Chemosphere 286 (2022) 131477

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Valorisation of biomass and diaper waste into a sustainable production of the medical mushroom Lingzhi *Ganoderma lucidum*

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ARTICLE INFO

Handling Editor: Derek Muir

Keywords:
Waste management
Ganoderma lucidum
Metabolomics
Circular economic
Diaper
Food waste

ABSTRACT

Global solid waste is expected to increase by at least 70% annually until year 2050. The mixture of solid waste including food waste from food industry and domestic diaper waste in landfills is causing environmental and human health issues. Nevertheless, food and diaper waste containing high lignocellulose can easily degrade using lignocellulolytic enzymes thereby converted into energy for the development and growth of mushroom. Therefore, this study explores the potential of recycling biomass waste from coffee ground, banana, eggshell, tea waste, sugarcane bagasse and sawdust and diaper waste as raw material for Lingzhi mushroom (*Ganoderma lucidum*) cultivation. Using 2% of diaper core with sawdust biowaste leading to the fastest 100% mushroom mycelium spreading completed in one month. The highest production yield is 71.45 g mushroom; this represents about 36% production biological efficiency compared to only 21% as in commercial substrate. The high mushroom substrate reduction of 73% reflect the valorisation of landfill waste. The metabolomics profiling showed that the Lingzhi mushroom produced is of high quality with a high content of triterpene being the bioactive compounds that are medically important for treating assorted disease and used as health supplement. In conclusion, our study proposed a potential resource management towards zero-waste and circular bioeconomy for high profitable mushroom cultivation.

1. Introduction

Global waste is expected to increase from 2 to more than 3 billion tons during the period 2016–2050 (The World Bank, 2018a). According to the statistics recorded by the World Bank, the distribution of solid wastes decreases as follows; food wastes (44%) > paper (17%) > plastic (12%) > glass (5%) > rubber and wood materials (2% each) (The World Bank, 2018b). Around 20 billion diapers are disposed yearly in the United States (US) encountering about 3.5 million tons of solid waste (Wright, 2018). Data from Malaysia shows that each child use about 6000–9600 diapers during its first 2.5 years, which equal 1.7 million tons of diaper waste annually (Khoo et al., 2019; Sheila, 2016). In a number of Europe and Asia countries, diaper waste is incinerated (Kim and Kim, 2018). Aside from diaper waste, about 1.3 billion tons of edible food is wasted word-wide each year (De Clercq et al., 2018).

Diaper and food waste disposal and incineration necessitate extensive spaces, which are frequently opposed by the surrounding community whereas the incineration through full oxidative combustion at high temperature ranged from 900 to 1000 °C emit toxic compounds such as Dioxin and PAHs as well as greenhouse gases (Haikal, 2016; Moya et al., 2017). The incineration system is more complex and expensive process compared to landfill therefore often used in developed countries (Khoo

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https://doi.org/10.1016/j.chemosphere.2021.131477
Received 8 March 2021; Received in revised form 21 June 2021; Accepted 6 July 2021
Available online 10 July 2021

et al., 2019), example of advance incineration system are sanitary landfill and modern waste incineration (Ali et al., 2021; Sadi and Arabkoohsar, 2019; Seibert et al., 2019). Sanitary landfill with specific features such as methane gas recovery well and leachate collection pipes had been developed to minimise the environmental issues (Imron et al., 2019; Millis, 2017). Modern waste incinerator systems are vary among countries such as in Japan, Sweden, Finland and Italy, the system are designed to manage disposed waste in the same time regenerate methane gases as free energy by-product (Adekomaya and Majozi, 2020; Dong et al., 2018; Falcone and De Rosa, 2020; Ritchie and Roser, 2020).

Sustainable waste management using naturally-occurring microbes for waste degeneration have been explored but unfortunately the process is slow and inefficient while posing a zoonotic risk (Gupta et al., 2018; Torrente-Velásquez et al., 2020). Macro-fungus such as mushrooms are new alternatives for efficient waste degradation as enzymes degrade recalcitrant macromolecules into many smaller fragments which essential to be use in the growth and development of mushroom (Gupta et al., 2018; Zavarzina et al., 2018). Among cultivated mushroom, Lingzhi mushroom (*G. lucidum*) represents one of the high-commercial value mushrooms found in more than 100 Chinese medical products (Li et al., 2016). The Lingzhi contains bioactive compounds, including polysaccharides, peptidoglycans and triterpenes as well as high nutritional values such as amino acids, fibre, low in fat, and vitamins B1, B2, B12, C, D, and E (Nakagawa et al., 2018; Wang et al., 2018). The species is rare in the wild and given their high demands growing the market for *in situ* cultivation using wood logs and substrates bags (Mehta et al., 2014; Rolim et al., 2014). The production of fruiting bodies in the wood logs produce a better quality but requirement of about six months to culture (Li et al., 2016). Therefore, the substrate bag cultivation is more favourable as mushroom hyphae grows faster and harvest of fruiting bodies happen more quickly (Li et al., 2016). Since biomass from the industry or agricultural waste contain high lignocellulose sources, including cellulose, hemicellulose, and lignin it is suitable as source for Lingzhi cultivation (Zhou, 2017). Nevertheless, feasibility of diaper waste and food waste as growth substrate for valorisation of medical mushroom and its quality and safety production have never been studied before. Therefore, this study offer an opportunity to increase the profit margin of farmers and creating a circular economic for sustainable mushroom industry.

2. Methodology

2.1. Activation of old Lingzhi mycelium stock on agar media

The mycelium of Lingzhi obtained from Gano Farm Sdn. Bhd., Tanjung Sepat, Malaysia was cultured in the potato dextrose agar (PDA) inside the laminar airflow. The mycelium allowed to develop on PDA in within 5–7 days in an incubator at 30 ± 2 °C.

2.2. Preparation of mother spawn

The arising mycelium is kept on dried corn for longer storage, also known as mother spawn. Dried corn and barley are widely used as the spawning agent because of its highly nutritive contents and spherical in shape, which can maximize the surface area in contact with the mycelium and result in a faster spreading of mycelium (Miles and Chang, 2004). First, dried corn was washed and soaked in water overnight to allow the imbibition of water to soften the grains. About 200 g of corn was weighed and put into a 250 mL conical flask. The conical flask was covered with cotton balls on the top of the flask and then autoclaved for 15 min at 121 °C (Nam et al., 2018). The PDA mycelium is then inoculated onto the corn media and kept in the incubator pre-set to 30 °C ± 2 °C for mycelium spreading. After one week, the fully colonized mother spawn was ready to be used for mushroom cultivation.

2.3. Collection of food waste and diaper waste

The food wastes such as sugarcane bagasse (SB), banana skin (BK), coffee grounds (CG), eggshell (ES), and tea waste (TW) are collected from local restaurants and allowed to dry in an oven preheat at 80 °C for 48 h to completely remove its water content (Ma et al., 2019). The raw waste materials are then blended into powder form with a conventional blender.

The diaper wastes are collected from a kid nursery, Taska Juhani from Gong Badak, Terengganu. This study only use disposable diapers contained urine without faeces of kids below 2 years old. For hygienic purposes, autoclave process of diaper wastes under 121 °C for 15 min is conducted to kill microbe before diaper core is separated from the outer layer plastic wool and dried subsequently for 3 days at 90 °C in the oven (Ma et al., 2019).

2.4. Chemical composition and nutrient profile of the food waste and diaper waste

The chemical composition of food and diaper wastes for element carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) were analysed by using element analyzer (Flash Smart, Thermo-Fisher Scientific, United States) according to the protocol as reported in Lam et al. (2019). Each waste samples were milled into smaller size using mortar and filtered through a 125 μm size sieve. The milled samples were dried at 105 °C to completely remove moisture. The waste samples are then loaded into furnace at 1000 °C with oxygen and Helium gas (He) as carrier gas. Quantitative detection of C, H and S contents in weight percent are obtained from the selective infrared absorption detectors, where the N contents was measured by thermal conductivity detector (Lam et al., 2018).

The lignocellulosic contents such as lignin, hemicellulose and cellulose are all analysed using Fibre-thermal analyser (Gerhardt Analytical System, Germany). About 1 g of milled waste sample is weighted and put into Gerhardt system fibre bag and loaded into the Fibre-thermal machine (Fettweis and Kühl, 2015).

Inductively Couple Plasma-Optical Emission Spectrometer (ICP-OES) (7300 DV, PerkinElmer PE Optima, America) is used for nutrient profiles analysis phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), calcium (Ca), iron (Fe), sodium (Na), and baron (B). About 1 g of each waste material is added to 2 mL of concentrated HCl in a crucible and allowed to evaporate on a hot plate. The dried samples in the crucibles were dissolved in 10 mL of 20% nitric acid and put in the water bath for 1 h. The mixture is diluted with 100 mL distilled water prior loading into the ICP-OES analysis (7300 DV, Perkin El).

2.5. Preparation of mushroom growth substrate

Different ratio of waste composition substrates were prepared as in (Table 1). To prepare the growth substrate with waste baby diaper formulation, the dried diaper cores were immersed in water to allow complete water absorption. The substrates were mixed and packed in triplicate with final weight of 600 g per block (Table 1a and b). The pH is adjusted to 6.8 ± 0.4 with addition of calcium carbonate by using soil pH meter (Takemura Denki Seisakusho, Japan) and the moisture is maintained at 55%–60% (Khan et al., 2013). All substrate blocks were then sterilized in autoclave machine at 121 °C for 60 min.

2.6. Inoculation of mother spawn onto growth substrate

The mother spawn of Lingzhi was transferred onto the growth substrate block and the mouth of substrate block was covered with a cap for limited air exchanged to support mycelium spreading and preventing other microbe contamination. The mushroom house was cleaned by 70% alcohol to prevent the occurrence of contamination by fungi or

Table 1
The formulation of Substrate Block.

a. Growth substrate plus waste baby diaper formulation

Treatment	Composition of growth substrate
C	Commercial growth substrate (sawdust: rice bran: calcium carbonate powder; 100:10:1)
D1	Commercial growth substrate +1% (10 g/kg) of diaper
D2	Commercial growth substrate +2% (20 g/kg) of diaper
D3	Commercial growth substrate +4% (40 g/kg) of diaper
D4	Commercial growth substrate +5% (50 g/kg) of diaper

b. Growth substrate formulated with food and diaper waste.

Treatment	Description					
	Waste Composition					
	Diaper (g/kg)	Coffee ground (g/kg)	Banana skin (g/kg)	Eggshell (g/kg)	Sugarcane bagasse (g/kg)	Other substrates (g/kg)
TA	500	200	100	100	100	–
TB	530	100	–	60	110	110 tea waste
TC	550	–	30	120	180	120 tea waste
TD	380	200	90	130	110	–
TE	250	100	50	50	50	500 commercial substrate block

bacteria from the environment. The Lingzhi substrate blocks were kept on the shelf of the mushroom house, the temperature was maintained at 25–29 °C and the humidity was maintained within 70–80% by spraying water once a day on the surrounding of mushroom house and constantly checked using a digital hygrometer and thermometer probe.

2.7. Growth performance and yield of Lingzhi mushroom

The growth performances of Lingzhi were observed by its cumulative mycelium spreading rate (CMSR), duration to complete colonization, duration to harvest and total mushroom yield (Sindhu et al., 2016). The cumulative mycelium spreading rate was observed in a weekly basis for 6 weeks and calculated using the formulation in Eq. (1):

$$\mathrm{CMSR} = \frac{\text{average mycelium length on mushroom growth substrate}}{\text{total length of mushroom growth substrate}} \times 100 \quad (1)$$

Duration to complete colonization is counted as day needed to achieve full mycelium colonization starting from the zero day of mother spawn inoculation. Once full mycelium colonization is achieved, removing the cover cap of substrate block to allow the emerge of primordia. The Lingzhi fruiting bodies were harvested once the colour of fruiting bodies turn reddish. The duration to harvest was calculated as the total days taken from the zero day of spawn inoculation to harvest. Total mushroom yield is the total weight of Lingzhi mushroom harvested once they reached maturation. The diameter, thickness and circumference of mushroom fruiting bodies from different substrate blocks were measured. Weighting the remaining substrate blocks after harvest the mushroom to calculate the weight reduction of every block. The biological efficiency of the fresh Lingzhi mushroom was calculated as in Eq. (2).

$$\text{Biological efficiency } (\%) = \frac{\text{weight of fresh mushroom harvested}}{\textit{weight of dry substrate}} \quad (2)$$

2.8. Analysis of bioactive compounds from Lingzhi mushroom

The fresh Lingzhi samples were harvested and deep-frozen in liquid nitrogen and immediately freeze-dried in Freeze Dry System (Labconco™ Benchtop 208/230v 60 Hz Model, Fisher Scientific, United State). For metabolites extraction, crushing about 500 mg of Lingzhi using mortar and pestle and extracting using 100% methanol and water in 1:1 ratio for polar metabolites, while 99% absolute chloroform (ACS graded, Sigma Aldrich) is used for organic metabolite extraction. The mixture was vortex prior centrifugation process at 4000 rpm for 20 min, the upper layer and lower layer samples were withdrawn carefully and kept in a separated vial. The samples were stored in −80 °C fridge prior to metabolomic detection.

2.9. Gas chromatography mass spectrometry (GC-MS) acquisition

The volatile extracts from chloroform were further analysed with online-linked GC-MS. The analysis was performed using Agilent GC-MSTM system linked with mass selective detector (6890 N + 5975C, Agilent Co., Ltd, USA). An elastic quartz capillary column HP-5MS (30 m × 250 μm x 0.25 μm) was used in the system with high purity helium as carrier gas at flow rate of 1 mL/min. Setting the temperature program from 50 °C and slowly increasing to 250 °C at the rate of 10 °C/min. The injection temperature was set on 250 °C and then continue raise the temperature to 280 °C at a rate of 5 °C/min. The program scan mass of MS was ranged from 30 to 600 atomic mass unit (amu) with the ionization current of 150 μA electron ionization and 70eV of ionization voltage. The program setting for the quadrupole and ion source temperature were 150 °C and 230 °C respectively.

2.10. Liquid chromatography mass spectrometry (LC-MS)-QTOF acquisition

Phytochemical analysis of Lingzhi extracts were carried out using auto sample AcquityUPLC system equipped with a binary solvent attach to a photodiode-array (PDA) detector. The column used is Acquity UPLC BEH C8 (2.1 × 100 mm, 1.7 μm particle size) coupled to a quadrupole time-of-flight (QTOF) mass spectrometer (Vion IMS LCQTOF MS, Waters, United State) equipped with electrospray ionization (ESI). Mobile phases are acidified water (0.2% acetic acid, v/v) plus acetonitrile with flow rate setting at 0.4 mL/min throughout all the gradients. The experiment temperature is set to 40 °C and the injection volume is 2 μL. The operating parameters are as below: source temperature of 120 °C, capillary voltage of 2.0 kV (ESI +), desolvation gas flow of 800 L/h under desolvation temperature of 550 °C. In MS^E mode, trap collision energy of low energy function is set at 4 eV, while ramp trap collision energy of high energy function is set at 10–40 eV. Spectral data are acquired by full scan in a mass range of m/z 50–1000. Data acquisition and processing are referred to elemental compositions of the precursors, the most rational molecular formula was sought in chemical databases such as Traditional Medicine Library (UNIFI 1.7) (waters, Manchester, U.K.).

2.11. Weight reduction of growth substrate

The weight reduction of growth substrate is an indicator to reflect the biodegradation rate of biomass materials from the food waste and diaper waste. The initial weight of growth substrate before mycelium inoculation and the final weight of growth substrate after harvesting the mushroom's fruiting bodies are measured and calculated by using Eq (3).

$$\text{Weight reduction } (\%) = (\text{IW} - \text{FW})/\ \text{IW} \times 100 \quad (3)$$

2.12. Statistical analysis

Statistical Package for Social Science (SPSS) software (SPSS Version 23, IMB Worldwide, USA) was used for the initial statistical analysis. In the SPSS software, all the triplicate data for each treatment obtained in the experiments such as mineral and lignocellulose content of waste materials, mycelium spreading rate, mushroom yield and size were firstly checked for descriptive normality test and then the data were reported as mean ± standard deviation (SD). The data were normally distributed when the skewness and kurtosis statistical data from descriptive test fall within z value = ± 1.96 (Faruk, 2019), where the skewness and kurtosis statistical data is calculated from each variable in the formula of mean/standard errors. The normally distributed data were then tested with one-way analysis of variance (ANOVA) for discrimination analysis. In the ANOVA analysis, the homogeneity variance test and Post Hoc Test using equal variances assumed Tukey's HSD, where n = 3 for each parameter were conducted. The treatments that fall in the different homogenous subset group and Tukey's HSD Post Hoc Test with $p < 0.05$ denoted for significantly difference among the substrate treatment.

The spectra obtained from GC-MS analysis were pre-processed for baseline and phrasing correction using MestReNova software (Mnova) and principal component analysis (PCA) was performed in SIMCA 13+ software (Ma et al., 2020).

3. Results and discussion

3.1. Chemical composition and nutrient profile of waste material

Lignocellulose and nutrient profile analysis of waste materials is shown in Table 2. The diaper waste also recorded high contents of minerals in both diaper and food wastes (Table 2). The high lignocellulose content of diaper cores suggests a potential for mushroom cultivation through lignocellulase enzymatic reactions (Koutrotsios et al., 2019). Previous studies report that the high lignocellulose content in cardboard and chopped office paper promote denser mycelium biomass and higher oyster mushroom yields (Mandeel et al., 2005). Shalahuddin et al. (2018) report a high NPK content in mushroom substrates significantly shortens mycelium-spreading rate and enhance the growth of fruiting bodies of oyster mushroom *Pleurotus ostreatus*. One of the basic criteria for a good mushroom substrate is the content of carbohydrate and N to support the growth of mushroom (Ogundele et al., 2014). Substrates with high nutrition content shorten the mycelium-spreading period due to a faster colonization of the whole mushroom substrate compared to those with low nutritional value (Sofi et al., 2014). Contrarily, deficiency of nutritional value could be the cause of poor mycelia densities and high risk of fungus contamination. Excess N value in mushroom substrate results in high lignin degradation lead to mycelium inhibition (Bellettini et al., 2019).

3.2. Mycelium spreading performance

Mycelium in substrate blocks containing diaper waste (D1-D4) is significantly faster in term of colonization (3–4 week) compared to the control (5 week) (Fig. 1a). As high humidity >80% inhibit the growth of mycelium and promote microbial contamination, the use of diaper waste containing superabsorbent polymers (SAP) hydrogels controls water content thereby promoting Lingzhi growth (Islam et al., 2017). Another study shows that adding SAP improves seedling and root growth of *Populus euphratica* and corncob (El-Rehim et al., 2004). In agriculture, SAP enhance plant growth through improved soil permeability and density and boosting the infiltration rate of water and increasing aeration and reducing irritation frequency of soil (Shahidian et al., 2010). The D3 and D4 substrates significantly shortens the period for mycelium to achieve full colonization and maturation period to harvest i.e. within 59 days (Fig. 1b). This is approximately 1–4 weeks shorter when comparing the harvest period of *G. lucidum* with previous studies and hence supplying diaper waste substrate with 4–5% of diapers as in D3 and D4 substrate could promote the growth of *G. lucidum* mushroom (Gurung et al., 2012; Peksen and Yakupoglu, 2009).

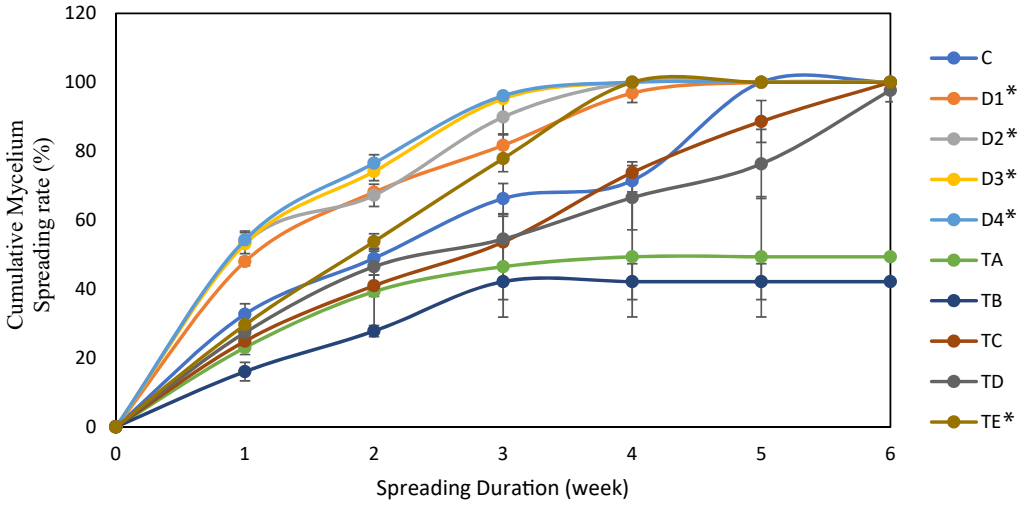
The TE substrate significantly boost mycelium spreading compared to the control and other substrate formulation (Fig. 1a). The highest

Table 2
Mineral, elemental compound and lignocellulose contents of waste collected from various type of waste. The data are presented in means ± SD (n = 3).

Type of Wastes	Diaper core	Coffee ground	Eggshell	Banana skin	Tea waste	Sugarcane bagasse
Mineral contents (mg/kg)						
Nitrogen (N)	23,997 ± 826	27,683 ± 650	21,510 ± 373	10,143 ± 1712	5168 ± 1236	1332 ± 176
Phosphorus (P)	2428 ± 130	895 ± 21	2837 ± 196	973 ± 68	4186 ± 1318	607 ± 137
Potassium (K)	40,516 ± 3371	4139 ± 252	6688 ± 587	734 ± 537	3698 ± 368	2120 ± 85
Sodium (Na)	91 ± 12	76 ± 15	45,938 ± 4504	913 ± 358	453 ± 64	29 ± 8
Magnesium (Mg)	1308 ± 103	1082 ± 35	325 ± 25	2410 ± 127	2015 ± 163	260 ± 12
Calcium (Ca)	1463 ± 35	895 ± 133	957 ± 82	70 ± 63	8456 ± 806	418 ± 39
Iron (Fe)	33 ± 2	39 ± 7	130 ± 67	122 ± 92	211 ± 22	40 ± 24
Zinc (Zn)	26 ± 2	11 ± 2	10 ± 2	9 ± 1	32 ± 1	22 ± 1
Copper (Cu)	3 ± 0	17 ± 1	2 ± 1	1 ± 0	20 ± 4	4 ± 0
Manganese (Mn)	159 ± 8	27 ± 2	2 ± 1	1 ± 1	930 ± 74	30 ± 1
Baron(B)	24 ± 5	7 ± 0	3 ± 1	1 ± 1	4.67 ± 1	−3± 0
Elemental analysis (%)						
Carbon (C)	36.85 ± 5	50.59 ± 1	11.53 ± 1	32.5 ± 6	43.55 ± 3	39.87 ± 0
Hydrogen (H)	5.68 ± 1	7.4 ± 1	0.44 ± 0	4.39 ± 1	5.55 ± 0	5.43 ± 1
Nitrogen (N)	1.11 ± 0	2.12 ± 0	0.86 ± 0	2.39 ± 1	2.25 ± 2	0.15 ± 0
Sulphur (S)	0	0	0	0	0	0
Lignocellulose contents (%)						
Hemicellulose	14.46 ± 3.5	34.43 ± 7.23	N/A	16.38 ± 2.81	18.79 ± 0.84	21.51 ± 0.72
Cellulose	66.69 ± 7.45	23.58 ± 0.27	N/A	18.40 ± 0.32	22.04 ± 2.47	30.40 ± 3.42
Lignin	4.59 ± 0.68	29.59 ± 2.82	N/A	23.60 ± 0.75	24.36 ± 3.25	14.16 ± 9.6
Others	14.26 ± 1.28	12.60 ± 2.75	N/A	41.62 ± 3.02	34.81 ± 0.21	33.93 ± 4.84

*The lignocellulose of eggshell is not available (N/A) as it doesn't contain any fibrous contents.

a



b.

Treatment	Mycelium spreading in six weeks (%)	Day to complete colonization (days)	Day to harvest (days)
C	**100±0.00** [a]	35±1 [c]	73±2 [c]
D1	**100±0.00** [a]	33±0 [b,c]	73±1 [c]
D2	**100±0.00** [a]	29±0 [a,b]	66±1 [b]
D3	**100±0.00** [a]	**24±0** [a]	**59±0** [a]
D4	**100±0.00** [a]	**25±0** [a]	**59±0** [a]
TA	49.37±1.42 [b]	N/A	N/A
TB	42.16±10.08 [b]	N/A	N/A
TC	**100±0.00** [a]	43±3 [d]	87±0 [d]
TD	97.67±3.33 [a]	53±1 [e]	91±1 [d]
TE	**100±0.00** [a]	**29±1** [a,b]	71±1 [c]

Fig. 1. Growth. **a)** Cumulative mycelium spreading rate of *G. lucidum* cultivation in different substrate blocks. **b)** The growth performance of mushroom (mycelium spreading rate, day to complete colonization and day to harvest). All data are presented in means ± SD, n = 3 and the alphabetic letters or * denotes for the significant different at $p \leq 0.05$.

mycelium rate of TE among all food waste derived substrates was probably due to the addition of the sawdust in the substrate composition. Sawdust is well known for the best substrate used in the mushroom cultivation and it had been commercially used by most of the mushroom developers (Liang et al., 2019; Ogundele et al., 2014). Contradict result was observed in formula TA and TB (no sawdust were added in the media formulation), the mycelium spreading was observed terminated after 3 weeks so no output was recorded (Fig. 1 a and 1b). This phenomenon might probably be related to unfavourable environment caused by excess nutrients that change the pH of subtract block (Simonic et al., 2008; Tang et al., 2009). The acidification of substrate is critically influenced by the available minerals and affecting the mycelial density (Anna et al., 2004). According to Tang et al. (2009), the valubale metabolite from medical Lingzhi mushroom is inhibited at pH below 3. Moreover, high nitrogen fertilizers might cause alteration of the metabolic composition of the mycelium biomass and hence cease the mycelium spreading process (Sperling et al., 2019).

Table 3
The yield performance of Lingzhi cultivated using different substrate blocks. The data are presented in means ± SD (n = 3) and showed significant difference at $p \leq 0.05$.

Treatment	Diameter of fruiting bodies (cm)	Thickness of fruiting bodies (cm)	Circumference of fruiting bodies (cm)	Mushroom Yield (g)	Biological efficiency (%)
C	9.3 ± 1 [b,c,d]	2.4 ± 0 [a]	25.8 ± 6 [a]	42.00 ± 5 [b,c]	21.01 ± 6 [b,c]
D1	11.2 ± 0 [a,b]	3.5 ± 1 [a,b]	27.2 ± 1 [a]	58.70 ± 2 [a,b]	29.12 ± 2 [a,b]
D2	**12.5 ± 0** [a]	**4.1 ± 1** [a,b]	**28.5 ± 4** [a]	**71.45 ± 4** [a]	**36.01**±3[a]
D3	8.7 ± 0 [b,c,d]	4.6 ± 1 [b]	23.3 ± 3 [a]	43.96 ± 2 [b,c]	21.98 ± 1 [b,c]
D4	7.7 ± 0 [c,d]	3.7 ± 1 [a,b]	19.5 ± 4 [a,b]	36.43 ± 11[c,d]	18.22 ± 5 [b,c,d]
TA	N/A	N/A	N/A	N/A	N/A
TB	N/A	N/A	N/A	N/A	N/A
TC	6.8 ± 0 [d]	2.5 ± 0 [a,b]	23.8 ± 1 [a]	31.01 ± 3 [c,d]	15.44 ± 3 [c,d]
TD	4.7 ± 2 [d]	3.4 ± 1 [a,b]	13.2 ± 2 [b]	17.43 ± 6 [d]	8.76 ± 5 [d]
TE	10.0 ± 2 [a,b,c]	3.3 ± 1 [a,b]	22.5±2[a,b]	48.4±2[a,b]	24.20 ± 21 [b,c]

3.3. Yield of Lingzhi mushroom

D2 substrate significantly yield more Lingzhi mushroom biomass compared to control with an average weight of 71.45 g per substrate block (Table 3). Even though the biomass mushroom harvest from D3 and D4 substrate blocks are faster than for D2, the growth potential of D3 and D4 is only 50% of D2. Higher biological efficiency in mushroom cultivation is the efficiency of mushroom production in relative to the mushroom substrate used. It is a critical parameter for mushroom farmers to check on the overall efficiency and manufactural benefit in mushroom production (Shields, 2018). The highest biological efficiency is seen in substrate block D2 (36%), which is significantly higher compared to commercial control substrate blocks that only recorded 21.01% growth. The biological efficiency comparison to other substrate such as 27.5% for poplar substrate (Jandaik et al., 2013), 10% for wheat bran (Rashad et al., 2019) and 5% malt substrate (Azizi et al., 2012) had further proved that D2 with sawdust and diaper core substrates are much productive. Overall, D2 was the most optimal growth substrates for mushroom growth in this study after consideration of all the growth aspects in term of mycelium spreading rate, shape and size of mushroom, mushroom yield, and biological efficiency.

3.4. Principle component analysis (PCA)

PCA is an unsupervised technique that used to reduce the dimensionality of high dimensional datasets such as mass spectra obtained from GC-MS analysis, in the meantime preserve the original distribution structure that showed inherent relationship to the original dataset (Packt, 2019). In this study, PCA performed to give an overview interpretation of metabolite profile of Lingzhi fruiting bodies cultivated in different substrate blocks (Fig. 2). The 2D score scatter plot reveals that the Lingzhi metabolic profile through the cultivation in subtract blocks C, D2, D3, D4 and TE are quite similar (Fig. 2a). The 3D PCA score scatter plot show that C, D2, D3 and D4 are closely clustered indicating that adding diaper waste leads to the production of secondary metabolites similar to the control (Fig. 2b). The stacking spectra of D2 being the most efficient growth substrate show no extra suspicious peaks indicating that using diaper waste does not cause any production of unwanted secondary toxins thereby being safe as human consumption (Fig. 2c). In addition, the consistency in metabolites production throughout three different batches of cultivation reflect D2 as a suitable growth media for Lingzhi production.

3.5. Metabolomic profiling of triterpenes

Lingzhi mushrooms contains various bioactive compounds including a wide range of polysaccharides, triterpenes, nucleosides, minerals and trace elements (Taofiq et al., 2017). Nevertheless, the inconsistency and low quantity of bioactive compounds is a challenge to the industry due to different production methods used including type of substrates and different quality of mushroom strain used especially the repeated subculturing mycelium and mushroom spawn are commonly seen in the mushroom industry (Galor et al., 2011; Hapuarachchi et al., 2018). By using UPLC-QTOF-MS Systems to profiling the triterpenes metabolites, at least 113 and 101 type of triterpenes metabolites detected from methanol and chloroform extract of D2 fruit body, respectively, which is slightly higher than found for methanol (100) and chloroform (78) in the fruiting body of control cultivated from commercial block. This is probably due to adding of diaper waste provide huge amount of degraded cellulose for fruit body formation. The quality of triterpenes metabolites in control and D2 were identified and compared showing that D2 is an ideal substrate formulation for the cultivation of Lingzhi mushroom (Fig. 3, Table 4).

Differences in triterpene functional groups including ganoderic acid (GAs), ganederic acid, lucidenic acid, poricoic acid, and saponin derivative triterpene among C and D2 extracts have been detected (Table 4 & Supplementary Material 1). These triterpenes have remarkable therapeutic effects that includes treatment of prostate cancer, fatigue syndrome and hepatitis among other (Liang et al., 2019). There are about 14 type of GAs detected in extracts of Lingzhi mushroom fruiting bodies seen in Table 4. GAs are classified based on their functional group including acetyl, carbonyl and hydroxyl group found in A, C2, U,V and X type were only detected in control treatment whereas GAs types such as B, Ma, R and β were detected in D2 treatment (Gill et al., 2018) (Table 4). The variation of triterpene compounds detected in control and D2 might probably due to the metabolite changes in D2 substrate with high nitrogen content seem to alter the bonding formation which trigger the different functional groups in ganoderic acid (Zhu et al., 2019). This high nitrogen contents influence nitrogen metabolism genes which is an important transcription factor for the biosynthesis of GAs (Zhu et al., 2019). GAs are the most significant triterpenoid produced from Lingzhi with antihepatotoxic, having antihypertensive properties, suppress proliferation of breast cancer cells (Liu et al., 2009; Xu et al., 2010; Zhou et al., 2006). GAs had reported to be useful in inhibiting the formation of tumor cells such as in colorectal carcinoma cell (HCT-116 cells) human hepatocellular cell (HuH-& cells), HepG2/ADM cells, and breast cancer cells (Li et al., 2005; Liu et al., 2015; Yang et al., 2018). To date, GAs are still mainly extracted from Lingzhi fruiting bodies (Dong et al., 2019; Ren et al., 2020). Therefore, the detection of a wide variety of GAs in D2 cultivation show a sustainable production of these natural compounds.

3.6. Waste reduction of mushroom substrate

Upon the completion of the mushroom cultivation, D2 showed the highest weight reduction of subtract block about 70% which indirectly reflected about 70% of waste reduction during the bio-degradation process in mushroom cultivation (Fig. 4). It is estimated that more than 104.2 billion tons of waste subtract block will be generated from mushroom industry which will be managed through dumping to the landfill in 2026 (Khoo et al., 2020), hence the high waste reduction achieved in this study represent that 70% reduction to landfill. The accumulation of spent mushroom substrate in landfills have been reported to cause severe environmental issue such as the release of greenhouse gas (Innocenti et al., 2017) and breeding ground for insect pests (Najafi et al., 2019). Therefore, the D2 cultivation using 2% of diaper core with sawdust is an alternative to current cultivation method that clearly reduces the impact to environment. In addition to this, the spent substrate mushroom blocks could also be used in plywood/bio-board production thereby creating a zero-waste circular--economy mushroom industry (Khoo et al., 2020).

4. Conclusion

This study shows the potential valorisation of recycling food and diaper waste to generate Lingzhi mushroom growth sustaining zero-waste production. The addition of diaper core up to 5% had resulted in higher mycelium spreading rate. The substrate D2 owned highest mushroom yield among the growth substrate, which indicate that addition of 2% diaper cores can act as good moisture control agent and nutrient booster for Lingzhi mushroom cultivation. Furthermore, the metabolite profiling from LCMS and GCMS proven that the high quality of Lingzhi produced for market demand. The market for powder Lingzhi is expected to increase at 8% from 2021 to 2027 but the output is reduced by the COVID-19 pandemic and shortage of workers. Therefore, the introduction of this method could provide site income for farmers while providing impressive 73% of waste reduction while reducing the pressure of solid waste generated to landfill and significantly contribute to the positive impact to our environment. Therefore, using mushroom cultivation as waste management strategy is a smart move as it is completely green and effective to degrade the recalcitrant plant cells that required chemical pre-treatment or otherwise need a long period to degrade in landfill. This formulation has also been applied in

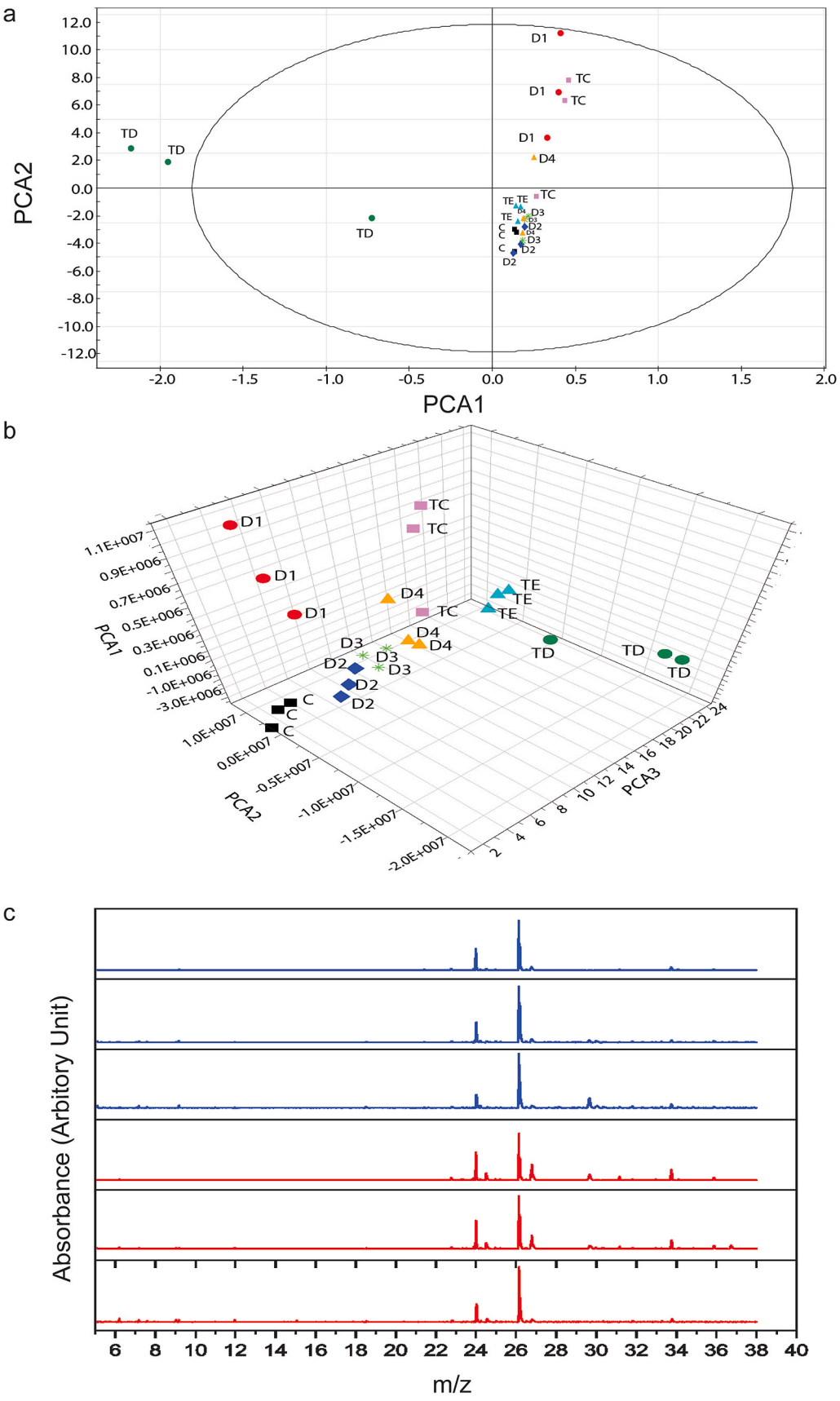


Fig. 2. Multivariate data analysis of GC-MS spectra from Lingzhi fruit body. a) PCA 2D scatter plot; b) 3D scatter plot; c) Stacking spectra of 3 D2 and 3 control replicates. PC1 and PC 2 represented the first two principal components of all 24 sample treatments.

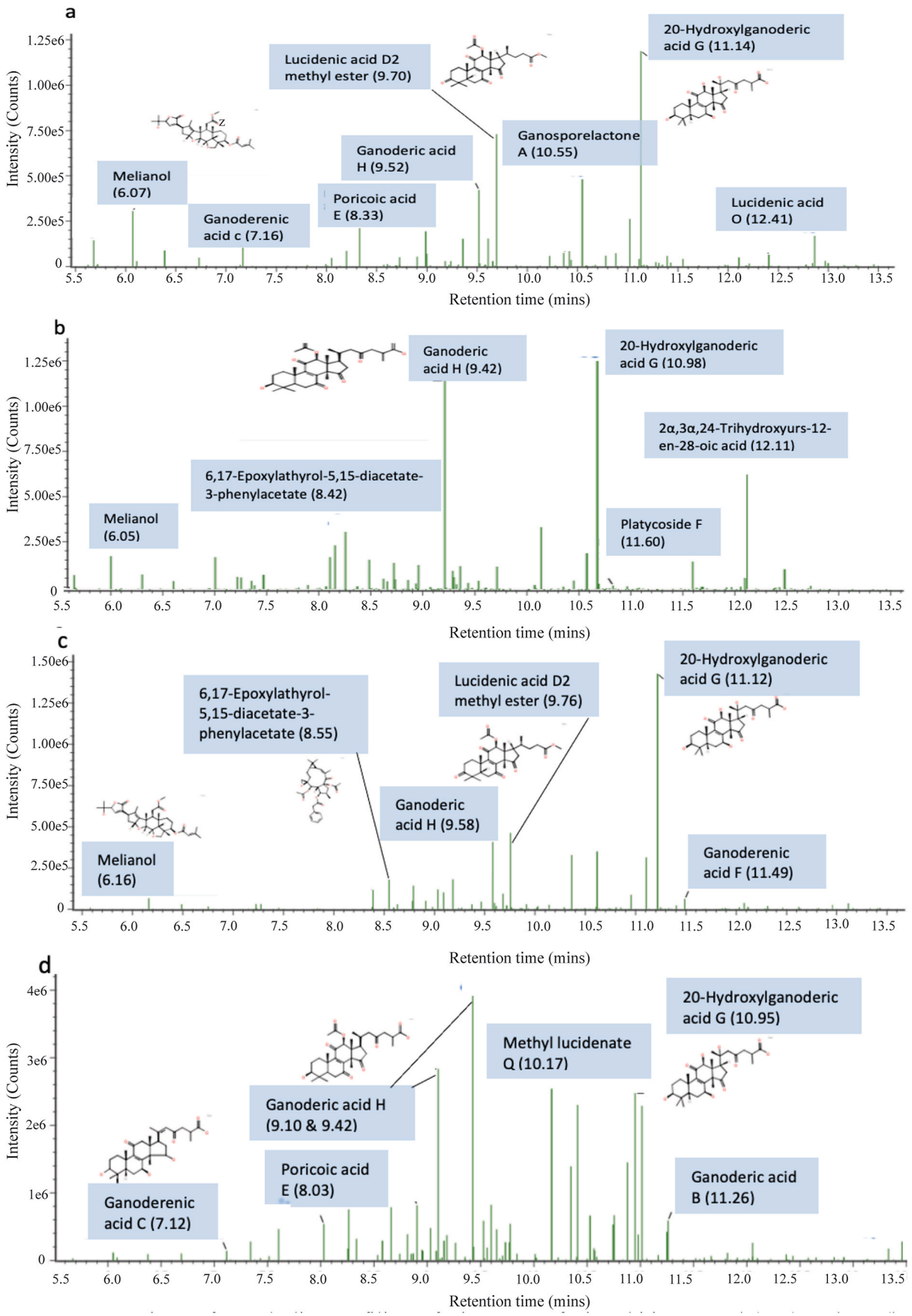


Fig. 3. Comparison of metabolite profiling of triterpene of Lingzhi in control (a, c) and D2 (b, d). a&b: chromatogram of methanol extract and c&d: chromatogram of chloroform extract detected by using UPLC-QTOF-MS System.

Table 4
Comparison of ganoderic acid (GAs) in Lingzhi harvested from Control and D2 by UPLC-QTOF-MS System.

Identified Triterpene compounds	Rt (min)	Measured *m/z*	Adducts	Molecular formula	Extract types			
					CC	CM	DC	DM
Ganoderic acid								
Ganoderic acid A	6.49	517.32	+H	$C_{30}H_{44}O_7$	–	✓	–	–
Ganoderic acid B	9.16, 11.26	539.30, 539.30	+Na, +Na	$C_{30}H_{44}O_7$	–	–	–	✓
Ganoderic acid C2	7.12	557.31	+H, +Na, +K	$C_{30}H_{46}O_8$	–	✓	–	–
Ganoderic acid G	7.60	555.29	+H, +Na, +K	$C_{30}H_{44}O_8$	–	✓	–	✓
Ganoderic acid H	9.10, 9.52, 9.58, 9.42	573.31, 573.31, 573.31, 595.29	+H, +H, +H, +Na	$C_{32}H_{44}O_9$	–	✓	–	✓
Ganoderic acid MA	10.38, 11.43, 11.49	573.38, 573.38, 573.38	+H, +H, +H	$C_{34}H_{52}O_7$	–	–	–	✓
Ganoderic acid R	12.91	577.35	+H, +Na	$C_{34}H_{50}O_6$	–	–	–	✓
Ganoderic acid S	12.11, 13.38	453.34, 453.34	+H, +Na, +H, +Na	$C_{30}H_{44}O_3$	–	✓	–	✓
Ganoderic acid U	12.06	473.36	+H	$C_{30}H_{48}O_4$	–	✓	–	–
Ganoderic acid V	11.15	529.35	+H	$C_{32}H_{48}O_6$	–	✓	–	–
Ganoderic acid X	12.06	513.35	+H	$C_{32}H_{48}O_5$	–	✓	–	–
Ganoderic acid α	5.58, 8.74	575.3238, 597.30	+H, +Na,+K	$C_{32}H_{46}O_9$	–	✓	–	✓
Ganoderic acid β	8.03, 8.26	501.32, 501.32	+H, +H	$C_{30}H_{44}O_6$	–	–	–	✓
Ganoderic acid ε	7.49	517.31	+H	$C_{30}H_{44}O_7$	–	✓	–	✓

Notes: CM denotes for the control from methanol extract; CC denotes for the control from chloroform extract; DM denotes for the D2 from methanol extract; DC denotes for the D2 from chloroform extract. Symbol √ denotes the detection of compounds whereas symbol - denotes that data is not available.

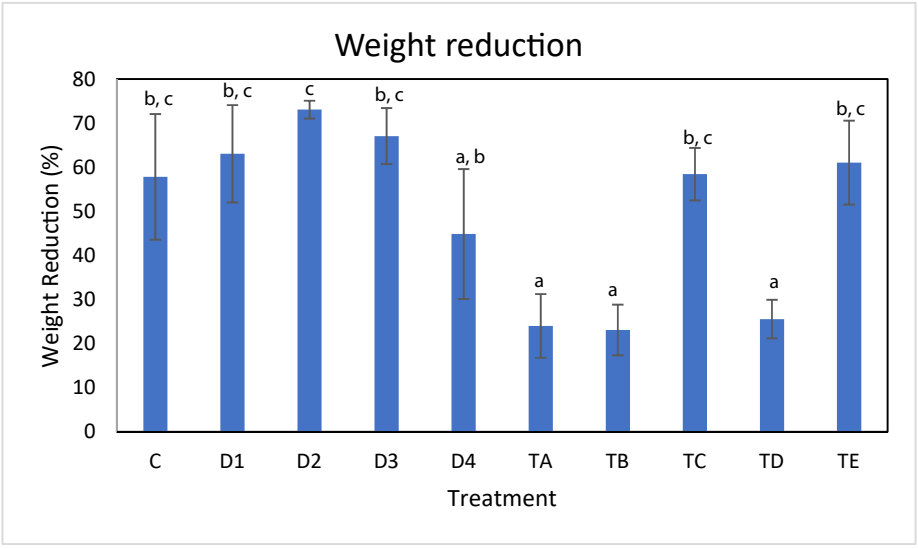


Fig. 4. The total weight reduction of substrate blocks after Lingzhi mushroom was harvested. The data are presented in means ± SD, n = 3 and the alphabetic letters denoted for the significant differences at $p \leq 0.05$.

horticulture, where growth has been detected in some explants (e.g. orchid). However, other variable elements (e.g. pH, temperature) still need to be optimised.

Credit author statement

Shing Ching Khoo: Writing – original draft preparation and main Project administration. Nyuk Ling Ma: Conceptualization, writing and editing. Wanxi Peng: Resources or Funding acquisition, Validation and Visualization. Kah Kei Ng, Meng Shien Goh & Hui Lin Chen: Project administration involved in experimental Formal analysis and Software Formal analysis. Suat Hian Tan & Vijitra Luang-In: Resources and Investigation. Chia Hau Lee: Metabolomic Formal analysis. Christian Sonne: Conceptualization, review, data Visualization and editing.

Declaration of competing interest

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.

Acknowledgement

The authors would also like to thank Ministry of Higher Education Malaysia for the financial support under Fundamental Research Grant Scheme (FRGS) (FRGS/1/2018/TK10/UMT/02/2, Vot 59512).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.131477.

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