horticulturae

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Impacts of Yield, Nutritional Value, and Amino Acid Contents during Short-Term Composting for the Substrate for *Agrocybe aegerita*

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Abstract: This investigation aimed to ascertain the efficacy of korshinsk peashrub as a viable substitute for cottonseed hull in the cultivation substrate of *Agrocybe aegerita*. The study incorporated korshinsk peashrub into the growth medium at incremental concentrations of 20%, 40%, and 60%, and subjected these blends to both fermentation and non-fermentation processes. Through rigorous assessment of yield of fruiting bodies, biological efficiency, nutrient profile, amino acid composition, and the integration of ecological and socio-economic advantages, an optimal substrate formulation was discerned. The findings revealed that the fermentation substrate FT2, with 40% korshinsk peashrub supplanting cottonseed hull, emerged as the superior blend following a comprehensive analysis. This formula notably yielded the highest crude protein and polysaccharide contents at 26.60% and 4.46%, respectively—an increase of 4.51% and 12.34% over the control. Consequently, these results suggest that korshinsk peashrub is a promising, cost-effective, and efficacious additive, capable of enhancing the yield and quality of *A. aegerita* and potentially replacing cottonseed hull extensively.

Keywords: *Agrocybe aegerita*; korshinsk peashrub; substrate; biological efficiency; nutritional value

Citation: Li, Z.; Zhou, Y.; Zhao, G.; Xu, C.; Pan, J.; Li, H.; Zou, Y. Impacts of Yield, Nutritional Value, and Amino Acid Contents during Short-Term Composting for the Substrate for *Agrocybe aegerita*. *Horticulturae* **2024**, *10*, 234. https://doi.org/10.3390/horticulturae10030234

Academic Editor: Domenico Ronga

Received: 31 December 2023
Revised: 13 February 2024
Accepted: 19 February 2024
Published: 28 February 2024

1. Introduction

Agrocybe aegerita, both an edible and medicinal mushroom, is endowed with an abundance of nutrients and confers significant health advantages [1]. Celebrated for its anticancer properties, aesthetic benefits, anti-diarrheal capacity, spleen fortification, and diuretic effects, it has been aptly dubbed the "Chinese God Mushroom" [2]. This species of woody mushroom thrives in the subtropical and temperate zones, with a distribution spanning southeastern North America, Japan, southern Europe, and China. It is a mushroom of choice for cultivation in numerous countries [3] and is extensively cultivated, particularly in southern China [4,5]. According to the statistics of China Edible Fungi Association, the yield of *A. aegerita* was estimated at 894,700 tons in 2021, and the scale of the edible mushroom industry continues to expand the output from 7.8 million tons in 2001 to 41.3396 million tons in 2021, a five-fold increase, resulting in a shortage of traditional raw materials. This surge has precipitated a scarcity of traditional raw materials, catapulting cottonseed hull prices to USD 290–300 per ton. Given that 80% cottonseed hull is the conventional substrate for cultivating *A. aegerita*, the imperative to innovate alternative agricultural waste into new cultivation substrates has become increasingly pressing.

Agricultural by-products are often abundant in lignocellulosic materials, the management and disposal of which pose significant challenges [6]. A variety of edible fungi have been adapted to exploit these lignocellulosic residues, deploying an arsenal of extracellular

enzymes, including cellulases, hemicellulases, pectinases, and ligninases, to decompose and utilize them [7–10]. The korshinsk peashrub, predominantly composed of cellulose, hemicellulose, and lignin, is one such agricultural residue. Classified within the Leguminosae family [11], this shrub is also referred to by its vernacular names: hairy stripe and white Caragana. As a quintessential species for reforestation projects on China's Loess Plateau [12], the korshinsk peashrub exhibits robust branching and regenerative capabilities, necessitating the pruning of its branches every four to five years [13]. This cyclical pruning generates a substantial yield of branches annually, which, if not utilized effectively, represents a considerable waste of biomass and a lost opportunity for environmental conservation. Cultivating edible fungi using these branches represents a valuable utilization pathway. This lignocellulosic waste is a large carbon source, which is considered to be a potential substrate for mushroom production, and it has been used for the cultivation of *Pleurotus tuoliensis*, *Pleurotus ostreatus*, *Flammulina velutipes*, and *Pleurotus eryngii*. Studies have shown that korshinsk peashrub can provide nutrients to mushrooms to improve growth and yield [14]. Prior research has indicated that the growth of *Pleurotus eryngii* and *Pleurotus tuoliensis* can be enhanced by incorporating korshinsk peashrub into the substrate. This practice not only augments the quality and yield of the fungi, but also serves as a substantial replacement for traditional substrates such as sawdust, sugarcane bagasse, and cottonseed hull [13,14].

Composting, particularly corncob composting, is currently a leading strategy for agricultural waste treatment. This method transforms the organic matter in solid agricultural wastes into nutrients and active substances. Fermentation material cultivation, due to its low pollution, low cost, simplicity, and high economic benefits, has gained worldwide application, surpassing raw material and clinker cultivation [15]. It has been used to cultivate *Pleurotus ostreatus*, *Agaricus bisporus*, and *Volvariella volvacea*. The mycelium from fermentation cultivation grows faster than that of raw material cultivation. However, the fermentation across the material pile is uneven, leading to significant differences in maturity and quality. This variation impacts the yield improvement of edible fungi [16].

This study evaluated the use of korshinsk peashrub as a novel substrate supplement for cultivating *A. aegerita*. It compared the effects of fermentation and non-fermentation on the growth of edible fungi. Developing and refining a substrate formula for *A. aegerita* is essential for its large-scale industrial production. The study evaluated the impact of various substrates on nutrient content accumulation to improve the nutrient profile of *A. aegerita* fruiting bodies. The findings advocate for the recycling of korshinsk peashrub to provide a valuable resource for *A. aegerita* cultivation, offering a beneficial alternative for mushroom production facilities to boost output and grower income.

2. Materials and Methods

2.1. Inoculum Source and Spawn Preparation

The cultivated *A. aegerita* strain B214 was sourced from the main producing area of Pingnan County, Fujian Province, China. The stock culture was preserved on PDA medium, and spawn was maintained on a substrate of 18% wheat bran, 80% sawdust, and 2% lime. The substrate was incubated for 30 days at 25 °C in the dark and stored at 4 °C for future use.

2.2. Composting Process and Sampling

Korshinsk peashrub for this study was provided by Ningxia Hui Autonomous Region Yanchi County Yuanfeng Grass Industry Co., Ltd. (Yanchi, China). The treatment process for korshinsk peashrub branches followed the procedure outlined in literature reference [13]. Auxiliary materials, including wheat bran, sugar, gypsum, and lime were market-purchased.

The control substrate consisted of 80% cottonseed hulls, with 60%, 40%, and 20% korshinsk peashrub replacing the corresponding sawdust concentrations in the substrates. The above four groups were named FCK, FT1, FT2, and FT3, respectively. A composting experiment was

carried out with the above composting formula including FCK, FT1, FT2, and FT3. The samples were collected at 0, 3, 6, 9, and 11 days and numbered as C0, C1, C2, C3, and C4, respectively. Each sample was collected from 9 random points (100 g wet weight each) in the upper, middle, and lower parts of the piles (3 points from each part), thoroughly mixed, divided into parts, and stored at 4 °C or −80 °C for further laboratory studies [17]. When the pile was turned, each bag was checked, weighed, mixed, and replaced.

2.3. Substrate Preparation

Table 1 presents the composite substrates used in this study. The fermented substrates including FCK, FT1, FT2, FT3 for composting correspond to CK, T1, T2, T3 substrate. All the treatments contained 18% wheat bran, 1% sugar, 0.5% each of lime and gypsum, dry-mixed, and then combined with 65% tap water [18]. The final moisture content was determined by oven-drying the substrate in triplicate. A total of 1000 g of moistened substrate was placed in polypropylene bags that were 17 cm wide and 35 cm long. Each treatment had 25 replicates. The bags were sealed with vent caps, and plastic rings were used to seal the bags, which were subjected to autoclaving for 120 min at 121 °C. All the other ingredients had not been pretreated before they were added to the substrate. The spawn were inoculated on a sterile table with 3 spoons per bag, and cultured at 25 °C.

Table 1. Composition of dry matter components of *A. aegerita* cultivated substrates with different additives.

Substrate	Cottonseed Hull (%)	Korshinsk Peashrub (%)	Wheat Bran (%)	Sugar (%)	Gypsum (%)	Lime (%)	TC (%)	TN (%)	C:N
CK	80	0	18	1	0.5	0.5	43.59	2.28	19.16
T1	60	20	18	1	0.5	0.5	39.80	2.16	18.40
T2	40	40	18	1	0.5	0.5	39.00	2.21	17.65
T3	20	60	18	1	0.5	0.5	36.71	2.21	16.58
FCK	80	0	18	1	0.5	0.5	44.17	2.34	18.87
FT1	60	20	18	1	0.5	0.5	41.76	2.41	17.36
FT2	40	40	18	1	0.5	0.5	40.22	2.48	16.52
FT3	20	60	18	1	0.5	0.5	35.98	2.27	15.82

Note: TC (total carbon), TN (total nitrogen), C:N: the carbon (C) and nitrogen (N) ratio.

2.4. Cultivation Methods of A. aegerita

A. aegerita was cultivated using the following method [19]. Spawn were inoculated into sterilized cultivation bags and grown for 50–60 days at 20–27 °C, in darkness, and at 60% relative humidity until fully covered with mycelia. Cultivation continued for an additional 5–7 days to ensure physiological maturity. The bags were then moved to a mushroom chamber for adaptive growth over 3 days under conditions of 20–25 °C and 85–95% relative humidity. The cultivation bags were opened and covered with the non-woven fabric to keep moisture, and periodically open the non-woven fabric for ventilation. The mushroom chamber should be ventilated 2 to 3 times a day, properly irradiate the scattered light, and the primordium will grow out after 5 to 7 days. It will be timely to harvest the mushroom according to the growth of fruit body. After removing the extreme values, a total of 25 replicates were obtained by weighing the fresh yields of fruiting bodies. The fresh yield of fruiting bodies was divided with the primary dry substrate in each bag to calculate the percentage of biological efficiency (BE) [13].

2.5. Physicochemical Properties of Composted Substrates

The loss of ignition and Kjeldahl method were used to estimate the contents of carbon (C) and nitrogen (N), respectively. The C:N ratio was established for each substrate as previously described [20].

2.6. Composition Analysis

Once the fruiting bodies had been harvested, different samples were dried to a constant weight at 60 °C in an oven and sealed for storage. The total proteins (%), ash (%), and fats (%) were measured using this powder as previously described [21–24]. The total soluble sugar content was determined using the sulfuric acid-anthrone colorimetric method [25].

The macro Kjeldahl method was used to measure the crude protein content (N × 4.38) of the mushroom [26,27]. Soxhlet extraction (GB5009.6) [28] was used to determine the mushrooms' crude content of fat. The standard procedure GB5009.4 [29,30] was used to measure their ash content. Crude fibre content is determined according to the method in GB 5009.10 [30]. The PONY Testing International Group (Beijing, China) conducted all the test analyses.

2.7. Assay of Amino Acid Composition

The amino acid composition of dried fruiting bodies was determined using an automatic amino acid analyzer (L-8800, Hitachi, Japan) [31]. In brief, the mushroom powder (0.03 g) was hydrolyzed at 110 °C for 22 h in threaded test tube containing 10 mL of 6 mol/L HCl and 5 mg/mL of phenol. Subsequently, the hydrolysate was filtered into a 50 mL volumetric flask and diluted with deionized water. The 2 mL hydrolysate was dried at 40 °C and then redissolved in 2 mL deionized water. After repeating the drying and dissolution processes three times, the dried samples were dissolved with 0.02 mol/L HC1 (2 mL) and filtered with 0.22 μm filter membrane for analysis.

2.8. Statistical Analysis

The original data were processed using Microsoft Excel 2016 (Redmond, WA, USA). Differences among the means of groups were assessed using Duncan's tests at a 95% confidence level ($p < 0.05$). SPSS 19.0 (IBM, Inc., Armonk, NY, USA) was used to conduct the statistical analyses and correlation analysis.

3. Results

3.1. Physicochemical Properties of the Substrate Materials

The physicochemical properties of the substrate materials were analyzed prior to the composting process, and the composition of the two primary materials is shown in Table 2. Cottonseed hull, a traditional cultivation material, exhibited a low total nitrogen (TN) content of 2.37% and a total carbon (TC) content of 44.51%. In contrast, korshinsk peashrub had a higher TN content of 2.45% and a lower TC content of 39.40%. It also contained high levels of hemicellulose, with cellulose, hemicellulose, and lignin contents of 21.74%, 26.36%, and 12.42%, respectively. However, the cellulose and lignin contents of the cottonseed hull (25.03% and 26.97%, respectively) were higher than those of the korshinsk peashrub. These findings confirm that the chemical composition of the korshinsk peashrub is a suitable carbon source for cultivating *A. aegerita*.

Table 2. Analysis of the lignocellulosic waste used to cultivate *A. aegerita*.

Material	Cellulose (%)	Hemicellulose (%)	Lignin (%)	TC (%)	TN (%)	C:N
Cottonseed hull	25.03	23.64	26.97	44.51	2.37	18.31
Korshinsk peashrub	21.74	26.36	12.42	39.40	2.45	16.08

3.2. Physicochemical Properties during Composting

The four formulations (FCK, FT1, FT2, and FT3) underwent composting treatment. Temperature, a crucial parameter for the digestion process, rapidly increased from 33 °C at the onset (C0 stage, day 0) and peaked on the third day. All treatments maintained a relatively stable thermophilic level of 52–65 °C (C2–C4 stages, days 6–11) until composting concluded (Figure 1a and Table S1). The thermophilic stage temperature in this study facilitated the biodegradation of organic matter by thermophilic microorganisms.

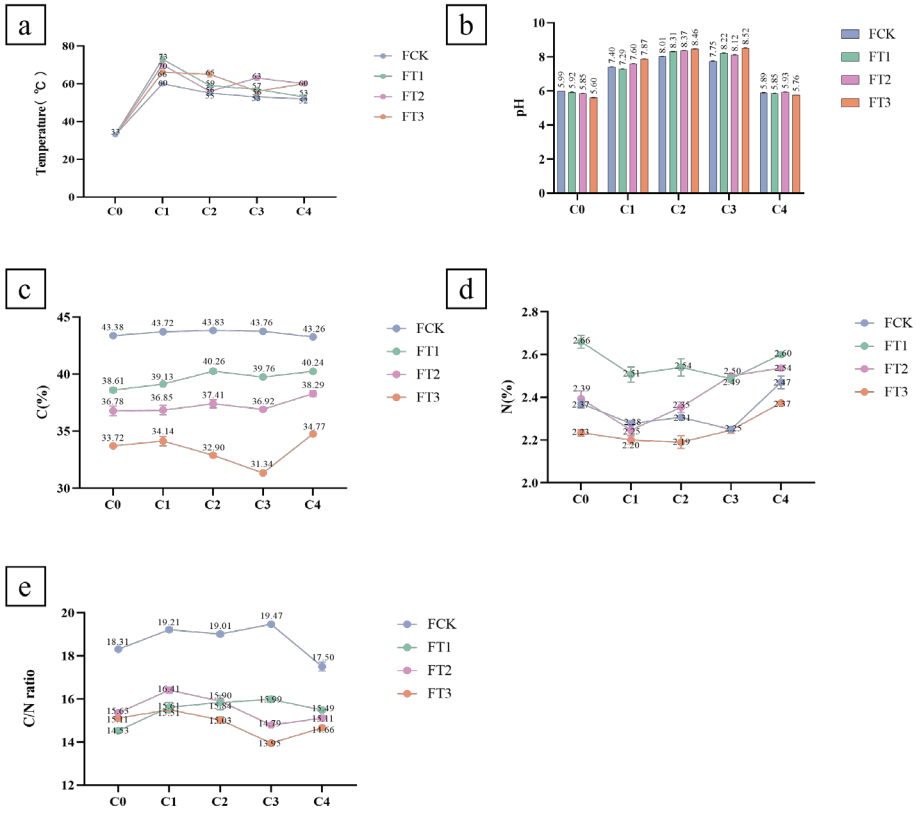

Figure 1. Physicochemical properties of different substrates and different time treatments during composting and the production characteristics of *A. aegerita* cultivation. C0, C1, C2, C3, and C4 represent composting treatments for 0, 3, 6, 9, and 11 days, respectively. All data were expressed as means ± SD (n = 3). (**a**) Temperature changes in four formulations (FCK, FT1, FT2, and FT3) at different fermentation stages (C0, C1, C2, C3, and C4), (**b**) pH changes in four formulations at different fermentation stages, (**c**) total carbon content changes at different fermentation stages, (**d**) total nitrogen content changes at different fermentation stages, (**e**) C:N ratio changes at different fermentation stages.

The initial pH values of the four composted substrates ranged from 5.60 to 5.99, and significantly increased on the third day (C1 stage). At this point, the pH values of FCK, FT1, FT2, and FT3 were 7.40, 7.29, 7.60, and 7.87, respectively. Finally, the pH values decreased to a range of 5.76–5.93 by the end of composting (C4 stage, 11th day) (Figure 1b and Table S2).

The TC and TN were measured at different fermentation stages. The four treatments initially had a TC range of 33.72–43.38%, a TN range of 2.23–2.66%, and a C/N ratio range of 14.53:1–18.31:1 (C0 stage) (Figure 1c–e and Tables S3–S5). As composting progressed, the TC increased to a range of 34.77–43.26% by the end of composting. Total nitrogen (TN) initially decreased, then increased, stabilizing at 2.37–2.60% by day 11 of phase C4. The final C/N ratios of FCK, FT1, FT2, and FT3 were 17.50:1, 15.49:1, 15.11:1, and 14.66:1, respectively.

3.3. The Fruiting Characteristics of A. aegerita

The yields of *A. aegerita* fruiting bodies grown on different non-fermented and fermented substrates were shown in Table 3. The fruiting body yields of CK and FCK were 208.05 ± 13.28 g/bag and 224.67 ± 4.71 g/bag, respectively, which did not differ significantly between the two treatments. The highest yield of fruiting bodies was FT1 (237.19 ± 19.35 g/bag), followed by FCK and FT2 (218.72 ± 7.42 and 224.67 ± 4.71 g/bag). There is no significant difference between the FT1 and FT2 treatments, which were no significantly different from the FCK group. T3 and FT3 had a lower yield than all other formulations, which did differ significantly compared with the other treatments. The biological efficiency (BE) across different substrates varied from 67.76% to 32.32%. T1 and

T2 did not differ significantly from the control group, while FT1 and FT2 also did not differ significantly compared with FCK. T3 and FT3 had similar yields, significantly different from those of the other samples tested. The results showed that different non-fermented and fermented substrates had an effect on the yields produced.

Table 3. Agronomic traits of *A. aegerita* when cultivated on different substrates (mean ± SD, n = 25).

Substrate	1st Plot Yield (g/25 Bags)	2nd Plot Yield (g/25 Bags)	3rd Plot Yield (g/25 Bags)	Average Yield of Plot (g/25 Bags)	Fruit Body Yield (g/Bag)	Biological Efficiency (%)
CK	4749	5391	4924	5021.33 ± 331.88 bc	208.05 ± 13.28 bc	59.44 ± 3.79 bc
T1	5043.2	4901.8	5038.3	4994.43 ± 80.26 bc	199.78 ± 3.21 bc	57.08 ± 0.92 bc
T2	4523	4428	4757.1	4569.37 ± 169.38 c	182.77 ± 6.78 c	52.22 ± 1.94 c
T3	3774.6	3879.6	3958	3870.73 ± 92.02 d	154.83 ± 3.68 d	44.23 ± 1.05 d
FCK	5503.6	5608	5738.4	5616.67 ± 117.64 ab	224.67 ± 4.71 ab	64.19 ± 1.35 ab
FT1	5762.2	5551.8	6475	5929.67 ± 483.85 a	237.19 ± 19.35 a	67.76 ± 5.52 a
FT2	5465.5	5283.6	5654.6	5467.9 ± 185.51 ab	218.72 ± 7.42 ab	62.49 ± 2.12ab
FT3	2168.8	2671	3645.2	2828.33 ± 750.67 e	113.13 ± 30.03 e	32.32 ± 8.58 e

Different lowercase letters denote significant differences in each column ($p < 0.05$).

3.4. The Nutrient Contents of the Mushrooms

Figure 2 and Table S6 shows that the nutrient contents of *A. aegerita* cultured on different substrates varied significantly. The crude protein content of values of *A. aegerita* was in the range of 25.40–29.60%, CK and FCK were 25.45% and 25.40%, respectively, showing no significant difference. The highest protein content among the different substrates of *A. aegerita* was observed in FT3 (29.6%), and the lowest protein content was observed in CK and FCK. This result indicated that the ingredient of the substrate affects the final protein content in the fruiting bodies; CK and FCK have identical compositions, except that FCK is fermented. The fat contents of CK and FCK were 3.55% and 3.60%, respectively, without significant differences, indicating that high cottonseed hull content in the substrate may lead to higher fat content. Ash content of values of *A. aegerita* was in the range of 7.20–7.80%, and T1 and T2 were the highest among the samples. Crude polysaccharide content of values of *A. aegerita* was in the range of 3.90–4.46%, with FT2 having the highest value. The crude fiber content of values of *A. aegerita* was in the range of 7.75–9.15%.

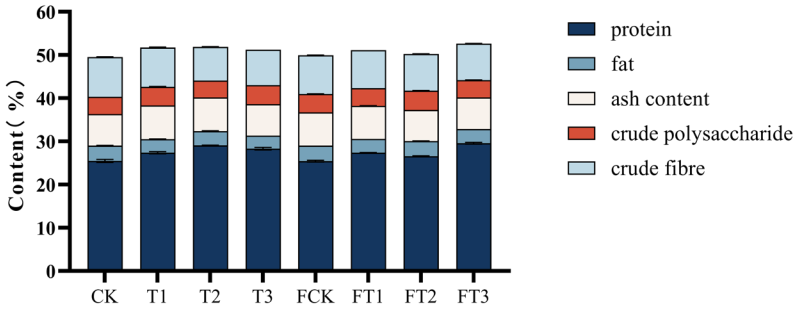

Figure 2. Nutritional value of *A. aegerita* when cultivated on different substrates. The contents of protein, fat, ash, crude polysaccharide, and crude fiber were determined under different treatments.

3.5. The Amino Acid Composition of Mushrooms

The composition of 16 amino acids of fruit body grown on different substrates was shown in Figure 3, Tables S7 and S8. The data indicate that as the proportion of korshinsk peashrub increased, both the total amino acid content and essential amino acid content first increased and then decreased (Tables S7 and S8). The total amino acid content of *A. aegerita* grown on various substrate formulas was in the range from 16.45 ± 0.35% to 20.05 ± 0.05%, and the essential amino acid content was in the range from 6.04% to 7.22%. Notably, mushrooms grown on FT2 had the highest levels of total amino acids and essential amino acids, at 20.05 ± 0.05% and 6.09 ± 0.06%, respectively, which were significantly different from those grown on CK and FCK. Among the 16 amino acids, glutamic acid consistently had the highest content for all of the formulas, followed by aspartic acid and leucine, while the content of other amino acids was affected by the substrate formula variation.

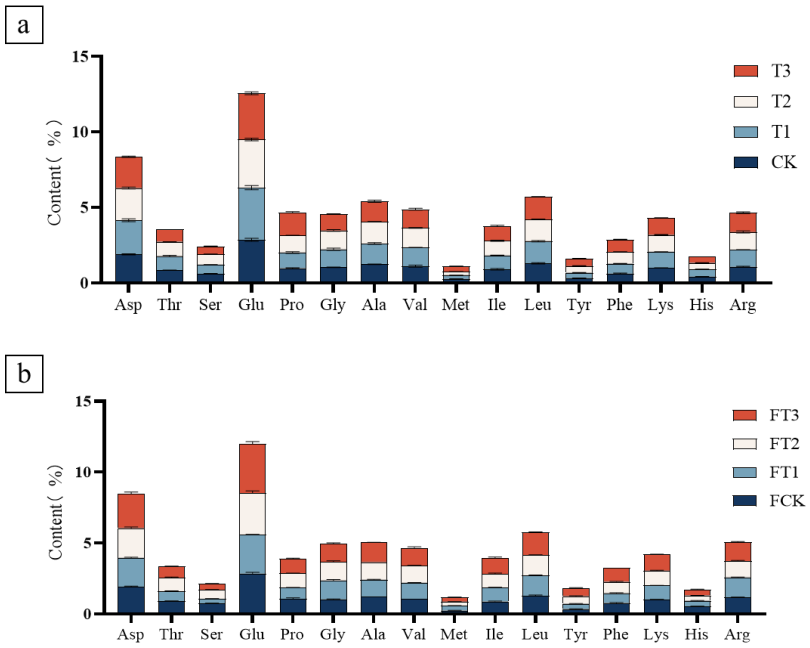

Figure 3. 16 amino acid composition of *A. aegerita* when cultivated on different substrates. (**a**) Amino acid content in the non-fermented groups, (**b**) Amino acid content in the fermented groups.

4. Discussion

Previous studies have shown that the biological efficiency of *A. aegerita* can reach 36% when grown on black tea residue [32]. Another result showed that cultivation of *A. aegerita* on a substrate consisting of 10–20% solid waste (SW) from poultry manure, 70–80% wheat straw and 10% millet increased the yield by 2–5 times compared to the control group, and the supplementation of SW also increased the crude protein content of *A. aegerita* [33]. This study utilized two common agricultural residues, namely, cottonseed hull and korshinsk peashrub, as primary ingredients. The experiment was divided into two groups: fermented and non-fermented. The ideal optimal C:N ratio for mushroom species varied, influencing mycelial growth and yield [26,34]. A composting matrix traditionally used for *A. aegerita* cultivation consists of 80% cottonseed hull, 18% wheat bran, 1% sugar, and 0.5% lime and gypsum, with a C:N ratio of 19:1. All treatments maintained a similar C:N ratio, ranging from 15.82 to 19.16. The yield of *A. aegerita* from non-fermented substrate compositions was in the order of CK > T1 > T2 > T3, while the yield from fermented substrate compositions followed the order of FT1 > FCK > FT2 > FT3. Previous research suggests that sawdust-based composting significantly improves *P. ostreatus* cultivation and the efficient use of lignocellulosic wastes [35]. Our results indicated that composting with cottonseed hull and korshinsk peashrub increased the yield of *A. aegerita*. In this case, the yield of the FT2 fermented substrate component increased by about 19.66% relative to the yield of the non-fermented substrate T2. Most substrates used for mushroom cultivation contain between 0.5% and 0.8% nitrogen, and adding nitrogen to the substrate can enhance the yield [36,37]. FT2 and FT1, with nitrogen contents of 2.48% and 2.41%, respectively, outperformed the other formulations, suggesting that an appropriate increase in nitrogen content in the medium may improve the yield of *A. aegerita*.

Table 3 illustrates that FT1, which contains 20% korshinsk peashrub, yielded the highest mushroom output, reaching 237.19 ± 19.35 g/bag. FT2, with 40% korshinsk peashrub, matched the productivity of both the fermented and non-fermented traditional formulas that contained 80% cottonseed hull, yielding 208.05 ± 13.28 g and 224.67 ± 4.71 g, respectively. A comprehensive analysis of the yield and biological efficiency (BE) of *A. aegerita* indicates a significant increase with the gradual rise in cottonseed hull content, showing a strong positive correlation with mushroom yield. In the Chinese market, korshinsk peashrub costs USD 200–210 per ton, while cottonseed hull is priced at USD 290–300 per ton.

Consequently, from an economic perspective, the cost of using mixed korshinsk peashrub chips is lower than that of using only cottonseed hull. Replacing cottonseed hull with mixed korshinsk peashrub chips for mushroom cultivation can reduce costs of production and enhance economic benefits. Therefore, based on the experimental results, FT2 formulation represents not only an excellent medium for growing *A. aegerita*, but also a cost-effective one for commercial use.

Numerous studies have demonstrated that various factors influence the protein content in mushrooms, including the type of substrate, its physical and biochemical properties, as well as the quantity and type of nutrients added to the substrates [38]. In this study, the protein content in fruit bodies was found to be higher when korshinsk peashrub was used to supplement the substrate, compared to the control. These findings align with previous research [39]. Edible mushrooms are an excellent source of digestible dietary fiber, especially β-glucans, which are widely recognized for their prebiotic properties and health benefits, such as anti-cancer and immunomodulatory effects, reducing the risk of bowel disorders, combating constipation, and decreasing blood cholesterol levels [40]. Polysaccharides, the main components of edible fungi, have attracted considerable attention due to their anti-tumor, anti-inflammatory, immunomodulatory, hypoglycemic, and hypolipidemic activities [41]. Compared to the control, supplementing with 40% korshinsk peashrub increased polysaccharide accumulation and reduced the crude fiber in *A. aegerita*. These results are consistent with findings in *P. eryngii*, where supplementation with a high concentration of korshinsk peashrub powder improved polysaccharide accumulation and reduced fiber accumulation [13].

This experiment revealed that substrates with higher cottonseed hull content produced fruiting bodies with more fat content. Although the fat content of *A. aegerita* is low, the concentration of unsaturated fatty acids is high, making it an important source of essential fatty acids in a healthy human diet [42]. Higher protein and polysaccharide content in *A. aegerita* grown with 40% korshinsk peashrub suggest the use of korshinsk peashrub as a new strategy to produce superior quality mushrooms to meet consumer demands.

Amino acids play a crucial role in protein metabolism and effectively repair human body metabolism [43]. Consequently, samples of *A. aegerita* fruiting bodies were analyzed for their content of 16 free amino acids (Figure 3). The supplementation of cottonseed hull with various concentrations of korshinsk peashrub significantly enhanced the accumulation of both total and essential amino acids in *A. aegerita*, compared to the control and FCK. The most abundant free amino acids identified were glutamic acid (Glu), leucine (Leu), aspartic acid (Asp), alanine (Ala), and valine (Val). These amino acids are produced early on through transamination processes, which are key to optimizing the body's amino acid supply. The findings lead to the conclusion that korshinsk peashrub supplementation boosts the production of free and essential amino acids in *A. aegerita* beyond what is achieved with traditional cultivation substrates.

5. Conclusions

This study provides a robust foundation for incorporating korshinsk peashrub into *A. aegerita* cultivation substrates, presenting a viable alternative to the reliance on cottonseed hulls. Our findings indicate that using a substrate composed of 40% korshinsk peashrub yields mushrooms of quality comparable to those grown in substrates with higher cottonseed hull content, while also enhancing their nutritional profile by increasing the crude protein and polysaccharide levels. Furthermore, *A. aegerita* cultivated on fermented FT2 substrates showed a significant yield increase of 19.66% compared to those grown on non-fermented substrates. Therefore, a 40% korshinsk peashrub fermentation substrate emerges as a strategic innovation for the commercial cultivation of *A. aegerita*, mitigating the impact of traditional substrate shortages and escalating costs. This fermentation approach also offers theoretical backing for the cultivation of other edible mushrooms. In future studies, data on the percentage of korshinsk peashrub added and the efficiency of substrate utilization by *A. aegerita* should be further investigated. Applying these research findings

could significantly boost the sustainability and economic viability of the mushroom industry. It ensures a steady production of high-quality *A. aegerita* and encourages the efficient utilization of agricultural by-products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10030234/s1, Table S1: Temperature changes of four formulations (FCK, FT1, FT2 and FT3) at different fermentation stages (C0,C1,C2,C3 and C4); Table S2: pH changes of four formulations at different fermentation stages; Table S3: Total carbon content changes at different fermentation stages; Table S4: Total nitrogen content changes at different fermentation stages; Table S5: C:N ratio changes at different fermentation stages; Table S6: Nutritional value of *A. aegerita* when cultivated on different substrates; Table S7: Amino acid composition of mushrooms cultivated on non-fermented substrates; Table S8: Amino acid composition of mushrooms cultivated on fermentation substrates.

Author Contributions: Conceptualization, Y.Z. (Yajie Zou); methodology, Y.Z. (Yajie Zou); software, Y.Z. (Yi Zhou) and Z.L.; validation, Y.Z. (Yi Zhou) and H.L.; data curation, C.X. and J.P.; writing—original draft preparation, Y.Z. (Yi Zhou) and J.P.; writing—review and editing, Y.Z. (Yajie Zou); visualization, Y.Z. (Yi Zhou) and C.X.; supervision and project administration, Y.Z. (Yajie Zou) and G.Z.; project administration and funding acquisition, Y.Z. (Yajie Zou). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Beijing Agriculture Innovation Consortium (BAIC03), and China Agriculture Research System, grant number CARS20.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Valverde, M.E.; Hernandez-Perez, T.; Paredes-Lopez, O. Edible mushrooms: Improving human health and promoting quality life. *Int. J. Microbiol.* **2015**, *2015*, 376387. [CrossRef]
2. Cheng, L.X.; Yang, M.Y.; Juan, J.F.; Qing, G.Z.; Ming, W.Y.; Liang, W.W. Research Progress on Nutrition, Health Value and Product Development of *Agrocybe aegerita*. *Edible Fungi China* **2019**, *38*, 1–4.
3. Herzog, R.; Solovyeva, I.; Bolker, M.; Lugones, L.G.; Hennicke, F. Exploring molecular tools for transformation and gene expression in the cultivated edible mushroom *Agrocybe aegerita*. *Mol. Genet. Genom.* **2019**, *294*, 663–677. [CrossRef]
4. Du, Q. Analysis of Nutritional Value of *Agrocybe aegerita* and its Anti-aging Effect. *Edible Fungi China* **2019**, *38*, 131–134.
5. Zheng, X.; Yin, W.; Zhang, S.; Lan, Y. Effects of Different Fermentation Conditions on Extracellular Polysaccharides Produced by Mycelia of *Agrocybe aegerita*. *Guangdong Agric. Sci.* **2019**, *46*, 25–32.
6. Philippoussis, A.; Zervakis, G.; Diamantopoulou, P. Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita*, *Volvariella volvacea* and *Pleurotus* spp. *World J. Microb. Biot.* **2001**, *17*, 191–200. [CrossRef]
7. Isikhuemhen, O.S.; Mikiashvili, N.A.; Adenipekun, C.O.; Ohimain, E.I.; Shahbazi, G. The tropical white rot fungus, *Lentinus squarrosulus* Mont.: Lignocellulolytic enzymes activities and sugar release from cornstalks under solid state fermentation. *World J. Microb. Biot.* **2012**, *28*, 1961–1966. [CrossRef] [PubMed]
8. Levin, L.; Diorio, L.; Grassi, E.; Forchiassin, F. Grape stalks as substrate for white rot fungi, lignocellulolytic enzyme production and dye decolorization. *Rev. Argent. Microbiol.* **2012**, *44*, 105–112.
9. Varnai, A.; Makela, M.R.; Djajadi, D.T.; Rahikainen, J.; Hatakka, A.; Viikari, L. Carbohydrate-binding modules of fungal cellulases: Occurrence in nature, function, and relevance in industrial biomass conversion. *Adv. Appl. Microbiol.* **2014**, *88*, 103–165. [CrossRef] [PubMed]
10. Wang, W.; Yuan, T.; Cui, B.; Dai, Y. Investigating lignin and hemicellulose in white rot fungus-pretreated wood that affect enzymatic hydrolysis. *Bioresour. Technol.* **2013**, *134*, 381–385. [CrossRef] [PubMed]
11. Jie, C.; Jibin, W.; Jimin, C.; Zongkuan, L. Spatial-Temporal Variability of *Caragana korshinskii* Vegetation Growth in the Loess Plateau. *Sci. Silvae Sin.* **2013**, *49*, 14–20.
12. Jia, X.; Shao, M.; Yu, D.; Zhang, Y.; Binley, A. Spatial variations in soil-water carrying capacity of three typical revegetation species on the Loess Plateau, China. *Agric. Ecosyst. Environ.* **2019**, *273*, 25–35. [CrossRef]
13. Zou, Y.; Du, F.; Zhang, H.; Hu, Q. Evaluation of korshinsk Peashrub (*Caragana korshinskii* Kom.) as a Substrate for the Cultivation of *Pleurotus eryngii*. *Waste Biomass Valorization* **2019**, *10*, 2879–2885. [CrossRef]
14. Du, F.; Qu, J.; Hu, Q.; Yuan, X.; Yin, G.; Wang, L.; Zou, Y. Maximizing the value of korshinsk peashrub branches by the integration of *Pleurotus tuoliensis* cultivation and anaerobic digestion of spent mushroom substrate. *Renew. Energ.* **2021**, *179*, 679–686. [CrossRef]
15. Hernandez, D.; Sanchez, J.E.; Yamasaki, K. A simple procedure for preparing substrate for *Pleurotus ostreatus* cultivation. *Bioresour. Technol.* **2003**, *90*, 145–150. [CrossRef] [PubMed]

16. Choi, I.Y.; Choi, J.N.; Sharma, P.K.; Lee, W.H. Isolation and Identification of Mushroom Pathogens from *Agrocybe aegerita*. *Mycobiology* **2010**, *38*, 310–315. [CrossRef] [PubMed]
17. Zhang, L.; Jia, Y.; Zhang, X.; Feng, X.; Wu, J.; Wang, L.; Chen, G. Wheat straw: An inefficient substrate for rapid natural lignocellulosic composting. *Bioresour. Technol.* **2016**, *209*, 402–406. [CrossRef] [PubMed]
18. Zeyang, J. Effects of Different Culture Materials on Mycelium Growth, Yield and Nutrients of *Lentinous edodes*. Master's Thesis, Anhui Agricultural University, Hefei, China, 2018.
19. Zhou, Y.; Liu, P.; Liu, B.; Su, R.; Zhou, J. Effects of Different Cultivation Formulas on Growth and Extracellular Enzyme Activity of Cultured *Agrocybe cylindracea*. *North. Hortic.* **2023**, *19*, 114–121.
20. Liu, M.; Xiao, Z.; Qiu, Y.; Qiu, G.; He, H. Optimization and Application on Liquid Spawns Cultivation Medium of *Agrocybe cylindracea*. *Edible Fungi China* **2016**, *35*, 39–42.
21. Carrasco-Cabrera, C.P.; Bell, T.L.; Kertesz, M.A. Caffeine metabolism during cultivation of oyster mushroom (*Pleurotus ostreatus*) with spent coffee grounds. *Appl. Microbiol. Biot.* **2019**, *103*, 5831–5841. [CrossRef] [PubMed]
22. Xiaobei, L.; Feng, Z.; Yan, Y.; Jie, F.; Yanfang, L.; Tao, F. Effect of Culture Media on Nutritional Value of Proteins in *Pleurotus eryngii* Fruit Bodies. *Food Chem.* **2015**, *36*, 262–267.
23. Chen, X.; Tan, Y.; Chu, W. Analysis on *Agrocybe aegerita*-Ws Sporophore Nutritional Components. *J. Anhui Agri. Sci.* **2013**, *41*, 11851–11880.
24. Muthu, N.; Shanmugasundaram, K. Effect of five different culture media on mycelial growth of *Agrocybe aegerita*. *Int. J. Pharm. Sci. Res.* **2015**, *12*, 5193–5197.
25. Feng, B.; Xu, Y.; Fu, W.; Li, H.; Li, G.; Li, J.; Wang, W.; Tao, L.; Chen, T.; Fu, G. RGA1 Negatively Regulates Thermo-tolerance by Affecting Carbohydrate Metabolism and the Energy Supply in Rice. *Rice* **2023**, *16*, 32. [CrossRef] [PubMed]
26. Lechner, B.E.; Alberto, E. Search for new naturally occurring strains of Pleurotus to improve yields: *Pleurotus albidus* as a novel proposed species for mushroom production. *Rev. Iberoam. Micol.* **2011**, *28*, 148–154. [CrossRef] [PubMed]
27. Xie, X.; Guo, X.; Zhou, L.; Yao, Q.; Zhu, H. Study of Biochemical and Microbiological Properties During Co-composting of Spent Mushroom Substrates and Chicken Feather. *Waste Biomass Valorization* **2019**, *10*, 23–32. [CrossRef]
28. Xu, L.; Wu, X.; Chen, G.; Wu, S.; Ren, K.; Zhou, Y.; Wu, Y.; Yan, L.; Guo, W. Effects of electron beam irradiation on the properties of frozen *Litopenaeus vannamei*. *J. Radiat. Res. Radiat. Process.* **2023**, *41*, 57–66.
29. Bao, Z.; Yi, Y.; Wan, X.; Yuan, B.; Li, C. Study on the selenium content and quality of bamboo shoots in Yichun Mingyue Mountain. *Spec. Econ. Anim. Plants* **2024**, *27*, 11–14.
30. He, Q.; Chen, W.; Xie, X.; Peng, H.; Deng, B.; Wang, Y.; Chen, Y. Analysis and evaluation of nutritional components in wild "Shuaba mushroom" in Foping, Shaanxi. *J. Northwest Univ. (Nat. Sci. Ed.)* **2018**, *46*, 51–57. [CrossRef]
31. Yu, W.; Lan, X.; Cai, J.; Wang, X.; Liu, X.; Ye, X.; Yang, Q.; Su, Y.; Xu, B.; Chen, T.; et al. Critical role of IL-1beta in the pathogenesis of *Agrocybe aegerita* galectin-induced liver injury through recruiting T cell to liver. *Biochem. Biophys. Res. Commun.* **2020**, *521*, 449–456. [CrossRef]
32. Kleofas, V.; Sommer, L.; Fraatz, M.A.; Zorn, H.; Rühl, M. Fruiting body production and aroma profile analysis of *Agrocybe aegerita* cultivated on different substrates. *Nat. Resour.* **2014**, *5*, 233–240. [CrossRef]
33. Isikhuemhen, O.S.; Mikiashvili, N.A.; Kelkar, V. Application of solid waste from anaerobic digestion of poultry litter in *Agrocybe aegerita* cultivation: Mushroom production, lignocellulolytic enzymes activity and substrate utilization. *Biodegradation* **2009**, *20*, 351–361. [CrossRef]
34. Cunha, Z.D.; Pardo-Gimenez, A.; de Almeida, M.M.; Villas, B.R.; Alvarez-Orti, M.; Pardo-Gonzalez, J.E. Characterization, feasibility and optimization of *Agaricus* subrufescens growth based on chemical elements on casing layer. *Saudi J. Biol. Sci.* **2012**, *19*, 343–347. [CrossRef] [PubMed]
35. Guo, Y.X.; Chen, Q.J.; Qin, Y.; Yang, Y.R.; Yang, Q.Z.; Wang, Y.X.; Cheng, Z.A.; Cao, N.; Zhang, G.Q. Succession of the microbial communities and function prediction during short-term peach sawdust-based composting. *Bioresour. Technol.* **2021**, *332*, 125079. [CrossRef] [PubMed]
36. KA, A.; TR, S.; KR, S. Cultivation of *Pleurotus sajor-caju* on certain agro-wastes and utilization of the residues for cellulase and D-xylanase production. *Agric. Food Sci.* **1990**, *10*, 21–26.
37. Gupta, Y.; Vijay, B. Post-composting supplementation in *Agaricus bisporus* under seasonal growing conditions. *Mushroom Res.* **1992**, *2*, 115–117.
38. Ye, D.; Hu, Q.; Bai, X.; Zhang, W.; Guo, H. Increasing the value of *Phragmites australis* straw in a sustainable integrated agriculture model (SIAM) comprising edible mushroom cultivation and spent mushroom substrate compost. *Sci. Total Environ.* **2023**, *869*, 161807. [CrossRef]
39. Li, G.; Liu, X.; Cong, S.; Deng, Y.; Zheng, X. A novel serine protease with anticoagulant and fibrinolytic activities from the fruiting bodies of mushroom *Agrocybe aegerita*. *Int. J. Biol. Macromol.* **2021**, *168*, 631–639. [CrossRef]
40. Cheung, P.C.K. Mini-review on edible mushrooms as source of dietary fiber: Preparation and health benefits. *Food Sci. Hum. Well* **2013**, *2*, 162–166. [CrossRef]
41. Guo, D.; Lei, J.; He, C.; Peng, Z.; Liu, R.; Pan, X.; Meng, J.; Feng, C.; Xu, L.; Cheng, Y.; et al. In vitro digestion and fermentation by human fecal microbiota of polysaccharides from *Clitocybe squamulose*. *Int. J. Biol. Macromol.* **2022**, *208*, 343–355. [CrossRef]

42. Kalac, P. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *J. Sci. Food Agric.* **2013**, *93*, 209–218. [CrossRef] [PubMed]
43. Murakami, H.; Shimbo, K.; Inoue, Y.; Takino, Y.; Kobayashi, H. Importance of amino acid composition to improve skin collagen protein synthesis rates in UV-irradiated mice. *Amino Acids* **2012**, *42*, 2481–2489. [CrossRef] [PubMed]