Bulgarian Chemical Communications, Volume 49, Number 1, (pp. 282 – 287) 2017

Optimization of fermenting conditions for antioxidant activity and yield of polysaccharides from mushroom solid fermentation

Z. Yuan, P. Yan*, D. Jing

School of Municipal and Environment Egineering, Harbin Institute of Technology, Harbin, 150001, China

Received January 23, 2016; Revised August 6, 2016

In order to establish a simple procedure for producing edible mushrooms with a high polysaccharides yield, antioxidant and hydroxyl radical scavenging activity, experiments were conducted to optimize the fermentation conditions for the mushroom, as well as the fermentation time. The results showed that mushrooms can grow on this medium and produce abundant polysaccharides which have the capacity for antioxidant and hydroxyl radical scavenging, which also provide a theoreticaland practical way to a large-scale production of *Pleurotuseryngii* with a high polysaccharides yield, high antioxidant and hydroxyl radical scavenging activity under an optimum fermenting time of 20 days' cultivating.

Keywords*:* polysaccharids, solid mushroom fermentation

INTRODUCTION

Mushrooms are able to convert lignocellulose biomass waste into human food and also produce medicinal and nutritional products which have many health benefits [1-3]. *Pleurotuseryngii* is an edible mushroom native to the Mediterranean regions of Europe, the Middle East and North Africa, but also grown in many parts of Asia [4]. It may naturally contain chemicals that stimulate the immune system [5]. Dietary intake of *Pleurotuseryngii* may function as a natural cholesterol lowering dietary agent [6].

Polysaccharides such as *Ganodermalucidum* polysaccharides [7], lentinan [8] and *Pleurotuseryngii* polysaccharides [9], from edible mushrooms have been reported as antioxidants in recent years. In this study, *Pleurotuseryngii* was fermented on a solid medium of kelp waste, a main oceanic nonproductive in related industries. The microparticles obtained from the incubation were investigated for antioxidant activity and hydroxyl radical scavenging activities.

The results showed that the *Pleurotuseryngii* can grow on the solid medium and produce polysaccharides. Meanwhile, the assays showed that an antioxidant activity and hydroxyl radical scavenging capacity of the microparticles obtained from *Pleurotuseryngii* incubation were significantly higher than those of the kelp waste without fermentation. The assays showed that antioxidant activity and hydroxyl radical scavenging capacity under the optimum conditions were significantly higher than those without optimization. It would be a beneficial pathway to recycle kelp waste by the fermentation of *Pleurotuseryngii* to produce potential natural antioxidants.

MATERIALS AND METHODS

Mushroom strains and solid fermentation

The kelp waste material was obtained from factories engaged in sodium alginate production in Weihai, China. The mushroom *Pleurotuseryngii* 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℃ 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 addictive glucose and wheat bran.

The medium without fermenting mushrooms 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. Uniform design and DPS software were employed to optimize the yield of polysaccharides from *Pleurotuseryngii* and the second-order regression model with five-factor five-level design was established. The factors and levels considered in the experiment are listed in table 1 and the test schemes were elaborated in table 2.

The experiments were conducted by the test schemes, the optional conditions were chosen and the verification experiment was then conducted under the optimum conditions. Single factor experiments were conducted respectively according to the relationship between the fermenting time and the yield of mushroom polysaccharides, antioxidant activity, as well as the hydroxyl radical scavenging activity. The EC_{50} value is the effective

* To whom all correspondence should be sent:
E-mail: 43359998@qq.com

concentration at which hydroxyl radicals were scavenged by 50% and obtained by interpolation from regression analysis.

Treatment of the experimental samples

A ferment powder suspension, hot water extracts, the polysaccharides solution and BHT solution was prepared for positive control. After fermentation for 20 days, 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 applied 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 50mg/mL.

Fourfold volumes of ethanol (95%) were added to the hot water extraction, centrifuged at 6,000 rpm for 30 min, left 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, BHT control experiments which is known as an antioxidant were carried out under the same conditions. All the chemicals used in the study were of analytical grade.

Assays for hydroxyl radical scavenging activity

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.

(a) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$

(b) $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\cdot + H^+$

Adding griess reagent to Fenton's reagent system, there is a maximum absorbance peak at 550 nm. In a definite concentration range, the light absorption of Fenton's reagent system is positively related to the concentration of HO·. Thus, the light absorption OD value will reduce when the inhibitor 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 OD_c is the absorbance without samples and OD_u is the absorbance in the presence of the samples of the ferment products. Hydroxyl radical scavenging activity (%)=$[(OD_c-OD_u)/OD_c]\times100$ (1)

Table1. Factors and Levels to be optimized.

Factor	Code	Level				
		1	2	3	4	5
Water content（%）	X1	50	55	60	65	70
Bran content（%）	X2	5.0	5.5	6.0	6.5	7.0
pH value	X3	0	0.25	0.5	0.75	1
Glucose content（%）	X4	0	0.04	0.08	0.12	0.16
Temperature（℃）	X5	15	20	25	30	35

Table2. Test schemes of the fermentation.

Numerical	code				
	X1	X2	X3	X4	X5
N1	4	3	5	3	1
N2	3	5	3	5	1
N3	1	2	3	4	5
N4	1	4	4	4	2
N5	4	4	1	4	2
N6	2	5	2	2	4
N7	2	1	5	5	3
N8	2	2	2	2	1
N9	5	1	3	1	2
N10	5	3	2	5	5
N11	5	5	4	3	3
N12	3	1	1	3	4
N13	4	2	4	2	4
N14	1	3	1	1	3
N15	3	4	5	1	5

Assays for antioxidant activity of the microparticles

The chemical kits used for total antioxidant capacity (T-AOC) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The measurement was followed by the manufacture's instruction.

T-AOC was described as the following equation:

T-AOC (U/mg) = [(*As*-*Ao*) /0.01 / 30]×*Vt* / Ms. (2)

Where *As* is the absorbance of the sample, Ao is the absorbance without the sample, *Vt* (ml) is the total volume of the reaction mixture and Ms (mg) is the mass of the sample.

RESULTS AND DISCUSSION

Optimization of the solid fermentation conditions from Pleurotuseryngii

Pleurotuseryngii can vigorously grow on the solid medium, as shown in Figure 1.

Fig.1. *Pleurotuseryngii* fermented on a kelp waste medium.

Uniform design and DPS software were employed to optimize the yield of polysaccharides from *Pleurotuseryngii*. Then a regression equation prediction model was established see formula (3) bellow, whose correlation coefficent R=1 e the coefficient=0.99994 a residual path coefficient =0.00805 , p=0.0254≤ regression equation was significant with a good fitting degree.

$$\hat{y} = 1.582 + 0.635X1 - 0.584X3 - 0.628X4 - 0.007X1 * X1 - 0.015X3 * X3 - 0.572X4 * X4 - 0.008X5 * X5 + 0.016X1 * X3 + 0.017X1 * X4 + 0.007X1 * X5 - 0.038X2 * X3 + 0.161X3 * X4 - 0.005X3 * X5 \quad (3)$$

The selected optimum solid fermentation conditions for the growth rate are shown in table 3. According to the optimization of the growth rate for *Pleurotuseryngii*.optimum conditions of the fermentation were: water content 70%, the PH value 5.0, the bran content 1. 0%, and the glucose content 0.16%, the temperature of the solid fermentation condition was 25.9℃. The maximum predicated growth rate for *Pleurotuseryngii* was 4.78mm/day. The verification experiments were conducted completed under the optimum conditions, which gave a growth rate of 4.93 mm/d. The fitting degree of 97.0% indicated a close agreement of the values predicted by the models and the values in the verification experiments.

Optimization of the fermentation time for a yield of mushroom polysaccharides, antioxidant activity and hydroxyl radical scavenging activity

After fully fermentation, the relationship between the fermentation time and the yield of mushroom polysaccharides, the antioxidant activity and the hydroxyl radical scavenging activity were investigated respectively. The results are shown in tables 4 to 6.

As is in table 4, the antioxidant activity affected by the fermentation time reached the top value 20 days after the bottles had fully grown mushrooms. Table 5 shows the yield of polysaccharides reached the top on that day too.

Table 6 shows that 15 days after fully grown, the hydroxyl radical scavenging activity was highest as EC_{50} was the lowest.

Table3. Optimal fermenting conditions

Optimal parameters		Results	
Water content (W_C, %)	70	Predictive value (mm/day)	4.78
PH value (P_H,1)	5.0	Actual value (mm/day)	4.93
Bran content (B_C, %)	1.0	Fitting rate (%)	97.0
Glucose content (G_C, %)	0.16	Traditional growth rate(mm/day)	3.15
Temperature(T_F, ℃)	25.9	Multiple of the increase(%)	58.1

Table 4. Antioxidant activity affected by fermenting time.

Fermenting time	Antioxidant activity(U/mg)
Kelp waste medium	0.252
0 day after fully grown	0.263
5 day after fully grown	0.327
10 day after fully grown	0.339
20 day after fully grown	0.387

Table 5. Yield of polysaccharides affected by the fermentation time

Fermenting time	Yield of polysacc-harides(mg/g)
Kelp waste medium	59.49
0 day after fully grown	88.79
5 day after fully grown	100.64
10 day after fully grown	107.89
20 day after fully grown	123.83

Table 6. Hydroxyl radical scavenging activity affected by the fermentation time

Fermenting time	EC_{50}(U/mg)
Kelp waste medium	122
0 day after fully grown	30.55
5 day after fully grown	17.44
10 day after fully grown	19.28
15 day after fully grown	12.83
20 day after fully grown	14.35

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 2 to 4.

The hydroxyl radical scavenging activity of the obtained samples from *Pleurotuseryngii* incubation (T in figures 2 to 4), BHT (C1) and the kelp waste alone(C2) were control tested. The Data was presented as the mean of three independent experiments ± SD.

The formula is listed below as formula (3), where OD_c is the absorbance without samples and OD_u is the absorbance in the presence of the samples of the fermentation products.

$$\text{Scavenging effect} = (OD_c - OD_u)/ OD_c \qquad (3)$$

In accordance with figures 2 to 4, a dose-dependent increase of the hydroxyl radical scavenging activities of the microparticles is apparently exhibited. The hydroxyl radical scavenging activity of the microparticles suspension was less than BHT but more than the kelp waste medium without fermentation. But the hot water extracts (figure 3), especially the polysaccharides (figure 4) drawn from the fermentation products had much more hydroxyl radical scavenging activity than the BHT and kelp waste medium without fermentation.

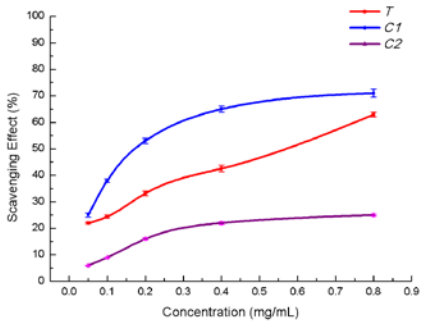

Fig.2. Hydroxyl radical scavenging capacities of the powder suspension (T) obtained from *Pleurotuseryngii*

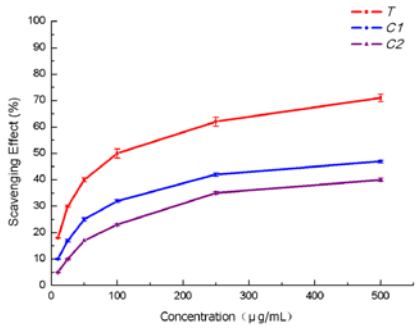

Fig.4. Hydroxyl radical scavenging capacities of the hot water extracts (T) obtained from *Pleurotuseryngii*

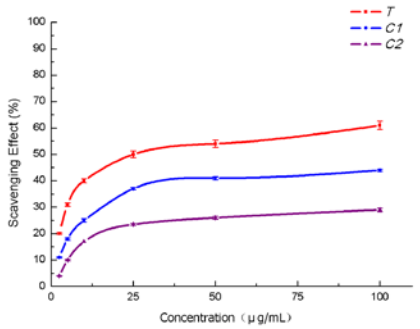

Fig.5. Hydroxyl radical scavenging capacities of the polysaccharides (T) obtained from *Pleurotuseryngii*

Compared with the data for hydroxyl radical scavenging, among the fermented powder suspension, the hot water extraction, the polysaccharides solution and BHT solutions (as positive control), *Pleurotuseryngii* solid ferment products have a high hydroxyl radical scavenging activity, which also increased with the higher concentration of polysaccharides which exist in both the suspension and extracts solution at different concentrations.

Antioxidant activity of the microparticles

As shown in table 7, the antioxidant activity of the mushroom sample, microparticles suspension obtained from *Pleurotuseryngii* fermentation was 0.141U/mg, which was slightly lower than that of the kelp waste without *Pleurotuseryngii* fermentation as control 2 and the BHT solution as control 1. The antioxidant activity for the hot water extracts obtained from fermentation products was 0.530U/mg, which was significantly higher than that of the kelp waste without *Pleurotuseryngii* fermentation and the BHT solution. The antioxidant activity for the polysaccharides obtained from fermentation was 1.433U/mg, significantly higher than that of the kelp waste without fermentation and the BHT solution, which means the hot water extracts and polysaccharides have more antioxidant activities.

Table 7. Antioxidant activity(U/mg)

Sample	Results
Powder suspension	0.141
Hot water extract	0.530
polysaccharides	1.433
BHT	0.218
Kelp waste medium	0.174

CONCLUISIONS

In this work, the *Pleurotuseryngii* can grow on the solid medium and produced polysaccharides. It was shown that the antioxidant activity and the hydroxyl radical scavenging capacity of the microparticles obtained from *Pleurotuseryngii* incubation were significantly higher than those from the kelp waste without fermentation. Likewise the assays showed that the antioxidant activity and hydroxyl radical scavenging capacity under the optimum conditions were significantly higher than those without optimization. A dose-dependent increase of the hydroxyl radical scavenging activities of the microparticles was apparently exhibited. The hydroxyl radical scavenging activity of the microparticles suspension was less than for BHT but more than for the kelp waste medium without fermentation. But the hot water extracts, especially the polysaccharides drawn from the fermentation products had a much higher hydroxyl radical scavenging activity than BHT and the kelp waste medium without fermentation. After optimization of the fermentation time, the optimum fermention time was chosen, which can affect the yield of polysaccharides, the antioxidant activity and hydroxyl radical scavenging activities. Thus, each component of the fermentation investigated of the antioxidant activity and hydroxyl radical scavenging activities obviously have encouraging results. It would be a beneficial pathway to recycle the kelp waste by fermentation of *Pleurotuseryngii* to produce potential natural antioxidants.

REFERENCES

1. B.Z.Zaidman, M. Yassin, J. Mahajna, S.P. Wasser *Appl. Microbiol. Biotechnol.*, **67**, 453(2005).
2. PY. M.ark, J.H.Won, Y.H. Kim, J.W.Choi, H.J.Park, K.Y. Lee, *Journal of Ethnopharmacol*., **101**, 120 (2005)
3. M. Shamtsyan, V. Konusova, Y. Maksimova, A. Goloshchev, A. Panchenko, A. Simbirtsev, N. Petrishchev, N. Denisova, *Journal of Biotechnol*., **113**, 77(2004)
4. Archived, May 18, 2006, at the Wayback Machine.
5. H. Nozaki, S. Itonori, M. Sugita, K. Nakamura, K. Ohba, A. Suzuki, Y. Kushi, *Biochemical and Biophysical Research Communications*, **373**(3), 435(2008)
6. N. Alam, K.N. Yoon,; J.S. Lee,; H.J. Cho,; M.J. Shim, T.S. Lee, *Saudi Journal of Biological Sciences*, **18**(4), 403 (2011).
7. B.S. Sanodiya, G.S. Thakur, R.K. Baghel, G.B. Prasad Bisen, *Current Pharmaceutical Biotechnology*, **10**(8), 17 (2009).
8. J.J. Zhang. R. Duan, K. Xu, Z.L. Li, *Journal of Fisher Science*, **29**, 10 (2010).
9. C.L. Hurd, P.J. Harrison, K. Bischof, S.C. Lobban, Seaweed Ecology and Physiology, Cambridge University Press, United Kingdom, 2014.

ОПТИМИЗАЦИЯ НА УСЛОВИЯТА ЗА ТВЪРДО-ФАЗНА ФЕРМЕНТАЦИЯ НА ГЪБИ ВЪВ ВРЪЗКА С ТЯХНАТА АНТИОКСИДАНТНА АКТИВНОСТ И ДОБИВА НА ПОЛИЗАХАРИДИ

Дж. Юан, П. Ян*, Д. Джън

Училище по социално и екологично инженерство, Технологичен институт в Харбин, Харбин 150001, Китай

Постъпила на 23 януари, 2016 г. ; коригирана на 6 август, 2016 г.

(Резюме)

Проведени са експерименти за установяване на оптималните условияза добиване на едливи гъби с висок добив на полизахариди и на антиоксиданти отстраняващи хидроксилните радикали. Резултатите показват, че могат да растат и да произвеждат обилно полизахариди с горепосочените свойства. Тези резултати дават възможност за мащабиране на процеса и за промишлено прилагане на тази твърдо-фазна ферментация с гъбите *Pleurotus eryngii* при оптимално време от 20 дни.