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Full Length Article

Camellia oleifera Seed Shell: An Effective Substrate for Producing *Flammulina velutipes* Fruit Bodies with Improved Nutritional Value

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Abstract

The rapid expansion of mushroom cultivation has resulted in a shortage of cottonseed hulls and other materials. *Camellia oleifera* seed shell (CSS) is one of the major by-products of tea oil processing and is available in large quantities in China and many other Asian countries, but it has yet not been utilized in mushroom cultivation. This study investigated the feasibility of using CSS for *Flammulina velutipes* cultivation. Results showed that fermented CSS (FCSS) was superior to non-fermented (NFCSS) for the cultivation. FCSS at 20 supplementation level as a substitute for cottonseed hull had positive effects on *F. velutipes* production, generating a yield of 445.84 g/bag (in 410 g dry matter of substrate), which was higher than the control (437.24 g/bag). Moreover, the commercial ratio and marketable quality of fruit bodies produced was almost unchanged. Conversely, using FCSS or NFCSS at 8%–28% supplementation range as a replacement of wheat bran considerably decreased yield by 29.2–213.88 g/bag and the commercial ratio by 2.29%-11.62%. The marketable quality of fruit bodies also became inferior because of the formation of big pileus and wide but short stipe. The fruit bodies produced on the substrate with 20% FCSS as a substitute for cottonseed hull had higher contents of protein (18.16%), amino acids (13.14%), linoleic acid (0.49%) and linolenic acid (0.2%) as compared with those (13.1%, 9.3%, 0.36% and 0.16%, respectively) from the control. In conclusion, FCSS could be used as an effective and economic substrate for *F. velutipes* cultivation, producing fruit bodies with improved nutritional value.

Keywords: Biological efficiency; *Camellia oleifera* seed shell; *Flammulina velutipes*; Mushroom cultivation

Introduction

Flammulina velutipes, commonly known as golden needle mushroom or winter mushroom, is a commercially important edible and medicinal mushroom that belongs to the family of *Tricholomataceae*, which is in the Agaricales. The fruiting body of this fungus has a delicious taste and has a high nutrient content (Jing *et al*., 2014; Kang *et al*., 2014). *F. velutipes* also possesses various biological and pharmaceutical activities, such as antioxidant, antitumor, anti-inflammatory, immunomodulatory and cholesterol-lowering effects (Wu *et al*., 2014; Yan *et al*., 2014; Chen *et al*., 2015; Xia, 2015). These nutritive and medicinal properties mean that *F. velutipes* a popular product in the market and demand is rapidly increasing.

Presently, *F. velutipes* cultivation mainly occurs in semi-automated factories in eastern Asian countries and many lignocellulosic solid substrates, such as softwood sawdust, cottonseed hull, sugarcane bagasse and corncob, are utilized as basic ingredients in its cultivation. Over recent years, there has been a rapid increase in mushroom cultivation worldwide, which has meant that supplying large amounts of substrates like cottonseed hull has become difficult. This has led to a continuous rise in the price of mushroom cultivation materials. These circumstances create an incentive to investigate alternative substrates for mushroom cultivation, and a large number of studies describing the utilization of novel biomasses for mushroom cultivation have been reported (Ohga and Royse, 2004; Liang *et al*., 2009; Yang *et al*., 2016).

Camellia oleifera is an edible oil producing woody species distributing specifically in the southern provinces of China (Kang *et al*., 2011). Presently, it is mainly grown in China, but it is also widely grown in some other Asian countries (Zhang *et al*., 2010). Its seed is mainly used for extruding nourishing oils enriched with unsaturated fatty acids, whose levels can be as high as 90% (Long *et al*., 2008; Huang *et al*., 2013). The unsaturated fatty acids mainly consist of oleic acid and linoleic acid (Lee and Yen, 2006). In recent years, the yield from *C. oleifera* seed has shown a yearly increase of 5.4% on average in China (Jian *et al*., 2011) and there are now around 5 million tons of tea oil

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processing byproducts, such as fruit hull, seed shell and oil cake produced each year (Su *et al*., 2014). Generally, these byproducts have always been discarded or incinerated by the tea oil processing industry. Thus, searching for new practical and economic applications for tea oil byproducts is potentially needed. To the best of our knowledge, there have been no previous reports on using *C. oleifera* seed shell (CSS) for mushroom cultivation.

The aim of the present investigation was to effectively utilize the CSS byproduct and to reduce *F. velutipes* production costs. For this purpose, the CSS potential as a substitute for cottonseed hull or wheat bran substrates was examined in *F. velutipes* cultivation in terms of its effect on mycelial colonization, yield, and the commercial ratio and marketable quality of fruit bodies produced. Furthermore, the change of the main nutritional components of fruit bodies in response to this substrate was also investigated. This study is useful for the effective utilization of CSS and economic *F. velutipes* cultivation.

Materials and Methods

M**ushroom Strain and Spawn Preparation**

The fungal strain *F. velutipes* F8801 used in this study was obtained from Sanming Institute of Edible Fungi, Fujian Province, China. It was grown on potato dextrose agar (PDA) at 25°C for 7 days, stored at 4°C and sub-cultured at regular intervals of four weeks. For preparing *F. velutipes* spawn, 750 mL glass bottles, filled with a sterilized substrate consisting of 60% sawdust, 18% cottonseed hull, 20% wheat bran and 2% gypsum (w/w, dry weight basis) with approximately 65% moisture content, were used. After inoculated with a mycelial agar disc of 1 cm diameter cut from the periphery of an actively growing plate colony, incubation was performed at 25°C and 60–70% relative humidity. Once the substrate had been completed colonized by the mycelia, it was used as spawn in fruiting test.

Pretreatment and Fermentation of CSS

The CSS was obtained from a local tea oil processing plant. It was ground using a hammer mill (Model-450, Gaoyou City Science Institute of Edible Fungi, Jiangsu Province, China) and screened with a 0.6 cm sieve so that the final sample components were 0.2–0.6 cm length, 0.2–0.6 cm in width and the seed shell particles were 0.1–0.15 cm thickness. For fermentation, the moisture content of the resulting particles was adjusted to around 65% using tap water and the moistened particles were stalked on a cement floor up to a height of 1.2 m, with a 1.5 m bottom width and an arbitrary length. Fermentation was carried out in the fall under natural environmental conditions and lasted 14 days. During this bioprocess, four turns were conducted at sequential intervals of 5 days, 4 days, 3 days and 2 days.

Substrate Preparation, Inoculation and Spawn Running

The fermented CSS (FCSS) and non-fermented CSS (NFCSS) were utilized as substitutes for cottonseed hull or wheat bran in a substrate. The twelve substrates, containing different percentages of FCSS and NFCSS are formulated and used, as listed in Table 1. Substrate CW was commonly used in local *F. velutipes* cultivation factories. Each substrate combination was completely mixed and the moisture content was adjusted to approximately 65%. The substrate mixture (1080 g) was put into 40 cm × 17.5 cm polypropylene bags, tightly packed and closed with plastic ring and vent. The sterilization was at 121°C for 2 h. After cooling, each bag was inoculated by spreading the spawn on the surface of substrate at 2% (w/w). Spawn running was at 18–24°C, 60–70% relative humidity under darkness condition. When the mycelium had fully colonized the substrate, the height from the bottom of the substrate to the top (*i.e*., the height of the mycelia) was measured and the mycelial growth rate (mm/day) was calculated as the height of the mycelia divided by the incubation time (days). Mycelial density was estimated by the naked eye. The colonization time was also recorded. There were 30 replicates for each substrate.

Cropping and Harvesting

The mature bags were moved into a fruiting room so that pinhead formation and fruit body development could be induced. The environmental conditions were as follows: 85–90% relative humidity, 8°C and a 12 h light cycle with a light density of 1000 lux. The necks and covers were removed from the bags when the primordia appeared. The mushrooms were harvested manually before the caps began to invert. Herein, only first flush was harvested before the spent substrates were discarded. Biological yield was determined by weighing the whole cluster of fruit bodies without removing the base of the stalks. Economic yield (g) was determined after removing the stalk bases. The commercial ratio was calculated as the economic yield divided by the biological yield, and is expressed as a percentage. The biological efficiency was calculated as follows:

$$BE\ (\%) = Wb/Ws \times 100$$

Where *BE* is the biological efficiency, *Wb* is the biological yield of the freshly harvested fruiting body and *Ws* is the dry matter weight of the substrate. Mushroom marketable quality was determined by measuring the pileus diameter, stipe length and stipe diameter.

Proximate Composition Analysis

Fruit bodies of *F. velutipes* were dried in an oven at 35°C for 3 h, 45°C for 3 h, 50°C for 2 h and 60°C to a constant weight. Protein content in the fruit bodies was determined by the Kjeldahl method and the nitrogen factor used for crude protein calculation was 4.38 (Chang and Miles, 1989).

Crude dietary fiber was determined by the acid-detergent method, expressed as percentage (Nyanga *et al*., 2013). Ash content (%) was determined by incinerating the dried sample in a muffle furnace (SX-2.5-10, Shanghai Hongji Instruments Co., Ltd., Shanghai, China) at 565°C for 3 h (Khan *et al*., 2008). Crude fat content was determined by extraction with petroleum ether for 8 h using a Soxhlet apparatus and expressed as percentage (Fernandes *et al*., 2014).

Amino Acid Analysis

The mushroom samples were hydrolyzed in sealed ampoules for 24 h at 110°C using 10 mL of 6 mol/L HCl containing 5 mg/mL phenol. The amino acids in the acid hydrolysate were analyzed by using an amino acid auto-analyzer (S433D, Sykam Company, Munich, Germany). The resolved peaks were identified and compared with a standard run under the same conditions (Cheung, 1997).

Fatty Acid Analysis

The fatty acid compositions of each sample were determined according to the method of Sukhija and Palmquist (1988). Five hundred milligrams of each dried sample was mixed with 2 mL of toluene and 3 mL of 5% methanolic hydrogen chloride, and incubated at 70°C for 2 h. Then, 5 mL of 6% potassium carbonate and 2 mL of toluene were added to the same tube to hydrolyze the samples; the samples were then centrifuged at 2500 ×*g* for 2 min. After transferring the upper layer to new tube, 1 g of anhydrous sodium sulphate and 1 g of activated charcoal were added to the samples, and fatty acid methyl esters were analyzed on a GC/MS-QP2010 plus system (Shimadzu Corporation, Kyoto, Japan), using a DB-5ms column (30 m × 0.25 mm × 0.25 μm). Injection volume was 2 μL and injector temperature was 250°C with 100:1 split ratio.

Statistical Analysis

Data are expressed as mean ± SD. The data were analyzed by one-way analysis of variance, and means showing statistical differences were compared according to Duncan's multiple-range test using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P<0.05$.

Results

Effects of FCSS and NFCSS on the Mycelial Colonization and Density

FCSS and NFCSS as substitutes for cottonseed hull: As shown in Table 2, the fastest mycelial colonization (2.68 cm/day) occurred on substrate FC20 among the six supplemented substrates, which was statistically similar to the control (2.63 cm/day). It also can be seen that when NFCSS was used to supplement the substrate, the colonization rate was considerably lower than that of the three FCSS treatments and the control. In the case of mycelial density, the FCSS at all three supplementation levels had little effect on it, but a negative effect was observed for NFCSS when the supplementation ratio exceeded 10%. Colonization time was shortest in substrate FC20 (41.04 d), but a slightly extended time (less than one day), compared to the control (42.23 d), was observed for FC10 (42.63 d) and FC30 (43.04 d). When NFCSS was used to supplement the substrate, an additional time of as long as 2.3–5.18 days was required for complete mycelial colonization compared to the control. These results showed that fermented CSS was superior to non-fermented CSS in terms of the mycelial colonization and density of *F. velutipes*.

Table 1: FCSS or NFCSS supplement ratios used when they replaced cotton seed hull or wheat bran in *F. velutipes* cultivation substrates (%, dry weight basis)

Substrate	Material			
	FCSS (%)	NFCSS (%)	Cottonseed hull (%)	Wheat bran (%)
CW	0	0	30	28
FC10	10	0	20	28
FC20	20	0	10	28
FC30	30	0	0	28
NFC10	0	10	20	28
NFC20	0	20	10	28
NFC30	0	30	0	28
FW8	8	0	30	20
FW18	18	0	30	10
FW28	28	0	30	0
NFW8	0	8	30	20
NFW18	0	18	30	10
NFW28	0	28	30	0

All substrates also contained 20% sawdust, 17% corncob, 3% corn powder and 2% calcium carbonate

Table 2: Mycelial growth rate, mycelial density, and colonization of *F. velutipes* on substrates containing different ratios of FCSS or NFCSS as a substitute for the cottonseed hull in the control (CW)

Substrate	Growth rate (mm/d)	Mycelial density	Colonization (days)
CW(control)	2.63 ± 0.12a	+++	42.23 ± 1.34a
FC10	2.58 ± 0.14ab	+++	42.63 ± 1.56ab
FC20	2.68 ± 0.11a	+++	41.04 ± 2.13a
FC30	2.56 ± 0.12ab	+++	43.04 ± 1.61ab
NFC10	2.32 ± 0.08d	+++	47.41 ± 2.07d
NFC20	2.38 ± 0.16cd	++	46.22 ± 1.71cd
NFC30	2.47 ± 0.13bc	++	44.53 ± 1.10bc

+ = sparse growth; ++ = moderate growth; +++ = dense growth
Data are means ± SD of thirty replicates; means followed by the same letters in each column are not significantly different ($P>0.05$) according to Duncan's multiple range tests

FCSS and NFCSS as Substitutes for Wheat Bran

As shown in Table 3, the growth rates on the FCSS supplemented substrates were always higher than the rates observed on the NFCSS supplemented substrates and the fastest mycelial colonization (2.76 mm/day) occurred on substrate FW28. But there were no statistical differences ($P>0.05$) among the seven test substrates.

Table 3: Mycelial growth rate, mycelial density, and colonization of *F. velutipes* on substrates containing different ratios of FCSS or NFCSS as a substitute for the wheat bran in the control (CW)

Substrate	Growth rate (mm/d)	Mycelial density	Colonization (days)
CW(control)	2.63 ± 0.12a	+++	42.23 ± 1.34a
FW8	2.75 ± 0.18a	++	40.00 ± 1.16a
FW18	2.69 ± 0.17a	++	40.89 ± 1.83a
FW28	2.76 ± 0.17a	+	38.46 ± 1.03a
NFW8	2.55 ± 0.08a	++	43.13 ± 1.71a
NFW18	2.55 ± 0.21a	+	43.13 ± 1.92a
NFW28	2.64 ± 0.19a	+	41.67 ± 1.47a

+ = sparse growth; ++ = moderate growth; +++ = dense growth

Data are means ± SD of thirty replicates; means followed by the same letters in each column are not significantly different (P>0.05) according to Duncan's multiple range tests

Table 4: Biological yield, commercial ratio, and biological efficiency of *F. velutipes* grown on substrates containing different ratios of FCSS or NFCSS instead of the cottonseed hull in the control (CW)

Substrate	Biological yield (g/bag)	Commercial ratio (%)	Biological efficiency (%)*
CW(control)	437.24 ± 23.12b	90.81 ± 3.48a	106.64 ± 5.64b
FC10	431.36 ± 24.32b	89.92 ± 5.61a	105.21 ± 5.93b
FC20	445.84 ± 27.03a	90.73 ± 4.31a	108.74 ± 6.59a
FC30	413.20 ± 19.20cd	88.68 ± 6.12a	100.78 ± 4.48cd
NFC10	416.08 ± 14.67c	86.74 ± 4.07a	101.48 ± 3.58c
NFC20	414.21 ± 28.15cd	86.15 ± 5.12a	101.03 ± 6.86cd
NFC30	408.43 ± 14.04d	85.53 ± 4.41a	99.62 ± 3.42d

*indicates that biological efficiency was calculated based on the biological yield

Data are means ± SD of thirty replicates; means followed by the same letters in each column are not significantly different (P>0.05) according to Duncan's multiple range tests

The mycelial density became sparse when FCSS and NFCSS were added to the substrate. Regarding the colonization duration, there was a slight reduction (1.34–3.77 d) in the time taken for the mycelia to grow when the FCSS supplemented substrates were compared to the control, while a little extension (less then one day) was observed in the NFCSS supplemented substrates as compared with that of the control. However, there were no statistical differences among these values. From these findings, it can be concluded that both FCSS and NFCSS had little influence on the mycelial colonization rate when they were used as replacements for wheat bran, but they did significantly affect mycelial density.

Biological Yield, Commercial Ratio and the Biological Efficiency of *F. velutipes* Grown on the Different Substrates

FCSS and NFCSS as substitutes for cottonseed hull: Table 4 shows that there were relatively wide variations in mushroom yield among the six supplemented substrates. The highest yield (445.84 g/bag) occurred on substrate FC20, which was statistically different (P<0.05) to the control (437.24 g/bag) and the other five supplemented substrates. It is also evident that the FCSS substrates always produced a higher mushroom yield than the NFCSS substrates when the supplement ratios were the same. There were no statistical differences in the commercial ratios of fruit bodies generated from the seven test substrates. The highest biological efficiency at 108.74% occurred on substrate FC20, and the lowest value at 99.62% was found on substrate NFC30.

FCSS and NFCSS as Substitutes for Wheat Bran

Table 5 shows that using FCSS or NFCSS as a replacement for wheat bran considerably decreased mushroom yield. The decrease content was positively related to the supplementation level. The lowest biological yield was on substrate NFW28 (223.36 g/bag), which was only 51.08% of the control (437.24 g/bag). The comparison indicated that the NFCSS substrates always produced lower yields than the FCSS substrates when the supplement ratios were the same. The commercial ratio of the fruit bodies decreased by 2.29%–11.62% when FCSS or NFCSS were added to the substrate as compared with that of the control. There was a considerable variation of biological efficiency ranging from 54.48% (for NFW28) to 99.52% (for FW8) among the six supplemented substrates, which was statistically lower than the control (106.64%).

Yield Attributing Characteristics of *F. velutipes* Grown on the Different Substrates

FCSS and NFCSS as substitutes for cottonseed hull: As shown in Table 6, there were no statistical differences (P>0.05) in pileus diameters between the seven test substrates. The maximum pileus (0.78 cm in diameter) was measured on substrate NFC10 and the minimum value (around 0.68 cm) was measured on substrates FC10, FC20, NFC30 and the control. A considerable variation of stipe diameter generated from seven different substrates was seen, with the smallest stipe diameter recorded on substrate NFC30 (0.26 cm) and the control (0.25 cm) and the largest observed on substrate NFC10 (0.37 cm). The *F. velutipes* stipe lengths were similar among the three FCSS supplemented and control substrates at nearly 20 cm, which were statistically longer than the stipe lengths (17.92–19.10 cm) recorded on the three NFCSS supplemented substrates.

FCSS and NFCSS as Substitutes for Wheat Bran

Table 7 shows that, with the exception of substrate NFW28, all supplemented substrates produced a larger pileus (the increased size between 0.03–0.14 cm) and a wider stipe (the increased size in the range of 0.04–0.07 cm) compared to the control (0.68 cm sized pileus and 0.25 cm stipe diameter).

Table 5: Biological yield, commercial ratio, and biological efficiency of *F. velutipes* grown on substrates containing different ratios of FCSS or NFCSS instead of the wheat bran in the control (CW)

Substrate	Biological yield (g/bag)	Commercial ratio (%)	Biological efficiency (%)*
CW(control)	437.24 ± 23.12a	90.81 ± 3.48a	106.64 ±5.64a
FW8	408.04 ± 19.64b	88.52 ± 5.20ab	99.52 ± 4.79b
FW18	350.23 ± 13.21c	87.06 ± 4.01abc	85.42 ± 3.22c
FW28	257.11 ± 14.37e	83.94 ± 5.66abc	62.71 ± 3.50e
NFW8	406.66 ± 17.35b	84.67 ± 5.89abc	99.18 ± 4.23b
NFW18	322.72 ± 19.12d	82.75 ± 3.42bc	78.71 ± 4.66d
NFW28	223.36 ± 10.04f	79.19 ± 4.63c	54.48 ± 2.45f

* Indicates that biological efficiency was calculated based on the biological yield

Data are means ± SD of thirty replicates; means followed by the same letters in each column are not significantly different (P>0.05) according to Duncan's multiple range tests

Table 6: Yield attributing characteristics of *F. velutipes* grown on substrates that contained different ratios of FCSS or NFCSS instead of the cottonseed hull in the control (CW)

Substrate	Parameters		
	Pileus diameter (cm)	Stipe diameter* (cm)	Stipe length (cm)
CW (control)	0.68 ± 0.03a	0.25 ± 0.03c	19.92 ± 1.23a
FC10	0.68 ± 0.04a	0.33 ± 0.03ab	19.86 ± 1.63a
FC20	0.67 ± 0.06a	0.30 ± 0.03bc	19.63 ± 1.26a
FC30	0.74 ± 0.04a	0.32 ± 0.02b	19.84 ± 1.67a
NFC10	0.78 ± 0.03a	0.37 ± 0.04a	19.10 ± 1.49b
NFC20	0.75 ± 0.07a	0.28 ± 0.02bc	17.92 ± 2.12cd
NFC30	0.69 ± 0.05a	0.26 ± 0.01c	18.55 ± 1.56bc

* indicates that stipe diameter was measured at the middle position of the stipe

Data are means ± SD of all the fruit bodies from three cultivation bags; means followed by the same letters in each column are not significantly different (P>0.05) according to Duncan's multiple range tests

Table 7: Yield attributing characteristics of *F. velutipes* grown on substrates that contained different ratios of FCSS or NFCSS instead of the wheat bran in the control (CW)

Substrate	Parameters		
	Pileus diameter (cm)	Stipe diameter* (cm)	Stipe length (cm)
CW(control)	0.68 ± 0.03c	0.25 ± 0.03b	19.92 ± 1.23a
FW8	0.73 ± 0.04abc	0.31 ± 0.02ab	18.13 ± 1.34b
FW18	0.82 ± 0.03a	0.32 ± 0.04a	17.43 ± 1.67bc
FW28	0.79 ± 0.03ab	0.29 ± 0.03ab	16.60 ± 2.21c
NFW8	0.71 ± 0.04bc	0.29 ± 0.02ab	17.92 ± 2.30bc
NFW18	0.71 ± 0.03bc	0.31 ± 0.03ab	17.13 ± 1.54bc
NFW28	0.68 ± 0.05c	0.25 ± 0.02b	14.57 ± 1.06d

* indicates that stipe diameter was measured at the middle position of the stipe

Data are means ± SD of all the fruit bodies from three cultivation bags; means followed by the same letters in each column are not significantly different (P>0.05) according to Duncan's multiple range tests

The stipe length varied from 14.57 cm (for NFW28) to 18.13 cm (for FW8) among the six supplemented substrates, which was statistically shorter than the control substrate stipe lengths (19.92 cm).

Approximate Compositions of the *F. velutipes* Grown on the Selected Substrate FC20

The substrate FC20 was considered to be a promising substrate for *F. velutipes* cultivation when the twelve supplemented substrates were compared using mushroom yield, commercial ratio and marketable quality as evaluation indexes. Thus, the content of the major nutritional compounds in the fruit bodies cultivated on this substrate were determined and compared to mushrooms growing on the control substrate. Table 8 shows that the protein, dietary fiber and ash contents were higher in the fruit bodies grown on substrate FC20 than in the fruit bodies grown on the control, with increases by 38.67%, 21.62% and 2.74%, respectively, whereas there was little difference in fat content, both at around 1.84%.

Amino Acid Profiles of *F. velutipes* Grown on the Selected Substrate FC20

Table 9 shows the amino acid composition and content of the mushrooms grown on the FC20 and the control substrates. Sixteen amino acids were detected in the mushrooms produced on the two substrates. The detected amino acid contents were significantly higher in the fruit bodies cultivated on substrate FC20 than in those grown on the control. The most abundant essential amino acid in the fruit bodies from both substrates was valine, and in the case of non-essential amino acids, it was glutamate. The essential amino acid and total amino acid contents of the mushrooms were 6.53% and 13.14% of dry matter, respectively, on substrate FC20, which were higher than those (4.86% and 9.30%) of the control.

Fatty acid Profiles for *F. velutipes* Grown on the Selected Substrate FC20

Table 10 shows that palmitic, oleic, and linoleic acids were the prominent saturated, monounsaturated and polyunsaturated fatty acids, respectively, in the fruit bodies produced on the two substrates. There was no significant difference (P>0.05) in saturated fatty acids content, but a higher unsaturated fatty acids content was measured in the fruit bodies grown on substrate FC20 (0.78 g/100 g dry matter) compared to the control (0.59 g/100 g dry matter). The linoleic and linolenic acid contents were significantly higher in the fruit bodies produced on substrate FC20 than in those produced on the control. The ratio of unsaturated to saturated fatty acids was 2.36 for FC20, but was only 1.74 for the control.

Discussion

As mushroom cultivation continues to expand rapidly in recent years, demand for the cultivation materials such as cottonseed hull and sawdust has increased considerably and correspondingly their price became expensive for mushroom growers. As a consequence,

Table 8: Comparison of approximate major nutrient compound compositions in *F. velutipes* cultivated on the selected substrate (FC20) and the control (CW)

Substrate	Protein (%)	Fat (%)	Fiber (%)	Ash (%)
FC20	18.16 ± 0.07a	1.83 ± 0.01a	9.17 ± 0.03a	6.27 ± 0.07a
CW (control)	13.10 ± 0.21b	1.85 ± 0.01a	7.54 ± 0.05b	6.10 ± 0.06b

Data are means ± SD of three independent samples; means followed by the same letters in each column are not significantly different (P>0.05) according to Duncan's multiple range tests

Table 9: Comparison of the different amino acid contents and compositions in *F. velutipes* grown on the selected substrate (FC20) and the control (CW) (g in 100 g of dried fruiting bodies)

Amino acids	FC20	CW (control)	Amino acids	FC20	CW (control)
Asparagine	0.98±0.02a	0.70 ± 0.02b	Isoleucine*	0.58± 0.01a	0.44±0.01b
Threonine*	0.64±0.02a	0.45 ± 0.01b	Leucine*	0.81± 0.01a	0.61±0.02b
Serine	0.59±0.01a	0.42 ± 0.01b	Tyrosine*	0.74± 0.02a	0.60±0.01b
Glutamate	2.27±0.02a	1.40 ± 0.02b	Phenylalanine*	0.68± 0.02a	0.52±0.01b
Glycine	0.52±0.01a	0.39 ± 0.02b	Lysine*	1.15± 0.01a	0.73±0.02b
Alanine	0.84±0.01a	0.63 ± 0.02b	Histidine	0.31± 0.01a	0.21±0.02b
Valine*	1.31±0.02a	0.97 ± 0.01b	Arginine	0.42± 0.01a	0.27±0.01b
Methionine*	0.62±0.01a	0.54 ± 0.01b	Proline	0.68± 0.01a	0.42±0.01b
Total essential amino acids	6.53±0.12a	4.86 ± 0.10b			
Total amino acids	13.14±0.22a	9.30 ± 0.23b			

* = Essential amino acids

Data are means ± SD of three independent samples; means for each amino acid content between two substrates followed by the same letters are not significantly different (P>0.05) according to Duncan's multiple range tests

Table 10: Comparison of fatty acid compositions in *F. velutipes* fruiting bodies grown on substrate FC20 and the control (g in 100 g dried fruiting bodies)

Fatty acids	FC20	CW
Capric acid $C_{10:0}$	0.07 ± 0.01a	0.07 ± 0.01a
Myristic acid $C_{14:0}$	0.05 ± 0.01a	0.05 ± 0.01a
Myristoleic acid $C_{14:1}$	not detected	not detected
Palmitic acid $C_{16:0}$	0.16 ± 0.02a	0.17 ± 0.01a
Stearic acid $C_{18:0}$	0.05 ± 0.01a	0.05 ± 0.01a
Oleic acid $C_{18:1}$	0.09 ± 0.01a	0.07 ± 0.01a
Linoleic acid $C_{18:2}$	0.49 ± 0.02a	0.36 ± 0.01b
Linolenic acid $C_{18:3}$	0.20 ± 0.01a	0.16 ± 0.01b
Total saturated fatty acid	0.33	0.34
Monounsaturated fatty acid	0.09	0.07
Polyunsaturated fatty acid	0.69	0.52
Unsaturated:saturated ratio	2.36	1.74

Data are means ± SD of three independent samples; means in each row followed by the same letters are not significantly different P>0.05 according to Duncan's multiple range tests

employing alternative materials for mushroom cultivation is potentially required. In the present study, CSS as an abundant byproduct of tea oil processing was first investigated for mushroom cultivation. Fermented CSS was superior to non-fermented CSS for *F. velutipes* cultivation in terms of its effect on mycelial colonization, yield and the commercial ratio and marketable quality of fruit bodies produced. This was probably because the CCS fermentation pretreatment can convert some inhibitory substances that are present in CSS into new compounds that do not inhibit *F. velutipes*.

In the present study, using FCSS and NFCSS as substitutes for cottonseed hull in the substrate for *F. velutipes* production, it was clearly shown that the commercial ratio of fruit bodies was unchanged. This indicates that the fruit body development of *F. velutipes* was not negatively affected by the two supplements. The biological efficiency results (99.62%–108.74%) recorded in this study were comparable to the results reported by Xie *et al*. (2017), but lower than those reported by Rezaeian and Pourianfar (2017), who reported a biological efficiency as high as 226.19% for one substrate. This variation in the biological efficiency may be caused by the differences in strains used, substrate formulation, and environmental conditions (Sánchez, 2004; Gaitán-Hernández and Salmones, 2008; Yang *et al*., 2013).

Supplementation of the basic substrate with nitrogen-rich organic materials is required if mushroom yield and quality are to improve and wheat bran is one of the most commonly used additives (Naraian *et al*., 2009; Moonmoon *et al*., 2011). In the present investigation, with FCSS or NFCSS being as a replacement of wheat bran as nitrogen source in substrates, the biological efficiency was greatly decreased by both additives and the decrease content was positively correlated with their supplement level. Furthermore, the commercial ratio of *F. velutipes* fruit bodies also decreased considerably, indicating that its development was negatively affected by the addition. These results strongly suggest that FCSS and NFCSS are not ideal substitutes for wheat bran when cultivating *F. velutipes*.

The supplements affecting the yield attributing characteristics of mushrooms have been well documented in many studies (Yang *et al*., 2013; Girmay *et al*., 2016). In the present investigation, the substrate NFC10 mushrooms had relatively larger pilei and wider and shorter stipes compared with the control. This may be mainly related to the smaller number of pinheads that had formed on the substrate, which allowed more space for each pinhead to grow in the relatively compact bag opening. The formation of a small number of pinheads in this substrate may be caused by the decreased nutrients due to the use of NFCSS to replace cottonseed hull. Similarly, the formation of fruit bodies of a small cap and short stipe on substrate NFC30 may be due to a severe deficiency of nutrients in the substrate caused by excess supplementation with NFCSS. As *Flammulina velutipes* bodies with relatively smaller pilei, longer stipes, and a median stipe diameter are preferred by consumers. The fruiting bodies produced on the FC20 and control substrates had a high marketable quality.

During mushroom cultivation, the chemical contents of mushroom fruiting bodies are easily affected by the strain genotype, substrate origin, and atmospheric conditions (Harada *et al*., 2004; Xu *et al*., 2016; Li *et al*., 2017). In the present investigation, higher contents of protein, amino acids,

fiber and ash in the fruit bodies were observed grown on the FC20 than on the control. The higher protein content obtained in the FC20 indicated that CSS promotes protein biosynthesis in *F. velutipes*. Different to the above nutritional compositions, fat contents obtained in the two substrates were similar. The observation that there was little variation in fat contents in the *F. velutipes* fruit bodies produced on the two different substrates support the earlier studies with the cultivation of *P. ostreatus*, where fat contents were almost unchanged, even when the substrate components varied considerably (Chang *et al*., 1981; Wang *et al*., 2001).

It is known that fatty acids are important constituents of fungal cells and they have recognized roles as storage materials and as components of the plasmalemma and cell organelle membranes (Pedneault *et al*., 2007). Palmitic, oleic and linoleic acids are the common major fatty acid components in mushrooms (Kavishree *et al*., 2008; Lee *et al*., 2011). Environmental factors, such as nutritional components, oxygen and temperature, are known to affect lipid content and composition in living organisms, including fungi (Losel, 1988; Suutari, 1995). In submerged cultures of medicinal mushrooms, such as *Ganoderma lucidum*, *Cordyceps militari*s and *Antrodia cinnamomea*, plant oils have been frequently used to enhance mycelial growth and exopolysaccharides production (Yang *et al*., 2000; Shih *et al*., 2006, 2007). It has been suggested that the stimulation effect by the oils was due to the partial incorporation of lipids into the cell membrane (Stasinopoulos and Seviour, 1990). In this present study, the linoleic and linolenic acid contents were significantly higher in the fruiting bodies produced on the FC20 substrate than in those produced on the control. This is useful because long-chain polyunsaturated fatty acids, such as linoleic and linolenic acids, are essential for human basal metabolism, and have many beneficial effects on human health (Parikh *et al*., 2005). The higher levels of linoleic and linolenic acids in the fruiting bodies of *F. velutipes* may be mainly due to incorporation into the cell membrane of the linoleic and linolenic acids present in fermented CSS.

Conclusion

The present study demonstrated that fermented CSS was always superior to non-fermented CSS for *F. velutipes* cultivation. The substrate FC20 produced a high mushroom yield while the commercial ratio and yield attributing characteristics of fruit bodies produced did not change significantly. Moreover, the contents of major nutritional component in the fruit bodies produced from this substrate were improved. In conclusion, fermented CSS can be served as an effective and economic substrate for partially replacing cottonseed hulls for *F. velutipes* cultivation, producing fruit bodies with improved nutritional value. The present study also provides an environmental and economic method for reusing the large amounts of CSS produced by tea oil processing plants.

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