Hindawi
Journal of Chemistry
Volume 2023, Article ID 9462748, 8 pages
https://doi.org/10.1155/2023/9462748

Research Article

Effects of Solid-State Fermentation with the *Ganoderma* spp. and *Coriolus versicolor* on the Total Phenol Contents and Antioxidant Properties on Black Soybean

Yanting Li, Lina Xu, and Shang Guo

Shanxi Institute for Functional Food, Shanxi Agricultural University, Taiyuan 030031, China

Correspondence should be addressed to Shang Guo; 15364619285@163.com

Received 10 April 2023; Revised 11 July 2023; Accepted 23 August 2023; Published 14 September 2023

Academic Editor: Luca Conti

Aim of this study was to evaluate the influence of solid-state fermentation of 4 strains ((*Ganoderma oregonense* (GO), *Ganoderma lucidum* (GL), *Ganoderma sinensis* (GS), and *Coriolus versicolor* (CV))) on the nutrients, functional ingredients, and antioxidant activity in black soybean. As a result, after fermentation by 3 strains of *Ganoderma* spp. and CV, the contents of crude protein, crude fat, reducing sugar, soluble protein, triterpenoid, and total phenol contents in black soybean significantly increased ($P < 0.01$), particularly in CV-black soybean. The contents of crude protein, crude fat, reducing sugar, soluble protein, and total phenol were 122.92%, 132.69%, 290.08%, 122.58%, and 142.47% higher than those of the control groups, respectively. But the total sugar and crude fiber contents decreased significantly ($P < 0.01$). Furthermore, analysis of antioxidant capacity showed that all the fermented products had scavenging effects on DPPH, hydroxyl, and superoxide anion radicals. In addition, the DPPH scavenging ability of CV fermented products was highest, the hydroxyl radical scavenging ability of GS fermented products was highest, and the superoxide anion radical scavenging ability of GO fermented products was highest, which were 9.43, 7.65, and 3.39 folds higher than those of control groups, respectively. All results evidenced that the fermented strains and the fermented time had remarkable influences on the nutritional ingredient and antioxidant activity of fermented products in black soybean. Also, there was a significant correlation between total phenolic content and antioxidant activities. In conclusion, fermented black soybean with fungus has the potential to be developed into a functional food and nutraceutical material.

1. Introduction

Ganoderma lucidum is a well-known edible and medicinal mushroom in Japan as Reishi, in China as Lingzhi, and in Europe and the United States as Youngzhi. The potential of those medicinal mushrooms in treating several diseases has been exploited by traditional folk medicines for centuries. Modern biochemistry and pharmacology studies have shown that it contains many active components, such as nucleosides, furans, sterols, alkaloids, triterpenoids, and polysaccharide [1]. Polysaccharide produced by *G. lucidum* is a type of carcinostatic agent, which has antitumor and hypoglycemic activities [2]. In addition, the usage of *Ganoderma* products is without any toxicity, and there are no apparent side effects, they have no particular effects on a specific organ in the human body, and they can improve normalization of organ function [3]. Therefore, *Ganoderma*-based products have attracted a great deal of attention for years. At present, the *Ganoderma*-based products could be generally divided into three types: fruit bodies-based, mycelia-based, and spore powder-based products [4]. Among these, mycelia-based products are the most promising for industrial production. Compared with the artificial cultivation of the fruit bodies, the production of *Ganoderma* products by fermentation technology holds obvious advantages [5, 6].

Solid-state fermentation (SSF) is defined as a fermentation process using a nonsoluble material that acts both as a physical support and as a source of nutrients in the absence of free-flowing liquid [7]. Many reports have shown that SSF of higher fungi would enhance the nutrition and antioxidant properties of the cereal extracts [8, 9]. Black soybean acted as

a kind of ideal comprehensive nutritional food of legume starches and has become an indispensable part of people's daily life. The most valuable compounds found were isoflavones, anthocyanins, and phenols [10]. This grain would be a good resource to provide much protein for the human body. However, neither cooking nor popping resulted in an effective utilization in the nutrition of soybean [11]. In the present study, we chose soybean acting as substrate to produce *Ganoderma* mycelia-based production. The objective of our work was to assay the influences of SSF with three strains of *Ganoderma spp.* and *Coriolus versicolor* on the total phenolic contents and antioxidant properties of soybean. In particular, the relationship between the indicators tested and the fermentation time was analyzed. This study would lay a foundation for phenolic contents and antioxidant properties production by fermenting soybean with different fungi.

2. Materials and Methods

2.1. Microorganisms. All the strains (Table 1) were obtained from Shanxi Agricultural University, China, and cultured on potato agar dextrose (PDA) at 25°C.

2.2. Preparation of Medium. Black soybean in our paper was obtained from a local supermarket. The grains dried to a constant weight. The basal medium for SSF consisted of 200 g of black soybean, moistened with 200 mL of nutrient salt solution which contained the following per liter of distilled water (g): 1% KH_2PO_4, 0.5% $MgSO_4 \cdot 7H_2O$, 0.01% $CaCl_2 \cdot 2H_2O$, and 3% $CaCO_3$. The initial pH of the salt solution was adjusted to 6.5 with 1M NaOH (aqueous solution). The medium is packed in polypropylene bags (12 cm × 24 cm × 0.05 cm). The bags of medium were sealed with plastic rings and sterilized for 60 minutes at 121°C (the moisture content was about 44%).

2.3. Preparation of Solid Fermentation Products. After sterilization, each bag was surface inoculated with mycelia from PDA medium (three discs, 10 mm in diameter) and incubated at 27°C in the dark. An uninoculated bag served as control. The fermented grains were harvested at 0, 7, 14, 21, 28, and 35 days after the mycelia extended into the whole medium. Then, the samples were dried to a constant weight at 45°C and then ground to pass through a 2 mm sieve prior to determining the nutritional components and antioxidant properties.

2.4. Determination of Main Nutritional Components of Solid Fermentation Products

2.4.1. Crude Protein Content. The crude protein content was determined by the Kjeldahl method [12].

2.4.2. Soluble Protein Content. The water-soluble protein contents were determined by the Coomassie brilliant blue method [13].

Table 1: Strain number of *Ganoderma* spp. and *Coriolus versicolor*.

Number	Strain name
1	*Ganoderma oregonense* (GO)
2	*Ganoderma lucidum* (GL)
3	*Ganoderma sinensis* (GS)
4	*Coriolus versicolor* (CV)

2.4.3. Reducing Sugar Content. The reducing sugar contents were determined by the 3 and 5-2 nitro salicylic acid method (DNS method) [13].

2.4.4. Total Sugar Content. With glucose as the standard substance, the total sugar content was detected according to the method described by Chen et al. [13].

2.4.5. Crude Fiber Content. The content of crude fiber was calculated after the sample was defatted by ethanol, nitrate boiled, filtered, cleaned, suction filtrated, dried, and weighed [13].

2.4.6. Crude Fat. The Soxhlet extraction method was used to extract the samples with ethyl ether [13].

2.5. Determination of Total Phenol Content. The content of total phenol was determined by Folin–Ciocalteu colorimetry with gallic acid as a standard [7, 9]. Each extract (150 mg) was dissolved in a solution of 5 mL of 3% HCl in methanol/deionized water (60 : 40), and the resulting mixture (100 μL) was added to 2 mL of a 2% aqueous sodium carbonate solution. After 2 minutes, 100 μL of 50% Folin–Ciocalteu's reagent was added to the mixture. After 30 minutes of standing, absorbance was measured at 750 nm against a blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid.

2.6. Determination of Triterpene Contents. Ursolic acid was used as a standard; the content of triterpene was determined by spectrophotometry [14].

2.7. Determination of Antioxidant Activity

2.7.1. DPPH Radical Scavenging Assay. A dried, ground fermented product (2 g) was extracted by stirring with 100 mL of 80% ethanol, at 25°C for 24 h, and then filtered by an ultrafiltration membrane. *Extracted Three Times*. The combined ethanolic extracts acted as test samples. 2,6-Ditert-butyl 4-methyl phenol (BHT) was used as the standard. The test sample 1 mL (0.5 mg/mL) in ethanol (4 mL) was mixed with 1 mL of ethanolic solution containing DPPH radical. The mixture was shaken vigorously and left to stand for 30 min without light. Then, the antioxidant activity of the mixture prepared using the methods was described by Xu et al. [12], and its absorbance was monitored at 517 nm. The scavenging activity (R_1) was calculated according to the following equation:

$$\frac{R_1}{\%} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100, \quad (1)$$

where R_1 is the radical scavenging of peptides, A_0 is the optical density of the control sample, A_1 is the optical density of the extract, and A_2 is the blank control.

2.7.2. Hydroxyl Radical (·OH) Assay. ·OH was generated by a Fenton-type reaction. The reaction mixture included 20 μL of $FeCl_2$ (1 mmol/L), 30 μL of 1,10-phenanthroline (1 mmol/L), 800 μL of carbonic acid-buffered saline solution, 50 μL of H_2O_2 (0.2 mol/L), and 20 μL of the test sample (deionized water as control). The scavenging activity was obtained according to equation (1).

2.7.3. Superoxide Anion Radical Scavenging Ability. The superoxide anion radical scavenging ability of ethanolic extracts was determined using the nitro blue tetrazolium (NBT) method as described by Xu et al. [12]. The test sample 1 g was combined with 1.0 mL of sodium carbonate (50 mM), 0.4 mL of NBT (24 mM), and 0.2 mL of EDTA (0.1 mM). 0.4 mL of hydroxylamine hydrochloride was added to initiate the reaction, and then the reaction mixture was incubated at room temperature for 15 min. Ascorbic acid was used as the reference compound, and its absorbance was monitored at 560 nm. The scavenging activity was obtained according to equation (1).

2.8. Statistical Analysis. Data are expressed as the mean ± standard deviation. Statistical significance between the groups was determined by a paired t test and one-way ANOVA for repeated measures. The results of $P < 0.05$ were considered statistically significant. The data were assessed using SPSS (version 15.0, SPSS Inc., Chicago, Illinois).

3. Results

3.1. Phytochemical Composition of Fermented Products. Table 2 shows the phytochemical composition of fermented products varied with the fermentation fungi. The crude protein content in the fermented black soybean with GO, GL, CV, and GS strains demonstrated 19.57%, 10.26%, 22.93%, and 12.7% higher than that in unfermented grains, respectively. The soluble protein of fermented products increased 15.03%, 20.04%, 32.69%, and 5.31% than that in unfermented grains, respectively. The contents of the triterpenoid fermented product enhanced 38.98%, 37.9%, 37.63%, and 31.45% than control. The significant increased contents (101.53%, 122.52%, 290.02%, and 119.08%) were found in the reducing sugar production of fermented product. In addition, the total polyphenol contents in fermented products indicated significantly higher (16.69%, 29.07%, 42.47%, and 32.73%) than those in the control, respectively. Furthermore, the crude fat content of fermented products improved, which were up to 20.82%~22.9%, whereas the content of total sugar in fermented products was significantly lower than that in unfermented grains. The dietary fiber in unfermented black soybean was 31.7%; after fermentation with 4 strains, it reduced 27.76%, 40.73%, 23.38%, and 35.24%, respectively.

In conclusion, the black soybean was fermented for 35 days at 27°C with the strains of GO, GL, GS, and CV; the contents of crude protein, soluble protein, reducing sugar, crude fat, triterpene, and total polyphenol were significantly higher than those observed in unfermented grains (the control). Meanwhile, the contents of total sugar and dietary fiber were significantly lower than those of the control group ($P < 0.05$).

3.2. Antioxidant Property Analysis of Fermentation Products

3.2.1. DPPH Radical Scavenging Ability of Fermentation Products. As shown in Figure 1, after fermented by 4 strains (GO, GL, GS, and CV), it was a significant positive correlation between DPPH radical scavenging ability in fermented products and fermentation time, and the correlation coefficient was 0.915, 0.912, 0.983, and 0.946, respectively. After being fermented for 35 days, DPPH radical scavenging ability in black soybean reached the maximum, which was 51.02%, 40.04%, 25.05%, and 37.73%, respectively, and it was 9.43, 7.19, 4.12, and 6.72 folds higher than that of unfermented black beans (control). By reason of the foregoing, the improvement degree of DPPH radical scavenging ability in fermented black beans of four strains was GO > GL > GS > CV.

3.2.2. Superoxide Anion Radical Scavenging Ability of Fermentation Products. After being fermented by four strains (GO, GL, CV, and GS), a significant positive correlation ($P < 0.01$, $r = 0.931$, 0.928, 0.963, and 0.914) was observed between superoxide anion radical scavenging ability and fermented time in Figure 2. The superoxide anion radical scavenging ability of black soybean reached the maximum value (25.98% and 18.84%) after fermented by GO and CV strains for 35 days, which was 3.39 and 2.18 folds higher than that of unfermented black soybean (control), respectively. Meanwhile, after being fermented by GL and GS strains for 30 days, the superoxide anion radical scavenging ability in black soybean reached the maximum value (15.63% and 22.38%), which was 1.64 and 2.78 times higher than that of unfermented black beans (control), respectively. In contrast, the degree of improvement of superoxide anion radical scavenging ability by fermented black soybean of four strains was GO > GS > CV > GL.

3.2.3. Hydroxyl Radicals Scavenging Ability of Fermentation Products. Variation of the hydroxyl radical scavenging ability of fermentation of black soybean by 4 edible fungi is presented in Figure 3. It evidenced a significant positive correlation (0.917, 0.918, 0.794, and 0.870) between the hydroxyl radical scavenging ability of fermented products and fermented time. After being fermented by four strains for 35 days, hydroxyl radical scavenging ability of black

Table 2: Phytochemical composition of fermented product.

Phytochemical composition	Unfermented	GO	GL	GS	CV
Total protein (g/100 g)	36.94 ± 0.37^{cD}	44.17 ± 0.38^{aAB}	40.73 ± 0.51^{bC}	41.63 ± 0.3^{bBC}	45.41 ± 0.86^{aA}
Total sugar (g/100 g)	15.40 ± 0.44^{aA}	7.30 ± 0.04^{cC}	8.90 ± 0.41^{bBC}	9.90 ± 0.23^{bB}	7.21 ± 0.03^{cC}
Soluble protein (g/100 g)	11.38 ± 0.18^{cC}	13.09 ± 0.04^{bB}	13.66 ± 0.04^{bB}	11.98 ± 0.27^{cC}	15.10 ± 0.04^{aA}
Reducing sugar (g/100 g)	2.62 ± 0.03^{cC}	5.28 ± 0.09^{bB}	5.83 ± 0.09^{bB}	5.74 ± 0.38^{bB}	10.22 ± 0.66^{aA}
Crude fat (g/100 g)	18.69 ± 0.02^{cD}	22.28 ± 0.22^{aAB}	20.82 ± 0.04^{bC}	21.17 ± 0.52^{bBC}	22.90 ± 0.11^{aA}
Dietary fiber (g/100 g)	31.70 ± 0.15^{aA}	22.90 ± 0.19^{cB}	18.79 ± 0.11^{eD}	20.53 ± 0.35^{dC}	24.29 ± 0.31^{bB}
Triterpenoid (g/100 g)	3.72 ± 0.32^{bB}	5.17 ± 0.16^{aA}	5.13 ± 0.05^{aA}	4.89 ± 0.1^{aA}	5.12 ± 0.08^{aA}
Total polyphenol (mg/100 g)	232.23 ± 0.7^{eE}	278.75 ± 0.32^{dD}	299.74 ± 0.08^{cC}	308.24 ± 0.03^{aA}	330.85 ± 0.04^{bB}

Note. Results marked with the superscript lowercase letter within each group differ, $P < 0.05$; results marked with the superscript capital letter within each group differ significantly, $P < 0.01$.

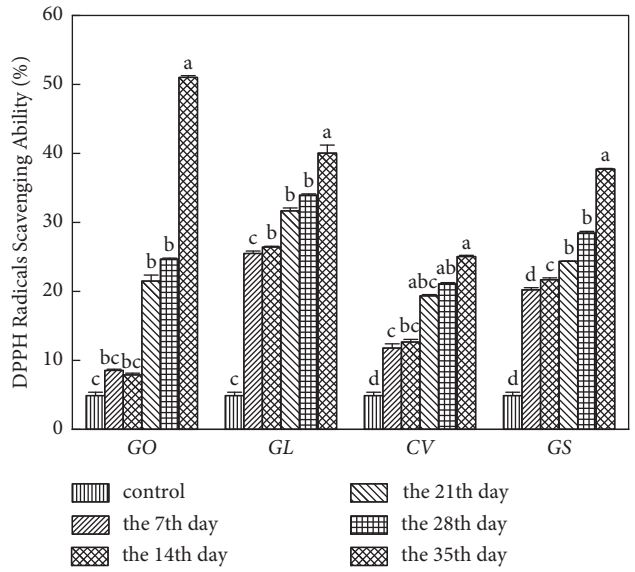

Figure 1: DPPH radical scavenging ability of fermentation products. Data were expressed as means ± SD ($n = 3$). Results marked with the superscript lowercase letter within each group differ, $P < 0.05$.

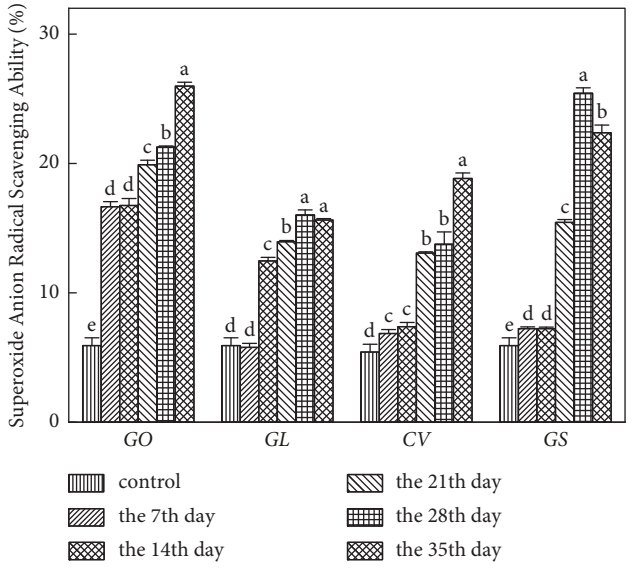

Figure 2: Superoxide anion radical scavenging ability of fermentation products.

soybean reached the maximum value (12.07%, 17.22%, 14.67%, and 36.31%), which was 1.87, 3.1, 2.49, and 7.65 folds higher than that showed in unfermented black beans (control), respectively. On the contrary, the improvement degree of black beans fermented by 4 strains on the hydroxyl radical scavenging ability was GS > GL > CV > GO.

3.3. The Correlation between the Total Phenolic Contents and Antioxidant Activity. Pearson's correlation coefficient can be used to measure the strength of a linear correlation between two continuous variables, and usually the absolute value of the correlation coefficient is less than 0.40. Moderate correlation was defined as the absolute value of the correlation coefficient between 0.40 and 0.69, and strong correlation is defined as an absolute correlation coefficient greater than 0.70. The correlation of total phenolic content, with DPPH, hydroxyl, and superoxide anion radical scavenging ability, was shown in Table 3. The correlation coefficient was higher ($r^2 = 0.878$ and 0.935, respectively. $P < 0.01$), with DPPH and superoxide anion fermented with GS than the others, while the correlation coefficient was highest ($r^2 = 0.987$, $P < 0.01$) between total phenolic content and hydroxyl radical scavenging ability fermented with GL. This means there was a significant correlation between total phenolic content and antioxidant activities. It could indicate the major contribution of phenolic compounds to the antioxidant activities of the extracts.

4. Discussions

After SSF on black soybean for 35 days by 3 strains of *Ganoderma* spp. (GO, GL, and GS) and CV, the contents of crude protein, crude fat, reducing sugar, soluble protein, triterpenoid, and total phenol significantly enhanced ($P < 0.01$); meanwhile, the total sugar and crude fiber content decreased significantly ($P < 0.01$). The ability of DPPH radical scavenging, superoxide anion radical scavenging, and hydroxyl radical scavenging of fermented products also increased significantly ($P < 0.01$), and there was significant positive correlation between the antioxidant capacity and fermentation time. Those results have been

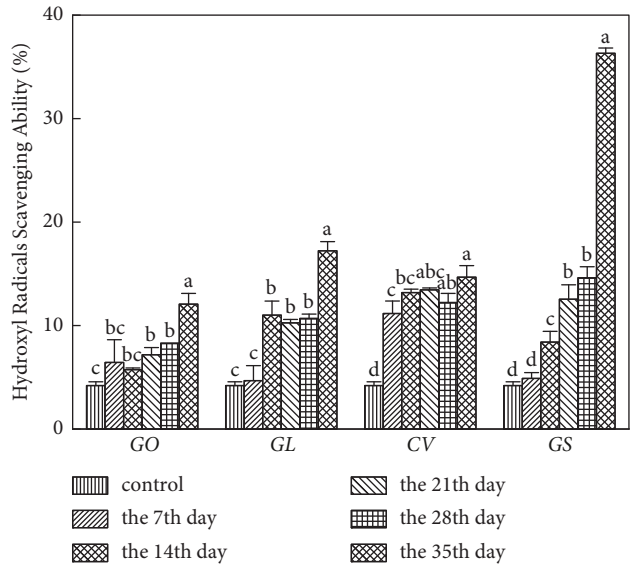

Figure 3: Hydroxyl radical scavenging ability of fermentation products.

Table 3: Correlation between total phenolic contents and antioxidant activity in the investigated extracts.

Items	DPPH radical scavenging ability	Superoxide anion radical scavenging ability	Hydroxyl radical scavenging ability
GO	0.771	0.738	0.745
GL	0.814	0.913	0.987
GS	0.878	0.935	0.596
CV	0.835	0.836	0.979

Note. $P < 0.01$.

found in the production of other substances, such as soybeans, buckwheat, and millet [12]. The phenomenon is in accordance with the exhibit on the higher fungus, *Agaricus* spp. [8, 9].

The total sugar and cellulose content of black soybean decreased significantly after fermentation by four strains, which were 35.7%–53.2% and 23.45%–40.7% lower than those of the control, respectively. Reducing sugar content increased significantly, 2–3.9 folds higher than that of unfermented black soybean. Those results have shown that the 4 strains in our study had the synthesis ability of reducing sugar. The strains could decompose macromolecular material, such as starch and cellulose, into small molecules (reducing sugar) of black beans through the amylase, cellulose enzyme in the growth of mycelium of the *Ganoderma* spp., and CV. Compared with 3 strains of *Ganoderma*, the total sugar content of fermentation products by CV decreased sharply (53.2%), while reducing sugar content increased obviously (390.1%); it showed that CV had the highest ability to break down macromolecule sugar into the reducing sugar. However, it was not easy to decompose the fiber of the black soybeans; therefore, crude fiber content was 23.4% lower than that of control after the fermentation. When cereals were fermented by edible fungi, the reducing sugar content in the fermentation products is 2–100 times than that of unfermented cereals, and the reducing sugar content in our study is 2–3.9 folds than that of the control; the phenomenon may be attributed to the different starch structures of black soybean and other cereals [8].

Black soybean is rich in protein. After fermentation, the protein content and soluble protein content of black bean increased significantly, which were 12.7%~22.93% and 5.31~32.69% higher than the control, respectively. CV could make the protein and soluble protein of black bean increase higher than other strains, which were 22.93% and 32.69%, respectively. Though the increasing rate was not the highest (compared with other cereal fermentation) [8], the protein content of fermented black beans could be up to 45.41%, soluble protein was 15.1% of the total weight, and soluble protein in the proportion of the total protein also increased from 30.81% to 33.25%. Some researchers reported that protein content of the fruit body of CV is 13.2% (wild versicolor) [15], the protein content of mycelium is 43.1% [16], and the protein content of the fruit body of *Ganoderma* spp. is 8.8%–17%, which were 27%–30% in mycelium [17]; it showed that the crude protein content of the fruit body of CV was higher than that in other strains of *Ganoderma*, so CV had a strong ability of protein synthesis in the solid-state fermentation process.

Black beans are rich in phytochemicals, such as anthocyanins, phenolic acids, flavanols, and saponins, which have good anti-inflammatory, antioxidation, and antitumor

activities and neuroprotective effects. Epidemiological and intervention studies have shown that there was positive correlation between regular consumption of soy products (including black beans) and the occurrence of multiple chronic diseases, such as Alzheimer's disease and diabetes mellitus [18–20]. The content of triterpenoids in black beans increased 31.45%–38.98% higher than control after fermentation, and there was no significant difference in the content of triterpenoids among the 3 strains (GO, GL, and CV) ($P < 0.01$), which were all above 5.1 g/100 g, but they were higher than that of GS. The content of total phenol after fermentation also increased significantly, and the fermentation effect of CV was the best, which increased 42.47% higher than the control, and the content reached to 330.85 mg/100 g. Our results indicated that the antioxidant properties of fermented black soybeans were stronger than that of the control. Furthermore, the total phenols of fermented cereals improved compared with the control. Mushrooms can produce a variety of secondary metabolites, such as polyphenols and polysaccharides, which have strong antioxidant activity [21, 22]. Generally, phenol compounds have strong antioxidant activity. The content of total phenols is one of the standards to evaluate the antioxidant activity of the edible and medicinal plants [23, 24]. So, the antioxidant properties of the samples should be in accordance with their total phenol contents. In our study, the total phenol contents were enhanced; meanwhile, the antioxidant activity increased after SSF. Those results showed that there was a positive correlation between polyphenol content and antioxidant activity during SSF in our study.

In this study, three methods were used to determine the antioxidant activity. The results showed that the scavenging ability of fermented black beans on DPPH•, O^{2-}•, and OH^{-}• was also significantly increased ($P < 0.01$), which was consistent with the results of Xu et al. [7], Chen et al. [8], and Kang et al. [9]. In our study, the DPPH scavenging ability of CV products was the highest, which was consistent with the result that the total phenol content of CV products was the highest. The ability of hydroxyl radical scavenging in GS products was the highest, and the superoxide anion radical scavenging ability of GO products was the highest during fermentation between 28 and 35 days. It showed that solid-state fermentation could improve the antioxidant performance of the substrate, and the activity changed with the fermentation time and strains.

The phenolic compounds act as antioxidants in plants [25]. Lee et al. [26] found that the contents of total phenols were moderately to highly associated with antioxidant properties. Theoretically, the antioxidant properties of the samples should be in accordance with their total phenols [9]. In our study, the correlations between antioxidant activities (measured by DPPH, hydroxyl, and superoxide anion assays) and total phenolics contents were analyzed by means of Pearson's correlation test. Results showed that the antioxidant activity has high correlation with the contents of total phenolics. Xu et al. [7] also suggested the highest correlation between total phenolic content and antioxidant activity, such as DPPH, hydroxyl, and superoxide anion. Our results agreed with those. Phenolic compounds are considered to be the most important antioxidants of plant materials. They constitute one of the major groups of compounds acting as a primary antioxidant or free radical terminators. The antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals [27]. In addition, they possess ideal structural properties for free radical scavenging properties [28]. On the contrary, different reports are found from the literature, whereby some authors suggested the phenolic compounds did not correlate with the antioxidant activity [8]. They believed that the type and quantity of phenolic compounds and the presence of nonphenolic antioxidants may also contribute to the antioxidant activity of the extracts. Nonphenolic antioxidants in fermented products with fungi, such as vitamin C, vitamin E, and beta-carotene might also be accountable in enhancing the antioxidant activity.

Soybean products play an important role in people's diets because they are high in protein, dietary fiber, and other micronutrients and low in fat. Due to their relatively low price and high protein quality, beans are an important plant protein substitute for animal protein in many developing countries [21]. Compared with other beans, black bean has the highest content of phenolic compounds and antioxidant activity [22], which has attracted extensive attention. In the fermentation process, microbes can use the protein and nonprotein nitrogen, glycogen of substrate compound, a large number of polysaccharides, and protein of edible fungus. At the same time, the exocrine, such as peptides, enzymes, proteins, and sugars, could increase the nutritional component contents in the fermented products. After fermentation,the lipid, protein, and carbohydrate retained, the vitamins increased, but the contents of cellulose and phytic acid decreased. Fermentation on grains could degrade nutrients into small molecules which could be easily eliminated by people, and the taste of the coarse grain was improved [23, 24].

At present, the solid fermentation technology of *Ganoderma* has been applied to the development of functional food in China, such as *Ganoderma lucidum* polysaccharide oral liquid [29], nutrition and health care fermentation tea [30, 31], *Ganoderma lucidum* sauce [32], and *Ganoderma lucidum* dietary fiber convenient food [33]. In those experiments, *Ganoderma spp.* and CV were used as the original strain, and black beans were used as the substrate for fermentation culture, and the nutrient composition and antioxidative activity of black beans were improved through the fermentation of *Ganoderma lucidum* mycelia. The fermentation products can be used as raw materials of functional food after drying and crushing.

5. Conclusion

This study was the first to report on the SSF of black soyabean by *Ganoderma* spp. and CV. The following conclusions can be drawn from this study:

(1) After solid-state fermentation by 3 strains of *Ganoderma* and CV, the contents of crude protein, crude fat, reducing sugar, soluble protein, triterpenoid, and total phenol in black beans increased significantly ($P < 0.01$),

while total sugar and crude fiber content decreased significantly ($P < 0.01$). (2) The fermentation effect of CV was the best; the contents of crude protein, crude fat, reducing sugar, soluble protein, and total phenol in black beans products all increased with highest rate except triterpenoid, which were 22.92%, 32.69%, 290.08%, 22.58%, and 42.47% higher than that of the control. (3) The fermentation time of black beans had remarkable influences on the antioxidant activity of fermented product. (4) Solid-state fermentation can improve the antioxidant capacity of black bean, the DPPH scavenging ability of CV fermentation products of black bean was the highest, the hydroxyl radical scavenging ability of GS fermentation products was the highest, and the superoxide anion radical scavenging ability of GO fermentation products was the highest, which was 9.43 times, 7.65 times, and 3.39 times higher than that of the control, respectively. (4) There was a significant correlation between total phenolic content and antioxidant activities.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The research was supported by the group project funds from the Shanxi Academy of Agricultural Sciences (YGC2019TD03), Key R&D Project of Shanxi Province (202102140601016), Special Project of Shanxi Forestry Key R&D Program (2023-Xu LN) and Scientific and Technological Achievements Transformation Guidance Project in Shanxi Province (202204021301014).

References

[1] Y. Liu, M. Y. Jin, P. W. Mao et al., "Statistical optimization of polysaccharides production by the lingzhi or reishi medicinal mushroom *ganoderma lucidum* (agaricomycetes) in solid-state fermentation using highland barley grains," *International Journal of Medicinal Mushrooms*, vol. 22, no. 11, pp. 1033–1041, 2020.

[2] X. W. Zhou, "Cultivation of *Ganoderma lucidum*: edible and medicinal mushrooms. Technology and application," *Hoboken*, vol. 1, pp. 385–413, 2017.

[3] C. Soccol, L. Y. Bissoqui, C. Rodrigues et al., "Pharmacological properties of biocompounds from spores of the lingzhi or reishi medicinal mushroom *Ganoderma lucidum* (Agaricomycetes): a review," *International Journal of Medicinal Mushrooms*, vol. 18, no. 9, pp. 757–767, 2016.

[4] X. Zhou, K. Q. Su, Y. M. Zhang, K. Q. Su, and Y. M. Zhang, "Applied modern biotechnology for cultivation of *Ganoderma* and development of their products," *Applied Microbiology and Biotechnology*, vol. 93, no. 3, pp. 941–963, 2012.

[5] C. Wang, S. C. Hsieh, H. J. Wang et al., "Concentration variation and molecular characteristics of soluble (1,3;1,6)-beta-D-glucans in submerged cultivation products of *Ganoderma lucidum* mycelium," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 3, pp. 634–64141, 2014.

[6] Z. Li, J. Zhou, Z. Lin, J. L. Zhou, and Z. H. Lin, "Development and innovation of *Ganoderma* industry and products in China," *Advances in Experimental Medicine & Biology*, vol. 1181, pp. 187–204, 2019.

[7] L. Xu, S. Guo, S. Zhang, S. Guo, and S. W. Zhang, "Effects of solid-state fermentation with three higher fungi on the total phenol contents and antioxidant properties of diverse cereal grains," *FEMS Microbiology Letters*, vol. 365, no. 16, pp. 1–8, 2018.

[8] Y. Chen, Y. Wang, J. Chen, H. Tang, C. Wang, and Z. Li, "Bioprocessing of soybeans (Glycine max L.) by solid-state fermentation with Eurotium cristatum YL-1 improves total phenolic content, isoflavone aglycones, and antioxidant activity," *RSC Advances*, vol. 10, no. 29, pp. 16928–16941, 2020.

[9] M. Kang, F. H. Zhai, X. X. Li, J. L. Cao, J. R. Han, and X. X. Li, "Total phenolic contents and antioxidant properties of buckwheat fermented by three strains of *Agaricus*," *Journal of Cereal Science*, vol. 73, pp. 138–142, 2017.

[10] N. Maneeploy, Y. Bundit, and H. Yuichi, "Improving the nutritional value and bioactivity of soybean meal in solid-state fermentation using bacillus strains newly isolated from the gut of the termite termes propinquus," *FEMS Microbiology Letters*, vol. 1, pp. 1–8, 2022.

[11] R. Shahzad, A. Shehzad, S. Bilal, I. J. Lee, and S. Bilal, "Bacillus amylolique faciens rwl-1 as a new potential strain for augmenting biochemical and nutritional composition of fermented soybean," *Molecules*, vol. 25, no. 10, pp. 23–46, 2020.

[12] L. Xu, S. Guo, Y. Li et al., "Ultrasound-assisted enzymatic extraction and bioactivity analysis of polypeptides from *Cordyceps militaris*," *Journal of Chemistry*, vol. 2023, no. 01, Article ID 1233867, 11 pages, 2023.

[13] J. H. Chen and J. Li, *Biochemistry experiment*, Science Press, Beijing, China, 5th edition, 2014.

[14] S. L. Jiang, S. M. Jiang, and L. C. Zeng, "Method for rapid quantification of total triterpenoids in *Ganoderma lucidum* by spectrometry," *Acta Agriculturae Universitatis Jiangxiensis*, vol. 04, pp. 634–636, 2006.

[15] X. Y. Chai, W. D. Zhu, and M. L. Chen, "The major nutrients of wild *Coriolus Versicolor* and the antibacterial," *Effect of its Polysaccharides Journal of Jining Normal University*, vol. 41, no. 06, pp. 10–15, 2019.

[16] J. Hu, Z. M. Lu, and G. H. Xu, "Nutritional analysis of *Coriolus versicolor* mycelia," *The Food Industry*, vol. 35, no. 11, pp. 268–270, 2014.

[17] X. Zhou, P. Hines, M. W. Borer, J. Lin, and L. Zhou, "Determine and analysis on primary nutritional components of *Ganoderma* spp," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 17, no. 2, pp. 219–225, 1998.

[18] R. Amarowicz, R. B. Pegg, and R. B. Pegg, "Legumes as a source of natural antioxidants," *European Journal of Lipid Science and Technology*, vol. 110, no. 10, pp. 865–878, 2008.

[19] R. Villegas, Y. T. Gao, G. Yang et al., "Legume and soy food intake and the incidence of type2 diabetes in the shanghai women's health study," *The American Journal of Clinical Nutrition*, vol. 87, no. 1, pp. 162–167, 2008.

[20] A. Fardet, E. Rock, C. Rémésy, and R. Christian, "Is the in vitro antioxidant potential of whole-grain cereals and cereal products well reflected in vivo?" *Journal of Cereal Science*, vol. 48, no. 2, pp. 258–276, 2008.

[21] S. Marathe, V. Rajalakshmi, S. N. Jamdar, A. Sharma, V. Rajalakshmi, and S. N. Jamdar, "Comparative study on antioxidant activity of different varieties of commonly

consumed legumes in India," *Food and Chemical Toxicology*, vol. 49, pp. 2005–2012, 2011.

[22] S. Furuta, M. Takahashi, Y. Takahata, Y. Nishiba, T. Oki, and M. Masuda, "Radical-scavenging activities of soybean cultivars with black seed coats," *Food Science and Technology Research*, vol. 9, no. 1, pp. 73–75, 2003.

[23] L. Xu, S. Guo, S. W. Zhang, S. Guo, and S. W. Zhang, "Effects of solid-state fermentation on the nutritional components and antioxidant properties from quinoa," *Emirates Journal of Food and Agriculture*, vol. 31, no. 1, pp. 39–45, 2019.

[24] Y. Xiao, C. He, Y. Chen et al., "UPLC–QQQ–MS/MS-based widely targeted metabolomic analysis reveals the effect of solid-state fermentation with *Eurotium cristatum* on the dynamic changes in the metabolite profile of dark tea," *Food Chemistry*, vol. 378, Article ID 131999, 2022.

[25] J. R. Han, C. An, and J. M. Yuan, "Solid-state fermentation of cornmeal with the basidiomycete *Ganoderma lucidum* for degrading starch and upgrading nutritional value," *Journal of Applied Microbiology*, vol. 99, no. 4, pp. 910–915, 2005.

[26] L. Lee, J. D. Park, C. H. Kim et al., "Contribution of flavonoids to the antioxidant properties of common and tartary buckwheat," *Journal of Cereal Science*, vol. 68, pp. 181–186, 2016.

[27] Z. Zin, A. A. Hamid, A. Osman, N. Saari, A. A. Hamid, and A. Osman, "Antioxidative activities of chromatographic fractions obtained from root, fruit, and leaf of Mengkudu (*Morinda citrifolia* L.)," *Food Chemistry*, vol. 94, no. 2, pp. 169–178, 2006.

[28] S. Kanatt, R. Chander, A. Sharma, R. Chander, and A. Sharma, "Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat," *Food Chemistry*, vol. 100, no. 2, pp. 451–458, 2007.

[29] W. G. Zhang, X. Liu, and Q. Cheng, "Research of nutritious and healthy *Ganoderma* polysaccharide oral liquid by solid fermentation," *Food Research and Development*, vol. 11, no. 09, pp. 94–96, 2006.

[30] Y. Q. Tang and J. Z. Xu, "Preparation of *Ganodema lucidum* and *Ginkgo biloba* solid fermented tea," *Edible Fungi of China*, vol. 13, no. 04, pp. 126–128, 2012.

[31] C. Zhang, M. Li, X. Chen, M. Li, C. Y. Xiong, and F. J. Yang, "Preliminary study on solid fermentation tea of wild poriacocos and *Ganoderma lucidum*in puer," *Ecotoxicology and Environmental Safety*, vol. 118, no. 04, pp. 126–132, 2015.

[32] R. Peng and H. G. Peng, "Study on the produce technics of *ganoderma soy*," *Journal of South China Normal University (Social Science Edition)*, vol. 06, pp. 1073–1076, 2004.

[33] L. Wang, N. Zhao, F. Zhang, W. Yue, and M. Z. Liang, "Development of *Ganodermalucidum*-fermented instant food with high-quality dietary fiber," *European Journal of Pharmacology*, vol. 616, no. 1-3, pp. 275–280, 2009.