

## Research Article

# Analysis of Nutritional Quality of Black Fungus Cultivated with Corn Stalks

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Black fungus is a homologous fungus of medicine and food. Its nutrient content determines the health value, and the accumulation of nutrients is easily affected by the substitute materials. The aim of this study was to analyze the effect of corn stalks as a cultivation substitution material for sawdust on macronutrients, micronutrients, and functional components of black fungus. The results reported that corn stalks could significantly increase the content of ash, protein, copper, and iron in black fungus, but reduce the content of zinc, magnesium, manganese, and colloidal substances; corn stalks had less effect on melanin and polyphenols; its effect on water, total sugar, reducing sugar, crude fiber, and total flavonoids was insignificant. Therefore, the study provided a theoretical basis for the cultivation of black fungus with corn stalks.

## 1. Introduction

Black fungus (*Auricularia auricula*) is one of the four most important cultivated edible fungi in the world [1]. It is rich in carbohydrates, amino acids, and trace elements. It also contains a large number of functional nutrients, such as polysaccharides, melanin, polyphenols, and flavonoids [2]. Black fungus has been consumed especially by the Chinese populations as a traditional Chinese medicinal mushroom since the 19<sup>th</sup> century for treating jaundice and sore throats. Therefore, black fungus, as a popular traditional Chinese medicine ingredient, is considered as one of the nutrient-rich and pharmacologically active edible fungi. Black fungus has been shown to possess immune-enhancing, anti-inflammatory, antiviral, anticoagulant, and antitumor properties [3–7].

Currently, the sawdust of broad-leaved trees is commonly used to cultivate black fungus in the forest area [8]. However, since most of the woods are banned for logging, the sawdust used for the cultivation of black fungus is obtained by crushing small-diameter wood powder. In addition, in recent years, the price of sawdust has been rising [9]. Therefore, searching for another alternative crop resource to cultivate black fungus has become an urgent issue to be solved by the industry [8].

Many nutritional and health benefits of black fungus are attributed to its own multinutrient composition [10]. However, there is currently no comprehensive understanding of the changes in nutrients in black fungus. Also, how a substitution material used for the cultivation of black fungus can affect the nutritional composition of the black fungus requires

a detailed investigation. Therefore, the aim of the study was to explore the effect of corn stalks as substitution material on the nutritional composition of black fungus including macro-nutrient, micronutrient, and functional components. The relationship between corn stalk and the nutritional composition of black fungus was further determined using correlation and principal component analyses.

## 2. Materials and Methods

**2.1. Materials and Reagents.** Reagents used were as follows: phenol (analytical grade), glacial acetic acid (analytical grade), perchloric acid (analytical grade), diatomaceous earth (analytical grade), fructose (analytical grade), hydrogen peroxide (analytical grade), methanol (chromatographic grade), minerals elemental standard salt solution, and hydrochloric acid (analytical grade).

The sample numbers and cultivation materials required for the test in Yichun Youhan, Heilongjiang Province, China, were as follows: sample no. 1 with sawdust, sample no. 2 with corn stalks 20% and sawdust 60%, sample no. 3 with corn stalks 40% and sawdust 40%, sample no. 4 with corn stalks 60% and sawdust 20%, and sample no. 5 with corn stalks 80%, and sawdust 20%.

**2.2. Instruments and Equipment.** Main laboratory apparatus used in the study were as follows: UV2550 Shimadzu UV spectrophotometer (Shimadzu Corporation, Japan); SPD-15C Shimadzu high-performance liquid chromatography (HPLC) (Shimadzu Corporation, Japan); 101-3A electric blast drying oven (Tianjin Tongli Xinda Instrument Factory, Tianjin, China); LD15-2A high-speed centrifuge (Beijing Medical Centrifuge Factory, Beijing, China); and HR2003 Philips shredder (Philips (Zhuhai) Co., Ltd., China).

The matrix formulations of the black fungus experimental samples were as follows:

- (1) Sawdust formula (as the control group): 80% broadleaf sawdust, wheat bran 17%, soy flour 2%, gypsum 0.7%, white ash 0.3%
- (2) Corn stalk 80% formula: 80% corn stalks, 17% wheat bran, 2% soybean powder, 0.7% gypsum, 0.3% white ash
- (3) Corn stalk 60% formula: 60% corn stalks, 20% broadleaf wood, 17% wheat bran, 2% soybean powder, 0.7% gypsum, 0.3% white ash
- (4) Corn stalk 40% formula: 40% corn stalks, 40% broadleaf sawdust, 17% wheat bran, 2% soybean powder, 0.7% gypsum, 0.3% white ash
- (5) Corn stalk 20% formula: 20% corn stalks, 60% broadleaf sawdust, 17% wheat bran, 2% soybean powder, 0.7% gypsum, 0.3% white ash

### 2.3. Experimental Methods

**2.3.1. Nutritional Composition Test Methods.** Protein content was determined according to the Kjeldahl method by the China National Food Standard GB 5009.5-2010. Crude fiber

content was determined according to the method of acid-base treatment by the China National Food Standard GB 5009.10-2003. Crude fat content was determined according to the Soxhlet extraction method by the China National Food Standard GB/T 15674-2009. Total sugar content was determined according to the China National Food Standard GB/T 15672-2009. Reducing sugar content was determined according to the direct titration by the China National Food Standard GB/T 5009.7-2008. Ash content was determined according to the high-temperature burning method by the China National Food Standard GB/12532-2008. Moisture content was determined according to the direct drying method the China National Food Standard GB 5009.3-2010.

**2.3.2. Determination of Micronutrient Test Methods.** Micronutrient content was determined using the inductively coupled plasma emission spectroscopy based on the method by the Agricultural Industry Standards of the People's Republic of China NY/T 1653-2008.

**2.3.3. Test Method for Measuring Functional Ingredients.** Polysaccharide content was determined according to the method by Shang et al. [11]. Melanin content was carried out in accordance with the method by Li et al. [12]. Total polyphenol content was performed according to the method by Zhou and Li [13]. Total flavonoid content was determined using the method by Li et al. [14]. Total gelatinous substance content was measured using the method by Yang et al. [15].

**2.3.4. Statistical Analysis.** Data processing and statistical analysis were conducted using SPSS 20.0 software. Statistical significance level of difference was determined at  $P < 0.05$ . Graphs were drawn using MS Excel software. All experiments were conducted in triplicate.

## 3. Results and Discussion

**3.1. Effect of Corn Stalk Addition on the Nutritional Composition of Black Fungus.** The nutritional composition of the black fungus was determined by a conventional nutrient analysis method. The results are shown in Table 1. With the increase of corn stalk addition, the changes of moisture, total sugar, reducing sugar, and crude fiber of black fungus were not significant. Compared with sawdust, the ash content of the black fungus cultivated with corn stalks was higher with a lower content of crude fat. When the amount of added corn stalks increased, the protein content of black fungus increased. Therefore, there are several advantages using the corn stalks as substituted material. For example, the use of corn stalks instead of sawdust can help to save wood resources. Also, they might be a promising base material because they are available at low cost and show limited adverse effects [16]. In addition, the yield of black fungus is also an important parameter because the black fungus cultivated with corn stalks in the study contained high protein and low fat, which was in line with the current concept of nutritious and healthy diet.

TABLE 1: Effect of different additions of corn stalks on the macronutrient components of the *Auricularia auricula*.

	Sawdust	Corn stalk 20%, sawdust 60%	Corn stalk 40%, sawdust 40%	Corn stalk 60%, sawdust 20%	Corn stalk 80%
Moisture (%)	13.91 ± 0.76	14.45 ± 0.80	14.22 ± 0.61	14.04 ± 0.98	14.23 ± 0.37
Ash (%)	3.55 ± 0.15	4.72 ± 0.88	3.67 ± 0.65	5.07 ± 0.34	4.36 ± 0.66
Total sugar (%)	59.90 ± 1.32	59.45 ± 1.91	59.60 ± 1.55	58.35 ± 1.93	59.22 ± 1.68
Total protein (%)	11.73 ± 0.52	11.91 ± 0.99	12.83 ± 0.36	13.19 ± 0.62	16.17 ± 0.80
Crude fat (%)	0.81 ± 0.02	0.69 ± 0.11	0.74 ± 0.32	0.59 ± 0.46	0.63 ± 0.22
Crude fiber (%)	5.63 ± 0.72	5.61 ± 0.69	4.55 ± 0.44	5.69 ± 0.81	5.28 ± 0.40
Reducing sugar (%)	0.76 ± 0.01	0.70 ± 0.03	0.62 ± 0.01	0.85 ± 0.04	0.75 ± 0.08

**3.2. Effect of Corn Stalk Addition on Micronutrients of Black Fungus.** The inductively coupled plasma emission spectroscopy method by the Agricultural Industry Standards of the People's Republic of China NY/T 1653-2008 was used to determine the micronutrients of black fungus. The results are shown in Table 2. Mineral elements are needed by the body tissues to maintain the normal body processes. They are also necessary for maintaining the body's acid-base balance and osmotic pressure. Calcium, iron, and zinc are essential elements of bone cell structure and function, promoting bone formation. In addition, magnesium is an activator of various enzymes in physiological and biochemical reactions, and magnesium also has the effect of regulating muscle excitability. Several studies have shown that the accumulation of mineral elements can be influenced by environmental factors and genetic factors of breeds [17, 18]. As shown in Table 2, corn stalks increased the content of copper and iron in black fungus and reduced the content of zinc, magnesium, and manganese compared with sawdust. Therefore, corn stalks could significantly affect the content of mineral elements in black fungus.

**3.3. Effect of Corn Stalk Addition on Functional Components of Black Fungus.** The functional nutrients of black fungus were measured for different addition amounts of corn stalks. The results are shown in Table 3. Compared with the sawdust, the different addition amounts of corn stalks had no significant effects on the crude polysaccharide content of black fungus, indicating that different addition amount of corn stalks had little effect on the accumulation of secondary metabolites of black fungus.

Compared with the black fungus cultivated with sawdust, corn stalks increased the total polyphenol content of black fungus, but with the increasing addition amount of corn stalks, the trend of total polyphenols was not obvious. This indicated that the different addition amounts of corn stalks had little effect on the total polyphenols of the secondary metabolites of black fungus. Also, when compared with the black fungus cultivated with sawdust, the addition of corn stalks increased the content of melanin in black fungus, but the change trend of melanin was not obvious with the increase in the addition amount of corn stalks. This indicated that the corn stalks had little effect on the melanin, a secondary metabolite of the black fungus.

Compared with the black fungus cultivated with sawdust, the addition of corn stalks had little effect on the flavonoid content of black fungus, indicating that the addition of corn

stalks did not increase the flavonoid content in black fungus. Overall, the content of flavonoids in black fungus cultivated with different addition amounts of corn stalks was relatively low. This might be because flavonoids accumulated mainly under stress conditions. The artificial management measures were suitable to grow the black fungus because the growth of black fungus was less affected by environmental stress, thereby resulting in a lower content of flavonoids.

Compared with the black fungus cultivated with sawdust, corn stalks increased the content of melanin in black fungus, but with the increasing addition amount of corn stalk, the trend of melanin change was not obvious. This indicated that the different addition amount of stalks added had little effect on the content of melanin in black fungus. Compared with the sawdust, the addition of corn stalks significantly reduced the content of colloidal substances in black fungus. As the addition amount of corn stalk added increased, the content of colloidal substances gradually decreased, indicating that the addition of corn stalks affected the accumulation of colloidal substances in black fungus to some extent.

**3.4. Effect of Corn Stalk Addition on the Nutritional Composition of Black Fungus.** Black fungus is rich in macronutrients, micronutrients, and functional ingredients. Although the macronutrient composition, micronutrient composition, and functional composition of black fungus can be affected by the addition of corn stalks, the relationship between the addition amount of corn stalk and the nutritional composition of black fungus was not obvious. Therefore, seven principal nutrients, seven micronutrients, and five functional components were analyzed by principal component analysis in order to find the limiting factors.

### 3.5. Effect of Corn Stalk on the Nutritional Composition of Black Fungus

**3.5.1. Principal Component Analysis (PCA) of Nutritional Composition of Black Fungus Cultivated with Corn Stalks.** PCA showed the effect of different addition amounts of corn stalks on the nutritional composition of black fungus. PC1 and PC2 explained the total variance of 47.49% and 26.34%, respectively (Figure 1). PCA clearly distinguished the black fungus cultivated with sawdust from those with different addition amounts of corn stalk. The black fungus cultivated with sawdust was positively correlated with PC1. Corn stalks 20%, corn stalks 40%, corn stalks 60%, and corn stalks 80% formulas were negatively correlated with PC1, indicating the

TABLE 2: Effect of different additions of corn stalks on the micronutrients of the *Auricularia auricula*.

	Sawdust	Corn stalk 20%, sawdust 60%	Corn stalk 40%, sawdust 40%	Corn stalk 60%, sawdust 20%	Corn stalk 80%
Copper (mg/kg)	1.44 ± 0.12	2.55 ± 0.23	2.59 ± 0.16	2.67 ± 0.07	3.54 ± 0.66
Zinc (mg/kg)	41.25 ± 1.03	27.38 ± 0.76	27.49 ± 0.98	28.89 ± 0.54	34.22 ± 1.46
Iron (mg/kg)	133.07 ± 5.42	119.79 ± 4.08	217.00 ± 6.11	325.74 ± 8.91	441.34 ± 9.11
Magnesium (mg/kg)	1918.71 ± 20.11	1359.71 ± 15.34	1445.45 ± 15.29	1383.45 ± 18.93	1235.37 ± 19.77
Manganese (mg/kg)	94.88 ± 2.88	27.07 ± 0.67	36.00 ± 1.32	34.47 ± 0.55	51.89 ± 1.53

TABLE 3: Effect of different additions of corn stalks on the functional components of the *Auricularia auricula*.

	Sawdust	Corn stalk 20%, sawdust 60%	Corn stalk 40%, sawdust 40%	Corn stalk 60%, sawdust 20%	Corn stalk 80%
Crude polysaccharide (g/100 g)	17.90 ± 0.90	18.00 ± 1.01	16.98 ± 0.78	18.68 ± 0.87	17.29 ± 1.11
Melanin (g/100 g)	3.56 ± 0.32	2.04 ± 0.22	3.19 ± 0.45	5.20 ± 0.64	6.40 ± 0.23
Total polyphenol (g/100 g)	13.94 ± 0.85	17.37 ± 1.07	18.23 ± 0.76	17.41 ± 1.13	16.42 ± 0.67
Total flavonoids (mg/100 g)	3.84 ± 0.11	2.82 ± 0.38	3.08 ± 0.45	2.90 ± 0.22	3.52 ± 0.30
Total gelatinous substance (g/100 g)	69.11 ± 2.76	59.34 ± 2.01	40.99 ± 1.93	33.14 ± 1.88	30.81 ± 1.35

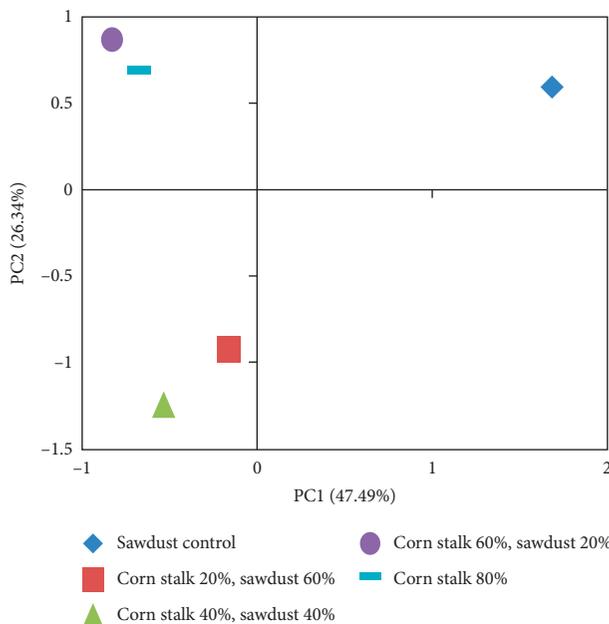


FIGURE 1: PCA of black fungus samples with different treatments under investigation of score plots.

presence of differences in nutritional composition in black fungus cultivated with corn stalks and sawdust. In PC2, with the increasing addition amount of corn stalks, the black fungus was more inclined to the positive direction of PC2, indicating that PC2 represented the difference in nutritional composition of black fungus cultivated by different addition amounts of corn stalks.

**3.5.2. Main Characteristic Components of Black Fungus Samples Cultivated with Different Addition Amounts of Corn Stalks.** Figure 2 shows the contribution of black fungus nutrients to two variables (PC1 and PC2) in PCA. The main variables positively related to PC1 were ash, moisture, and reducing sugar, while the main variables negatively related to

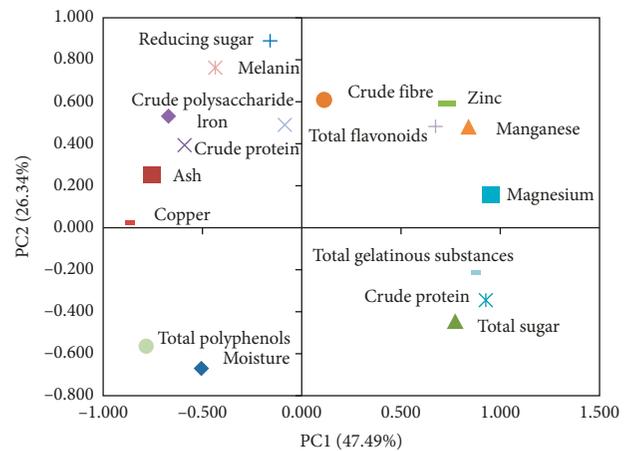


FIGURE 2: Loading plots representing the degree of contribution of each nutrition component to PC1 and PC2.

PC1 were gelatinous substances and flavonoids. The main variables significantly correlated with PC2 were all mineral elements such as copper, iron, zinc, manganese, some essential nutrients, ash, reducing sugar, some functional components, total flavonoids, and melanin, while the main variables negatively correlated with PC2 including gelatinous substances, total sugar, moisture, and total polyphenols.

#### 4. Conclusion

In this study, corn stalks were used as substitutes for sawdust to cultivate black fungus, and the effects of corn stalk addition on the nutrient composition of black fungus were investigated. The results showed that different addition amounts of corn stalks increased the content of ash, protein, copper, and iron in black fungus, but reduce the content of zinc, magnesium, manganese, and colloid substances. Different addition amounts of corn stalks had little effect on melanin and total polyphenols and no significant effect on water, total sugar, reducing sugar, crude fiber, and

flavonoids. Therefore, the cultivation of black fungus with corn stalks used as a substitute substrate could significantly affect the macronutrients, micronutrients, and functional components of black fungus, thereby providing a theoretical basis for the cultivation of black fungus with different substitute materials.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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