 \pm 32	151 \pm 13

As showed in table 5, the EC_{50} value of the *C.sp.* sample, the polysaccharides obtained from *Cantharellus sp.* fermentation was 21.75 $\mu\text{g/mL}$, which was significantly lower than that of the kelp waste without *Cantharellus* fermentation (2910 $\mu\text{g/mL}$) as control1, and the BHT solution(151 $\mu\text{g/ml}$) as control2. According to the EC_{50} values, the polysaccharides obtained from *Cantharellus sp.* fermentation exhibited significantly higher hydroxyl scavenging capacity than BHT and the kelp waste medium.

Compared with the hydroxyl radical scavenging activity of the positive control BHT and negative control kelp waste medium, the activity of the hot water extracts and polysaccharides from *Cantharellus sp.* were higher than both of them, while the fermented microparticles suspension has more hydroxyl radical scavenging activity than the negative control. It indicated that the hydroxyl radical scavenging results were scientific and reliable, and the product of the kelp waste fermentation had high useful value. The biological conversion of kelp waste is a practical and environmental friendly resource recycling method.

4. Conclusions

In this study, *Cantharellus sp.* could grow on the solid medium mainly composed of kelp waste, which indicated kelp waste could be employed as an alternative component for fermentation of *Cantharellus sp.* The optimization of the conditions for *Cantharellus sp.* fermentation shows more growth rate of the solid kelp waste fermentation system, and more potential comprehensive utilization of kelp waste.

Compared with datas of hydroxyl radical scavenging, among the fermented powder suspension, hot water extraction and polysaccharides solution and BHT solutions(as positive control),shows *Cantharellus sp.* fermented products has high hydroxyl radical scavenging activity, which also increase with higher concentration of polysaccharides from *Cantharellus sp.*.

The efficient and high biological activity conditions of biological conversion of kelp waste fermented of medicinal fungi has provided a potential pathway for the biological transforming kelp waste into high value-added industrial product of medicine, food and feed as natural hydroxyl radical scavenging.

Reference

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