# Corncob as a Substrate for the Cultivation of Lentinula Edodes

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# Corncob as a Substrate for the Cultivation of *Lentinula edodes*

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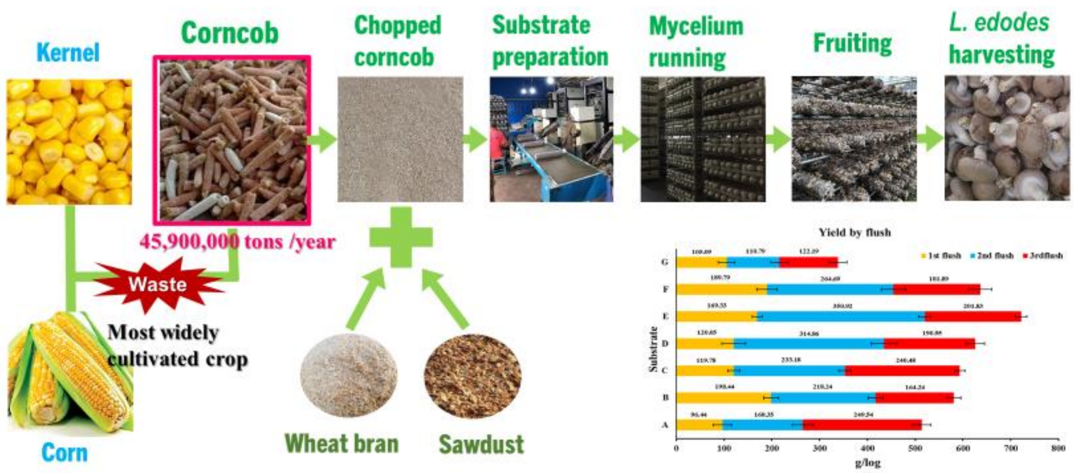
**Abstract** Corncob is a major agricultural waste in the world. The study aims to evaluate the effect of using corncob as a substrate for *Lentinula edodes* cultivation, so as to provide an economic and eco-friendly approach to transform waste biomass into high quality edible mushrooms (whose original substrate - wood - is becoming difficult to obtain these days). 6 formulas containing gradient proportions of corncob and a sawdust control were applied (100 cultivation bed-log per group), and substrate chemical profile were monitored. Effects of formula on mycelia growth, yield, biological efficiency, and agronomic traits and nutritional profile of mushrooms were investigated. The formula composing of 50% corncob, 28% sawdust, 20% wheat bran, and 2% gypsum showed the best performance with the fastest mycelia growth, better log browning, the highest yield (722.08 g/log) and summit biological efficiency (80.23%). The determined carbon/nitrogen in this substrate was 66.84. Yields in groups using 18 ~ 58% corncob were significantly improved comparing to the sawdust control, indicating a strong boosting effects of corncob. Agronomic traits of fruit bodies, such as pileus and stipe sizes, were not much affected by tested formulas. However, addition of corncob had an influence on nutritional composition of mushrooms, the polysaccharide content in mushrooms peaked (4.51g/100g) when the substrate contains 40% corncob. These results revealed an excellent potential of corncob when used as a main substrate ingredient for *L. edodes* cultivation.

**Keywords** *Lentinula edodes*; corncob; substrate formula; biological efficiency

Graphic Abstract



**Statement of Novelty**

Corncob is a major agriculture waste due to large corn consumption. This study provides an economic and ecofriendly approach to utilize corncob for *L.edodes* production, by which the corncob biomass can be effectively transformed into high quality edible mushrooms. Using corncob to cultivate *Lentinula edodes* also helps ease the increasing difficulty in obtaining its original substrate wood, whose supply is dwindling. This study reveals the feasibility of using corncob as main substrate to produce *L.edodes*, and provides substrate formulas with yield, biological efficiency and nutritional advantages. Corncob modifies chemical profile of substrates, boosts mycelial growth, and improves fungal polysaccharide. This work would probably be well adopted by plant factories and farmers.

## 1Introduction

*Lentinula edodes* (Berk.) Singer (Shiitake in Japanese or Xiang-gu in Chinese) is a nutritious edible and medical mushroom. They provide a nutritionally significant content of protein, indispensable amino acids, dietary fibers and vitamins (B1, B2, B12, C, D, and E), and are also reported to present medicinal molecules such as polysaccharides, terpenoids, sterols and lipids, that participate actively in modulating several human disorders and boosting the immune system [1]. As a world's leading cultivated mushroom, *L. edodes* has an annual output over 7 billion kg, contributing 22% of the world's mushroom supply [2]. China has a history of artificial cultivation of Xiang-gu as early as 800 years ago, and is now the major producer of this species, many communities in China benefited from economic profits of *L. edodes* production and lifted themselves from poverty.

In early days, *L. edodes* was usually grown on natural logs of the shii tree (*Castanopsis cuspidate*). Nevertheless, the sawdust-based substrate invented in late 1960s in China - a breakthrough technique in *L. edodes* cultivation - largely increased the production efficiency by shortening crop cycle and improving nutrient utilization, and it is now upgraded and widely adopted by growers in all producing countries. Currently, most shiitake in the United States are cultivated on nutrient-supplemented sawdust substrate, using a 16 - 20 day spawn run and then browning outside or inside the bag [2]. Approximately 90% of the shiitake production in Japan is using blocks made from solidified woodchips [3]. In China, a typical substrate formula for *L. edodes* cultivation composes of 78% sawdust, 20% wheat bran and 2% gypsum, which highly relies on wood supply [4]. However, the new logging bans since 2015 and the forest resource control regulation implemented in 2018 , as preventive measures against deforestation and related environmental impacts, have resulted in dwindling supply of wood products and soaring of wood prices, it is therefore necessary to explore alternative substrates with ecological and economic advantages.

*L. edodes* is reported to grown on several agricultural and forest products and byproducts, such as ground wheat straw [5] and hazelnut husk [6], enabling biotransformation of wastes. As compared with reference oak sawdust (52%) formula, yields were 11% higher when 8% wheat straw (44% sawdust in the formula) was applied in the substrate and 19% higher when substitute proportion increasing to 16% (32% sawdust in the formula), while mushroom sizes were not affected by formula change, predicting ground wheat straw's promising potential for *L. edodes* cultivation [5]. Mycelial growth measurements revealed adding cereal brans to eucalyptus residues supported fast growth of *L. edodes*, formulations with soya bran had better performance than rice or wheat brans, suggesting mycelium running is related to the bioavailability of nitrogen [7]. Hazelnut husk was reported as a competent new basal ingredient in substrate for *L. edodes* cultivation, the highest yield (202.96 g/kg substrate) was achieved when using 75% hazelnut husk:15 beech wood-chip:10 wheat bran formula, which was not significant different from the control group (60 beech wood-chip:20 wheat straw:20 wheat bran), though the biological efficiency (BE) was lower [6].

Corn is a major crop in the world. As the most widely produced feed grain in the U.S., it accounts for more than 95% of total production and use (USDA, https://www.ers.usda.gov/topics/crops/corn-and-other-feedgrains/). Corncob is a major byproduct from corn industry, being easy to collect from the field. China is a world's major corn producer that produces 45,900,000 tons of corncobs after corn harvesting, among which 8,000,000 are utilized as forage or burned as fuel, leaving a huge research and development space for other effective applications. Corncobs contain 32% - 36% cellulose, 35% - 40% hemicellulose, 17% - 20% lignin and 1.2% - 1.7%, with water holding capacity and solidity showing a potential for fungi cultivation [8]. Previously, *Pleurotus eryngii* (oyster mushroom) was reported to grown on a substrate containing 70 % corncob and other ingredients including cotton hull, bran, corn flour, lime, gypsum, and potassium dihydrogen phosphate, the optimal biological efficiency reached 60.5 %, and mushroom products contained 1.56% polysaccharides [9]. A comparative study investigated *Agaricus brunnescens* cultivated using corncob substrate or rice straw substrate, and found that mushrooms harvested from corncob substrate showed higher content of protein, while those harvested from rice straw substrate showed higher content of fiber and free amino acids; further nutritional analysis showed amino acid composition of *A.brunnescens* cultivated on corncob substrate were more close to the WHO/FAO recommended reference pattern and got higher amino acid score, indicating better nutritional value [10]. In this study, corncobs were assessed as a substrate ingredient for *L. edodes* cultivation at various proportions substituting sawdust, the effects of different formulas on production efficiency and agronomic traits were investigated, and nutritional quality of mushrooms were determined, hence to provide a useful alternative approach to grow *L. edodes* and enhance biotransformation of agriculture wastes.

## 2 Materials and Methods

### 2.1 Isolate and spawn

Isolate of *L. edodes* (Shenxiang215，strain number CCMJ2806) was provided by the National Engineering Research Center of Edible Fungi, Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences. The isolate was maintained on potato-dextrose agar (PDA) at 23 oC in the dark before use. Then spawn was prepared by cultivating isolates on substrates composing of 78% oak wood sawdust, 20% wheat bran and 2% gypsum at 23 oC in the dark, running for 25 days to allow substrates get fully colonized by the selected strain.

### 2.2 Substrates and preparation

Substrates containing different ratios of corncobs were prepared using formulas in Table 1. The formulas were designed referring to the traditional formula (A) and substituting corncob for sawdust at gradient ratios (B ~ G). Corncobs (dried and chopped to particle size of 0.5 ~ 0.8 cm) were supplied by corn growers from Xiayi county, Henan province, China. Sawdust, wheat bran and gypsum were prepared as described in our previous publication [4]. Ingredients were mixed thoroughly, after which tap water was added until moister content reaching 55%. Homogeneous substrate mixtures were prepared after fully stirred.

Table 1 Substrate formula

| Formula | Sawdust % w/w | Corncob % w/w | Wheat bran % w/w | Gypsum % w/w |
|---|---|---|---|---|
| A | 78 | 0 | 20 | 2 |
| B | 58 | 20 | 20 | 2 |
| C | 48 | 30 | 20 | 2 |
| D | 38 | 40 | 20 | 2 |
| E | 28 | 50 | 20 | 2 |
| F | 18 | 60 | 20 | 2 |
| G | 0 | 78 | 20 | 2 |

### 2.3 Cultivation and fruiting

The well-mixed substrates were put into mushroom bags (17 cm × 55 cm × 0.05 cm), 2300 ~ 2400 g per bag, to make artificial bed-logs. Then bed-logs were sterilized in an autoclave for 16 hours at 100 ℃. After cooled down to below 28 ℃, the sterilized substrates were inoculated with 40 ± 1 g prepared spawn. The inoculated bed-logs were maintained in a ventilated and darkened spawn running room at 23 ℃, 60 - 70% relative humidity (Rh) until mycelium running accomplished (approximately 35 ~ 40 days). In order to reduce impact of environmental differences, bed-logs of each group were randomly distributed and set on the shelves in different areas of the cultivation room. Bed-logs were pricked to allow inflation of oxygen as we reported before [11]. Mycelium then gradually matured, forming tumor-shape nodules. After browning of bed-logs and forming primordia, the environmental conditions inside growing room were controlled as 18 ± 2 ℃, 85% - 90% Rh to encourage fruiting. Fresh *L. edodes* fruit bodies with pilei not fully unfolded were picked. The harvesting period lasted up to 120 days, a total of 3 flushes of mushrooms were acquired. 100 bed-logs were applied for each substrate group (a total of 700 bed-logs).

### 2.4 Chemical analysis of substrates

Before and after sterilization, pH of substrates was determined by a handheld pH meter (Horiba, Fukuoka, Japan). Homogenized substrates (100g for each formula) were dried in an oven at 50 ℃ until reaching a constant weight. Total carbon content was determined using a commercial test kit (Comin biotechnology, Suzhou, China) based on ferrous sulfate reaction and spectrophotometry, all procedures were conducted following manufacturer's instruction. Total nitrogen content was determined using the Kjeldahl method (Kjeltec™ 8000, Foss, Hilleroed, Denmark). Determination of lignin, cellulose, hemicellulose was performed according to the manuals of commercial test kits (Comin biotechnology, Suzhou, China). Experiments were performed in triplicates.

### 2.5 Assessment of mycelial growth and yield

Mycelia growth rate was determined at 23 ℃ in glass tubes using the method modified from Zou et al. [12], substrates were equally distributed to each tube by weight and pressed to the same height, mycelia growth rate ( mm / d) was calculated as the average growth rate between the day once mycelia began to grow (Day 1) and 6 days after day 1 (Day 7). Data were measured in pentaplicates.

Yields were calculated as the sum of the weight of 3 flushes of fresh mushrooms, weighing using an electronic balance with an accuracy of 0.01 g. BE (%) was calculated as the ratio of grams of fresh mushroom that harvested per gram of dry substrate [5]. All 100 bed-logs of each substrate group were investigated for yield and BE.

### 2.6 Evaluation of fruit body quality

Fruit bodies harvested in the second flush were applied for morphological and chemical tests. 60 Fruit bodies from 20 bed-logs were randomly selected (excluding deformities), and their pileus thickness, pileus diameter, stipe length and stipe diameter were measured using a vernier caliper. 500 g of fruit bodies (randomly selected) were dried in an oven at 50 ℃ until reaching a constant weight. Samples were grinded and kept at 4 oC for later use. Ash, protein, and fat were determined according to national standard methods issued by National Health Commission of P. R. China: Standard GB 5009.4 - 2016 for ash analysis, Standard GB5009.5 - 2016 for protein analysis, and Standard GB 5009.6 - 2016 for fat analysis. Polysaccharides were determined as outlined by Haltiwanger [13]. Calcium content, potassium content, and sodium content were determined using commercial detection kits (Comin biotechnology, Suzhou, China) and flame spectrophotometry, all procedures were performed following the manufacturer's manual. Phosphorus content was determined adopting a commercial detection kit (Comin biotechnology, Suzhou, China) and spectrophotometry, according to a protocol provided by manufacturer. Tests were performed in triplicates.

### 2.7 Statistical Analysis

Data were expressed as mean ± S.D.. The original data were processed using Microsoft Excel (Microsoft Inc., Redmond, WA, USA). Differences among the means of groups were analyzed by analysis of variance (ANOVA) invoking SPSS 17.0 (IBM Inc., Armonk, NY, USA). Duncan's multiple range tests was performed at 95% confidence level ($p<0.05$).

## 3. Results and Discussion

### 3.1 Chemical profile of substrates

Before sterilization, substituting corncob for sawdust significantly increased pH of the substrate, reaching a peak at 8.23 when sawdust was totally replaced by corncob, however no significant difference was observed in pH after sterilization (6.27 ~ 6.67), suggesting an excellent buffer effect of the substrates (Table 2). Total carbon contents of substrates were 46.12% ~ 49.58%, total nitrogen contents were 0.61% ~ 0.77%, increasing of corncob significantly reduced substrate's carbon level while elevating nitrogen level even more steeply, leading to a notable decline in C/N (Table 2). Biopolymers in woody biomass such as lignin and cellulose are essential aliment for fungi growth, *L. edodes* is known for efficient capacity of degradation of lignocellulosic materials in nature [14]. Thus, we determined lignin, cellulose and hemicellulose in substrates, showing that lignin content was not significantly varied with formula, however significant decrease in cellulose and increment in hemicellulose were found when changing sawdust to corncob (Fig.1).

Table 2 Chemical profile of substrates

| Substrate | pH before sterilization | pH after sterilization | Total carbon (%) | Total nitrogen (%) | C/N |
|---|---|---|---|---|---|
| A | 7.27±0.25$^{a}$ | 6.57±0.15$^{a}$ | 49.58±0.45$^{b}$ | 0.61±0.01$^{a}$ | 81.28 |
| B | 7.60±0.15$^{b}$ | 6.57±0.06$^{a}$ | 48.95±0.78$^{ab}$ | 0.63±0.01$^{b}$ | 77.62 |
| C | 7.77±0.06$^{b}$ | 6.27±0.06$^{a}$ | 48.76±0.88$^{ab}$ | 0.68±0.01$^{c}$ | 71.71 |
| D | 7.60±0.15$^{b}$ | 6.60±0.12$^{a}$ | 46.77±0.77$^{a}$ | 0.69±0.00$^{cd}$ | 67.78 |
| E | 7.80±0.17$^{b}$ | 6.60±0.11$^{a}$ | 46.79±0.94$^{a}$ | 0.70±0.01$^{d}$ | 66.84 |
| F | 7.80±0.12$^{b}$ | 6.47±0.15$^{a}$ | 46.50±0.74$^{a}$ | 0.71±0.01$^{e}$ | 65.49 |
| G | 8.23±0.21$^{c}$ | 6.67±0.12$^{a}$ | 46.50±0.25$^{a}$ | 0.77±0.00$^{f}$ | 60.39 |

Data are expressed as mean ± S.D.. Values with no letters in common are significantly different ($p < 0.05$).

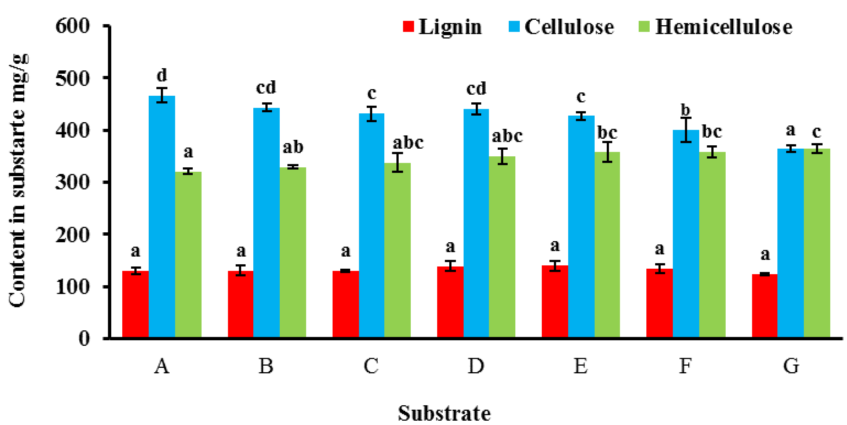



Fig.1 Lignin, cellulose and hemicellulose contents of different substrates. Values with no letters in common are significantly different ($p < 0.05$).

### 3.2 Effect of substrate formula on mycelia growth, log browning and mushroom yield

Different chemical profile of formula resulted in significant variance of mycelial growth rate (Table 3). Among all tested groups, the fastest growth rate was found when adopting formula E, reaching 2.96±0.13

mm/d, which was 30.97% higher than the control (A)， indicating supplementation of corn cob at an appropriate proportion effectively boosted mycelia growth.

Browning, a critical developmental process that contributes to the quantity and quality of *L. edodes* production, largely affects resistance potential against disease and insect, yield, and number of fruiting flushes [15]. Our study suggested that partly substituting corncob for sawdust promoted log browning. However, the optimal browning effect was not obtained when only corncob existing in substrate, this may relate to influence of corncob on substrate's physical properties, such as air permeability and water holding capacity.

Yield is the crucial factor determining the profit of plant industry. 3 flushes of mushrooms were collected, whose total yield and biological efficiency both peaked when applying formula E (Table 3). In group E, 722.08 g/log of mushroom were harvested, which increased by 40.39% as compared with the control, showing an excellent boosting effect. Besides, other substrates supplemented with corncob (B, C, D, F) also exposed various increase in yield, suggesting that partial substitution with corncob probably improved the C/N of substrate and made it more suitable for *L. edodes* growth. Most of the corncob substrates had maximum mushroom harvesting in the second flush, while sawdust control substrate showed a growing yield per flush (Fig.2). Due to current needs for intensive production, 2 ~ 3 flushes of mushrooms are usually collected when grown in *L. edodes* factories, in order to avoid high time and labor cost caused by log recuperation and water supplementation. However, even corncob was well decomposed and utilized in the harvesting period as discussed in this study ( the first to the third flush), there is also a possibility that yield decline may occur in the later harvesting period, which may affect individual growers who sometimes harvest 5 flushes of mushrooms.

Table 3 Effect of substrate formula on mycelia growth, log browning and mushroom yield

| Substrate | Mycelia growth （mm/d） | Browning | Yield （g/log） | Biological efficiency （%） |
|---|---|---|---|---|
| A | 2.26±0.05$^{a}$ | *** | 514.33±26.87$^{b}$ | 57.15±2.99$^{b}$ |
| B | 2.31±0.05$^{a}$ | **** | 580.92±17.09$^{c}$ | 64.55±1.90$^{c}$ |
| C | 2.71±0.07$^{b}$ | **** | 593.44±15.01$^{cd}$ | 65.94±1.067$^{cd}$ |
| D | 2.78±0.02$^{b}$ | **** | 625.86±27.21$^{cd}$ | 69.54±3.02$^{cd}$ |
| E | 2.96±0.13$^{c}$ | ***** | 722.08±11.02$^{e}$ | 80.23±1.22$^{e}$ |
| F | 2.50±0.06$^{a}$ | **** | 636.37±26.46$^{d}$ | 70.71±2.94$^{d}$ |
| G | 2.45±0.03$^{a}$ | ** | 338.07±19.73$^{a}$ | 37.56±2.72$^{a}$ |

Data are expressed as mean ± S.D.. Values with no letters in common are significantly different ($p < 0.05$).

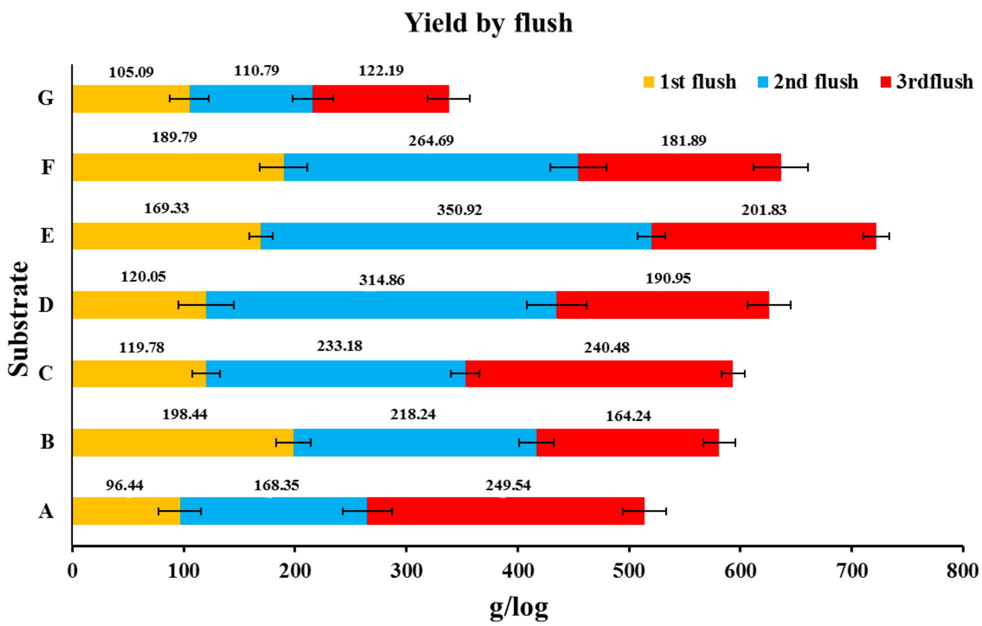
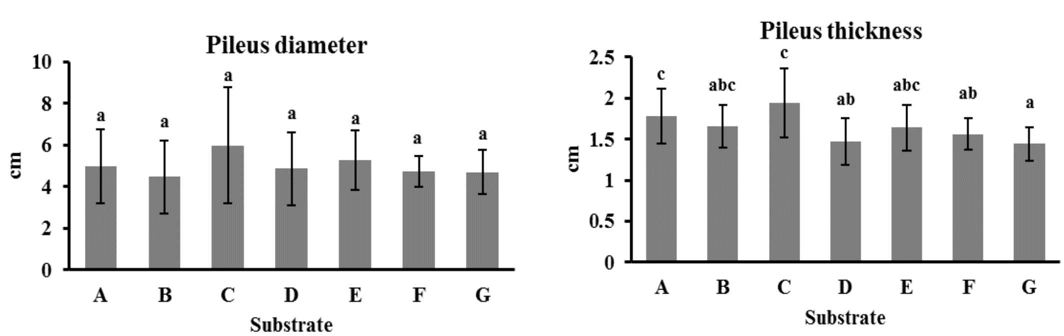


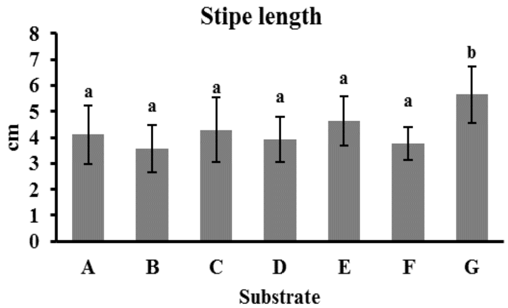

Fig.2 Mushroom yield of different substrates in the first, second and third flush.

### 3.3 Agronomic traits of mushrooms

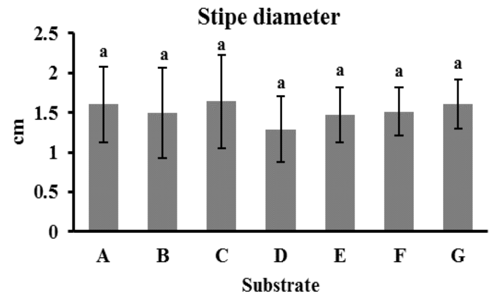
Agronomic traits of mushrooms hugely affect their commercial value. The results showed using corncob did not affect diameters of mushroom pileus or stipe (Fig.3). Average stipe length was not significantly varied either, except that when using corncob only in substrate G, remarkably long stipes were observed. The thickness of pileus was most affected by the formula among agronomic traits, while thickest pileus and thinnest pileus were observed in group A and G, respectively. Previous studies showed that the formula sometimes affected the morphology of fruit bodies [12,16], however in our study, application of corncob did not notably affect the shape of fruit body, this may attribute to that our bud management during fruiting was conducted strictly referring to protocols of scale production, and fruit bodies on each log were controlled.




A B




C




D

Fig.3 Effect of substrate formula on agronomic traits of mushrooms, including pileus diameter (A), pileus thickness (B), stipe length (C), and stipe diameter (D). Values with no letters in common are significantly different ($p < 0.05$).

### 3.4 Nutritional profile of fruit bodies

Edible fungi are excellent source of polysaccharide and protein, and they do not contain much fat. It was found that different formulas in this study significantly affected contents of ash, polysaccharide, protein, and fat in mushrooms (Fig. 4). The ash content in harvested mushrooms ranged from 6.19 ~ 6.94 g/100g, increasing with the concentration of corncob in the formula, and group G had 12.12% more ash than control. The mushrooms also contained 3.56 ~ 4.51g/100g polysaccharides, and substrate D produced mushrooms with highest polysaccharide level, which was 26.69% more than control. Protein content in mushrooms (186.45 ~ 221.36 g/kg ) decreased with increasing of corncob, and protein content in mushrooms produced by substrate G was 18.72% lower than control. The fat content in mushrooms (1.57 ~ 1.89 g/100g) also differed with formula, mushrooms cultivated by substrate G showed lowest fat level while mushrooms produced using formula E or F showed highest fat levels. As for minerals, phosphorus and potassium levels were not significantly affected by formula, while sodium and calcium contents were increased with the increase of corncob proportion (Fig. 5). *L. edodes* is an important edible fungus for both culinary and clinical use, its polysaccharide has been developed as functional foods and medicine [17]. As consumers generally have more pursuit on health and wellness today, differential product supply, especially emphasizing on higher bioactive contents would stimulate customer interest and purchase. Our results revealed substrate formula had great impact on nutritional profiles of mushroom products, it is probably an effective and feasible approach to obtain high-yield and high-quality products through formula improvement.

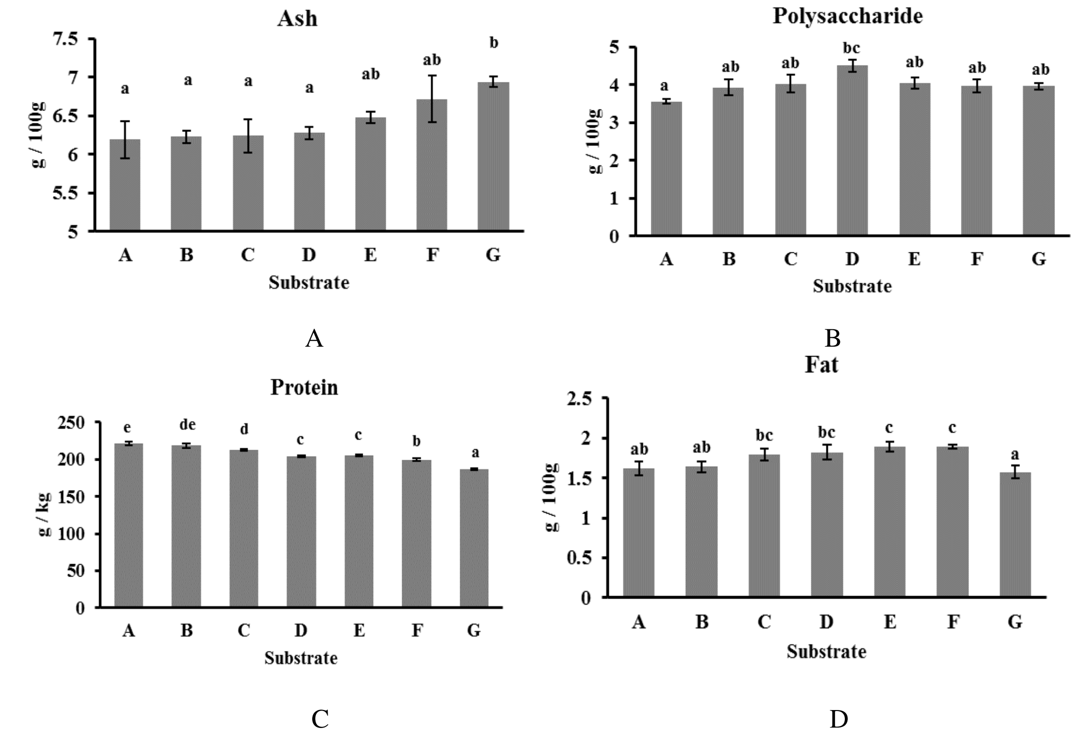



Fig.4 Effect of substrate formula on ash (A), polysaccharide (B), protein (C), and fat (D) of mushrooms. Values with no letters in common are significantly different ($p < 0.05$).

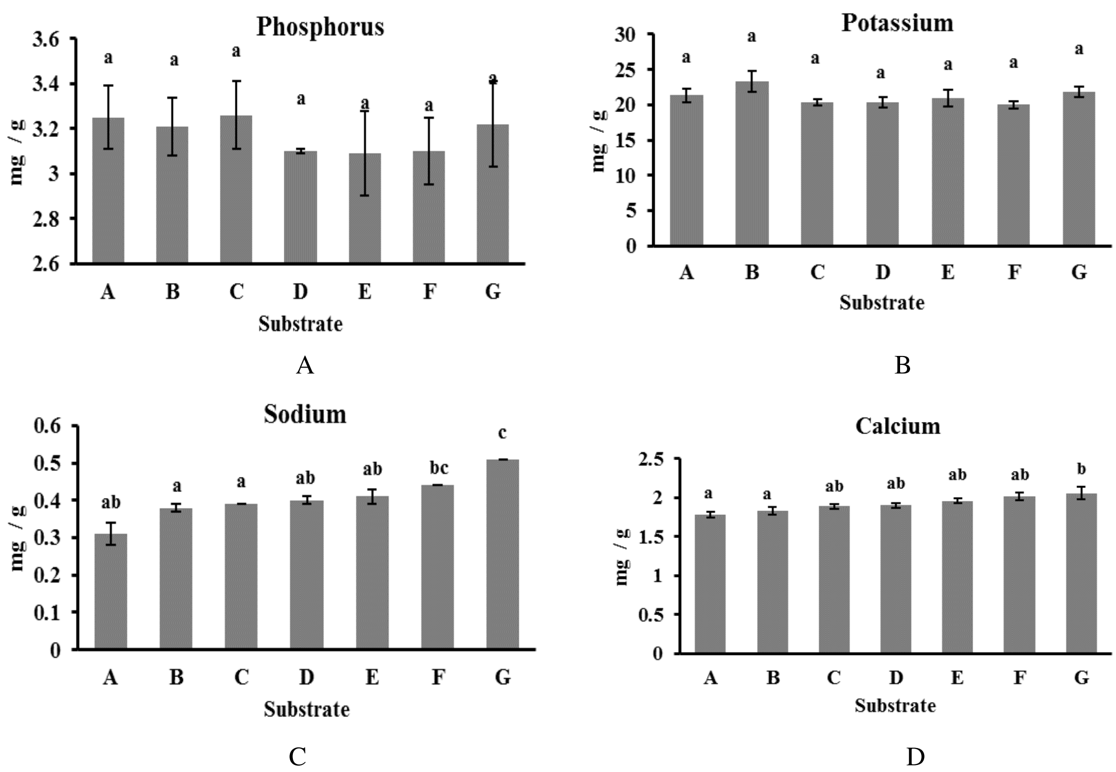



Fig.5 Effect of substrate formula on contents of minerals in mushrooms, including phosphorus (A), potassium (B), sodium (C), and calcium (D). Values with no letters in common are significantly different ($p < 0.05$).

## 4 Conclusion

Corn is the most widely produced crop in the world, leaving corncob as a major agriculture waste. In this study, we assessed the possibility of using corncob to cultivate *L. edodes* in this study, monitored the chemical profile and pH of substrates, and investigated the influence of formula on mycelia growth, log browning, mushroom yield, BE, and agronomic traits and nutritional profile of mushrooms. The results revealed the feasibility to substitute corncob for sawdust in *L. edodes* production in terms of ecological and economic benefits, and a high-yield formula (E: 50% corncob, 28% sawdust, 20% wheat bran, 2% gypsum) was obtained. Using formula E to produce *L. edodes*, the single log yield was as high as 722.08g/log, increased by 40.39% comparing to the control, showing an excellent boosting effect. The proportion of corncob in this formula reaches 50%, which can effectively reduce the use of sawdust and make good use of agricultural waste, thus lowering material cost and reducing environment burden.

In recent years, consumer's demand for *L. edodes* has gradually shifted from an adorable appearance to health benefits. The mushrooms produced by corncob - sawdust mixture substrates were with competent agronomic and nutritional quality. Among our tests, using formula D (40% corncob, 38% sawdust, 20% wheat bran, 2% gypsum) produced mushrooms with highest polysaccharide level, which was 26.69% higher than the control, hence this formula may serve as an alternative approach to obtain *L. edodes* with higher polysaccharide content.

## Declaration of Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Funding

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# Figures

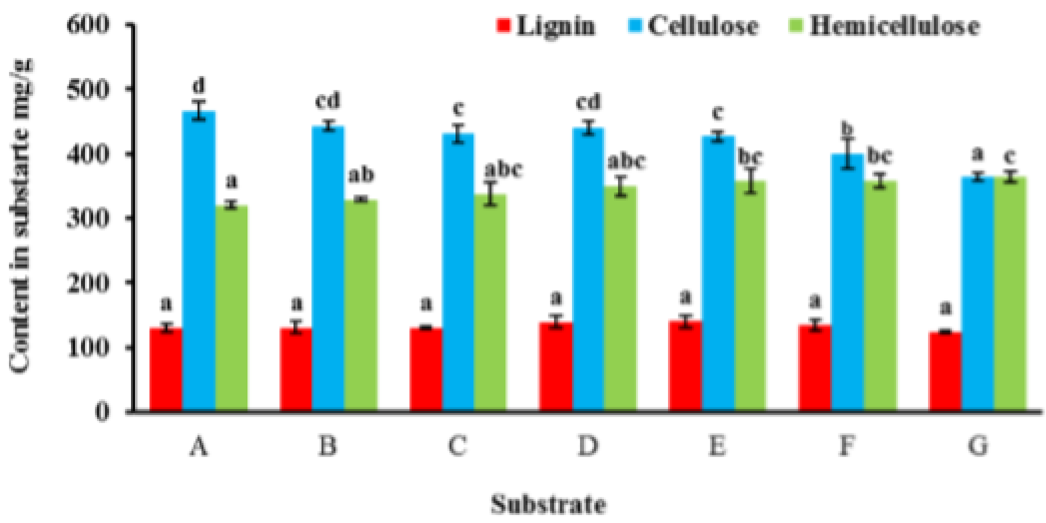


**Figure 1**

Lignin, cellulose and hemicellulose contents of different substrates. Values with no letters in common are significantly different ($p < 0.05$).

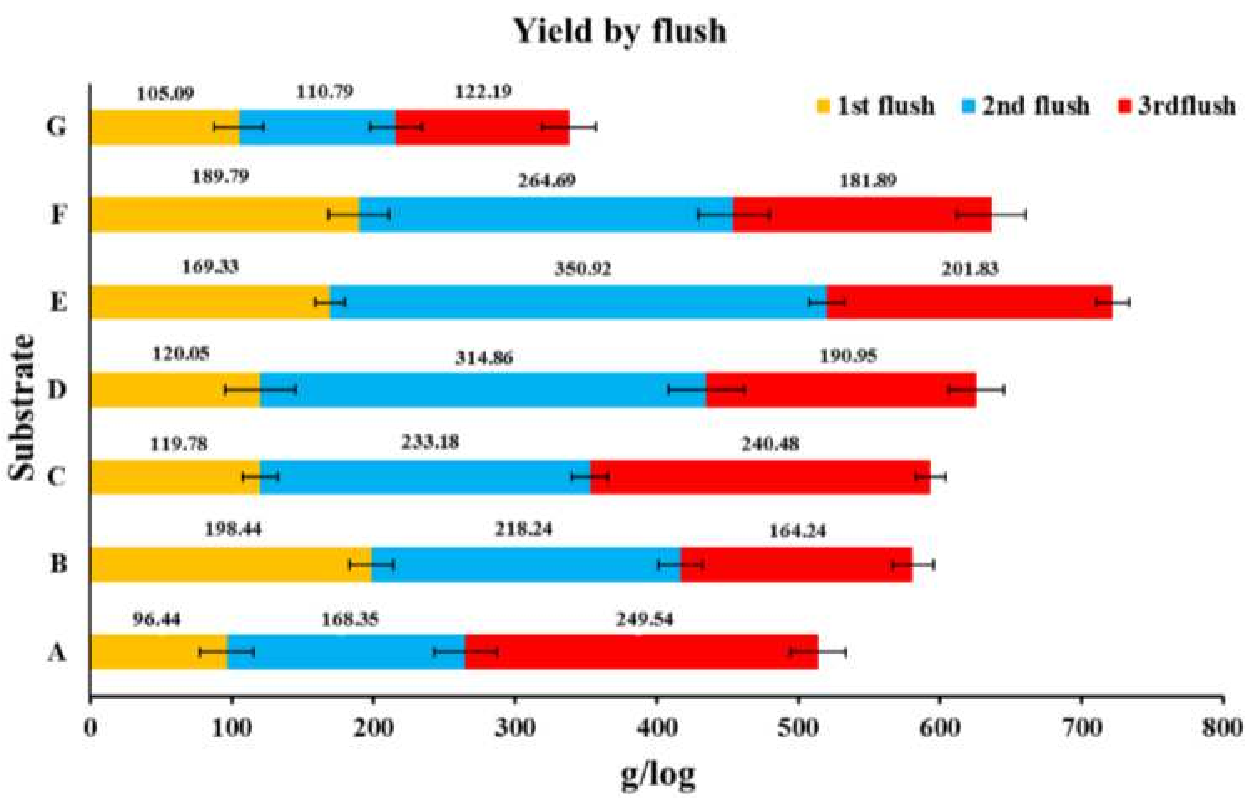


**Figure 2**

Mushroom yield of different substrates in the first, second and third flush.

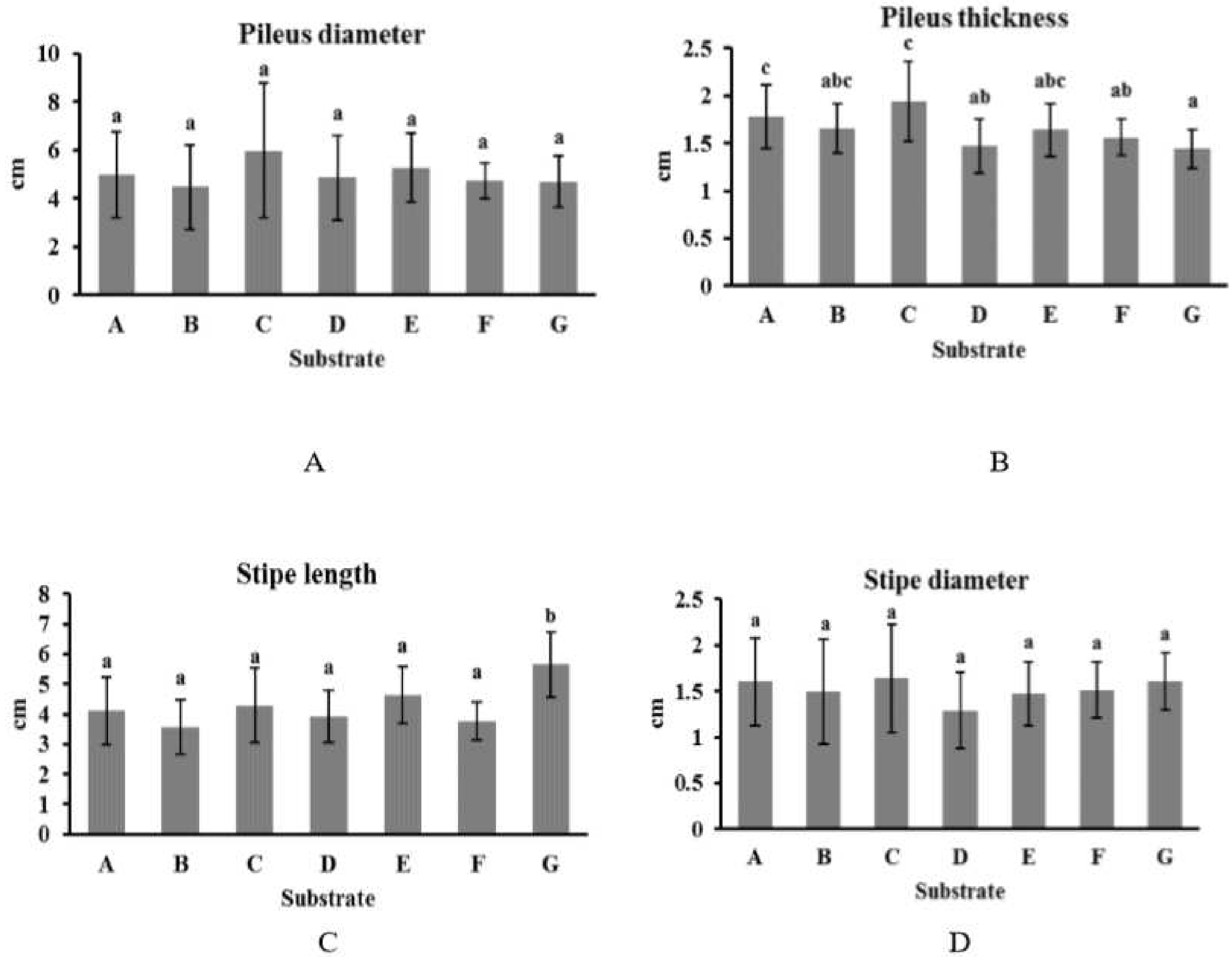


**Figure 3**

Effect of substrate formula on agronomic traits of mushrooms, including pileus diameter (A), pileus thickness (B), stipe length (C), and stipe diameter (D). Values with no letters in common are significantly different ($p < 0.05$).

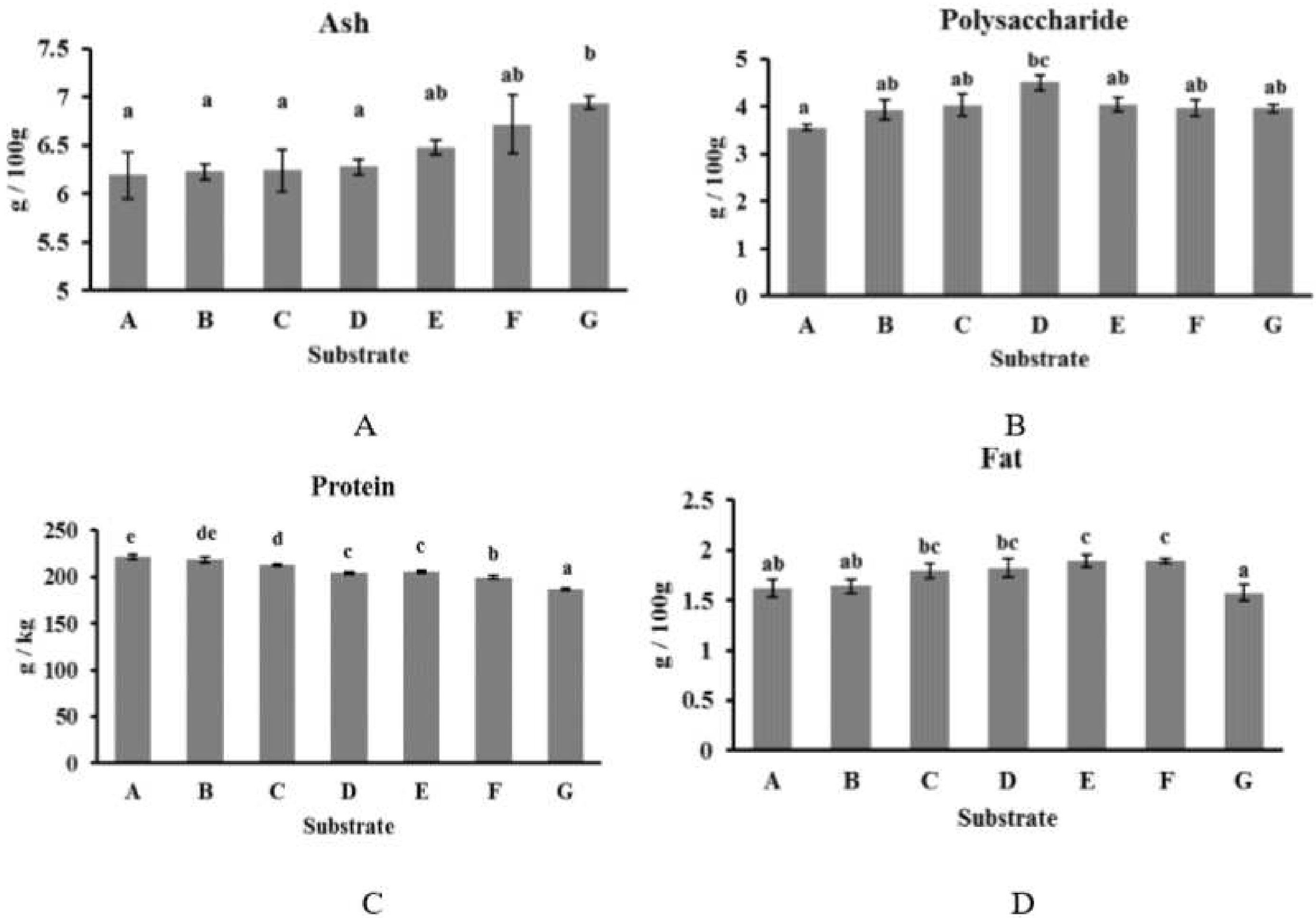


**Figure 4**

Effect of substrate formula on ash (A), polysaccaride (B), protein (C), and fat (D) of mushrooms. Values with no letters in common are significantly different ($p < 0.05$).

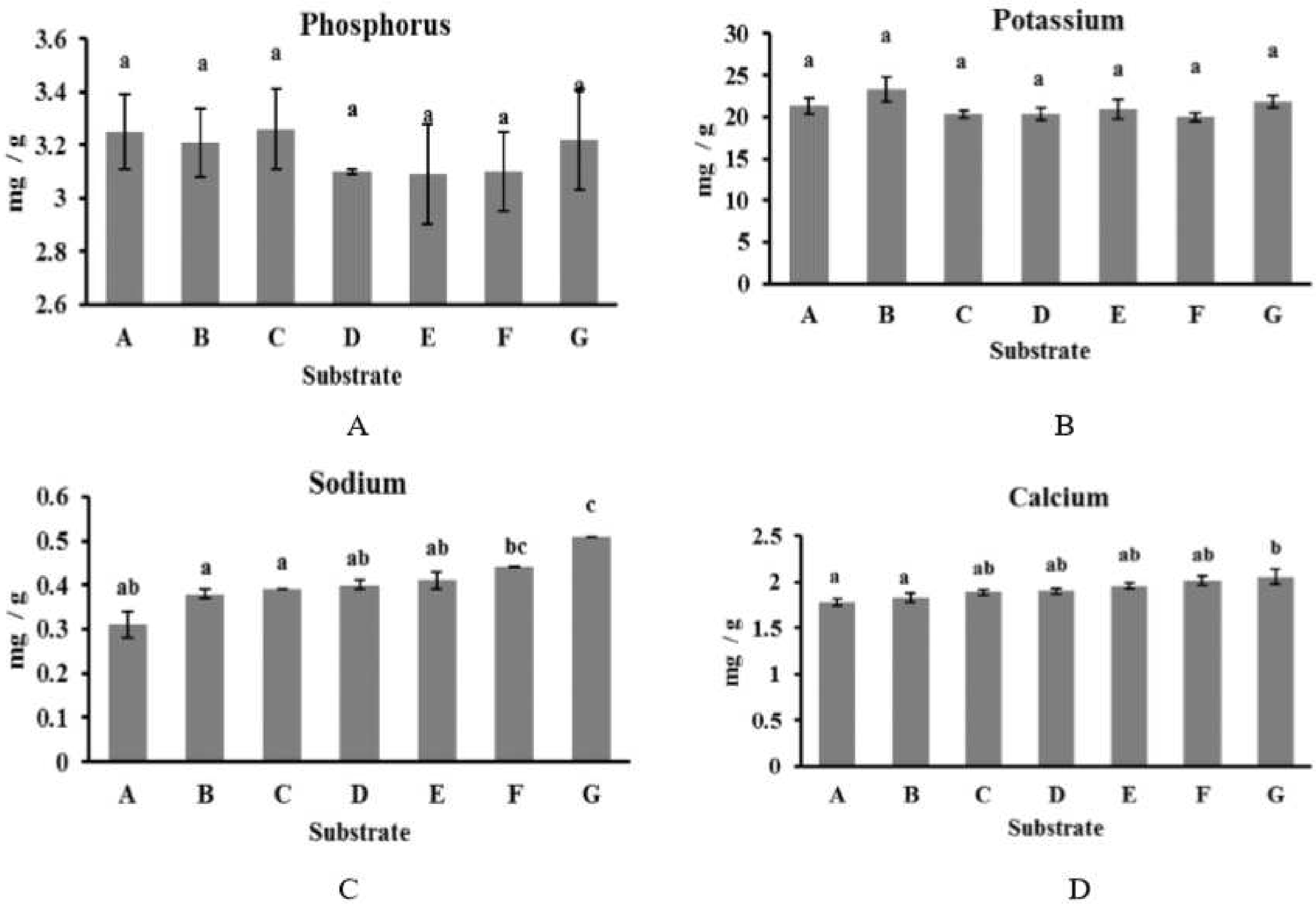


**Figure 5**

Effect of substrate formula on contents of minerals in mushrooms, including phosphorus (A), potassium (B), sodium (C), and calcium (D). Values with no letters in common are significantly different ($p < 0.05$).

# Supplementary Files

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- GraphicAbstract.png