Abstract

In recent years, there has been an unprecedented increase in interest in more efficient utilization of agro-industrial residues because it provides an alternative way to reduce production costs and solve many environmental hazards. Soybean curd residue, a by-product of tofu, soymilk or soy protein manufacturing, is discharged as an agro-industrial waste. 0.7 million tons of SCR is disposed in Japan annually, and the most of SCR was incineration, which has caused severe environmental pollution.

In fact, SCR is rich in nutrients, such as proteins, fat, starch and sugar, which could allow SCR to be potentially utilized as a high quality media for the microbial fermentation.

Ganoderma lucidum (*G. lucidum*) is one of the most famous traditional Chinese medicines. Modern pharmaceutical research shows that *G. lucidum* polysaccharide has several physiological and health effects, including strong antioxidant activities, immuno-modulating activities, and anti-tumor activities.

Lentinus edodes (*L. edodes*), commonly known as the shiitake mushroom, is the second most widely used traditional Chinese medicinal mushroom in the global market. And *L. edodes* phenolic compound has been found to be an excellent antioxidant and synergist that is not mutagenic.

However, there were few reports about the production of *G. lucidum* polysaccharide and *L. edodes* phenolic compound using agricultural waste.

In this study, the optimal fermentation conditions of *G. lucidum* polysaccharide

and *L. edodes* phenolic compound using soybean curd residue as a substrate were investigated. Furthermore, the antioxidant activities and immunomodulatory activities of *G. lucidum* polysaccharide and *L. edodes* phenolic compound were assessed.

1. The effects of fermentation conditions on the production of polysaccharides from *G. lucidum* using soybean curd residue as a substrate were investigated. Based on the optimum conditions of solid-state fermentation, the fermented time, the inoculum size and the C/N ratio were optimized by response surface methodology. The optimal fermentation conditions for *G. lucidum* polysaccharide were determined to be the following: 14.53% of the inoculum size, 10.49 of the C/N ratio and 21.18 days incubation. The maximum polysaccharide yield of 48.14 ± 1.47 mg/g was obtained in the verification experiment.

2. The production of total polyphenol from *Lentinus edodes* using soybean curd residue were investigated. Based on the results of single-factor experiments, the inoculum size, the moisture content and the fermented time were optimized using central composite design in response surface methodology. As results, the optimal fermentation conditions of the total polyphenol production were determined as following: 12.13% of the inoculum size, 76.96% of the moisture content and 24 days incubation. Compared with unfermented soybean curd residue, the total polyphenol yield increased from 3.12 ± 0.02 to 22.93 ± 0.41 milligram gallic acid equivalent per gram, polysaccharide, proteins and various amino acid of the fermented SCR were increased significantly.

3. *G. lucidum* polysaccharide was extracted from fermented soybean curd residue

by ultrasonics assisted extraction. The optimal extraction conditions were 30 min, 80 ℃, 80 watt of the power with 10 of the water to solid ratio and *G. lucidum* polysaccharide of 115.47 ± 2.95 mg/g was obtained. Furthermore, the antioxidant and immunomodulatory activities of *G. lucidum* polysaccharide were investigated. The results showed that *G. lucidum* polysaccharide exhibited strong scavenging activities against DPPH radical, hydroxyl radical, hydrogen oxide, ABTS radical cation decolorization and reducing power, moderate ferrous chelating effect, and weak SOD-like activity. For immunomodulatory activities, *G. lucidum* polysaccharide was demonstrated to strongly stimulate the proliferation of the macrophage, the production of the nitric oxide, phagocytosis and protective effect on the macrophages from Doxorubicin (DOX) damage in a dose-dependent manner. *G. lucidum* polysaccharide seemed to play an important role in the exploration of natural antioxidants in food industry and pharmaceuticals.

4. *L. edodes* phenolic compounds (LEPC) were extracted from fermented soybean curd residue by ultrasonics assisted extraction. The optimal extraction conditions were 10 min, 40% ethanol concentration, 100 watt of the power with 30 of the ratio of water to solid and *L. edodes* phenolic compounds of 44.16 ± 2.35 mg GAE/g was obtained. *L. edodes* phenolic compounds (LEPC-I) were further purified by macroporous adsorption resins column SP825 (donated as LEPC-II). Antioxidant activities of LEPC-I and LEPC-II were investigated. The results showed that LEPC exhibited strong scavenging activities against DPPH radical, hydroxyl radical, hydrogen oxide, ABTS radical cation decolorization and reducing power, as well as

weak ferrous chelating effect and weak SOD-like activity. Furthermore antioxidant activities of LEPC-II were higher than those of LEPC-I. Therefore, LEPC-I and LEPC-II should be explored as the natural antioxidants in food industry and pharmaceuticals.

Briefly, reusing SCR for solid-state fermentation by *G. lucidum* and *L. edodes* was not only a simple and practicable method but also could reduce the effects on the environment. The fermented SCR was rich in nutritious substances and low in cost, resulting in excellent economic efficiency.