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Development of Antimicrobial and Antioxidative Chicken Patties Using Liquid-Fermented *Ganoderma lucidum* and *Pleurotus djamor* Fruiting Body Biomass

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Academic Editor: Camelia Elena Luchian

Received: 19 May 2025
Revised: 26 June 2025
Accepted: 30 June 2025
Published: 9 July 2025

Citation: Zahia-Azizan, N.A.; Yee, C.S.; Ushidee-Radzi, M.A.; Ilham, Z.; Abd Rahim, M.H.; Raseetha, S.; Hamid, N.; Jamaludin, A.A.; Wan-Mohtar, W.A.A.Q.I. Development of Antimicrobial and Antioxidative Chicken Patties Using Liquid-Fermented *Ganoderma lucidum* and *Pleurotus djamor* Fruiting Body Biomass. *Fermentation* **2025**, *11*, 393. https://doi.org/10.3390/fermentation11070393

Abstract

Medicinal mushroom production utilising rural cultivation (solid state fermentation) requires approximately six months compared to culinary mushroom production (7 days). Urban cultivation (submerged liquid fermentation) can be used as a sustainable method of producing medicinal mushroom biomass. In this study, chicken patties were fortified with liquid-fermented *Ganoderma lucidum* flour (GLF) and *Pleurotus djamor* mushroom biomass flour (PDF) at concentrations of 3%, 6%, and 9%. These were compared to a negative control (0% mushroom flour chicken patty) and a commercial patty. Chicken patties fortified with 3% PDF and 9% GLF recorded the lowest cooking loss, at 5.55% and 10.3%, respectively. Mushroom chicken patties exhibited lower cooking losses and significant changes in colour and texture compared to control samples. Notably, 3% GLF chicken patty achieved the highest overall acceptability score of 6.55 followed by 9% PDF chicken patty (6.08) ($p < 0.05$). Biomass flour of liquid-fermented *Ganoderma lucidum* (ENS-GL) and *Pleurotus djamor* (ENS-PD) were extracted for their endopolysaccharide and analysed for their functional properties. All elemental, FT-IR, and NMR spectroscopy analyses revealed the existence of a comparable beta-glucan polymer structure, linkages, and absorptions when compared to the Laminarin standard. In addition, ENS-GL also proved to possess higher antimicrobial activities and significant antioxidant levels (DPPH-scavenging activity, ferric reduction potential and total phenolic content) compared to ENS-PD. Overall, this study revealed that sustainable liquid-fermented *Ganoderma lucidum*, a medicinal mushroom, outperformed *Pleurotus djamor*, a culinary mushroom, as a potential alternative flour for combating hunger in the future.

Keywords: biomass flour; chicken patty; endopolysaccharide; *Ganoderma lucidum*; *Pleurotus djamor*; zero hunger

1. Introduction

Since ancient times, Greeks, Romans, and Chinese have included mushrooms in their diet, and the popularity of mushrooms has increased over time, notably in many Asian and European countries [1,2]. Edible fungi are a great source of nutrients, promoting health and preventing diseases such as hypercholesterolemia, cancer, and hypertension [3,4]. In addition, they comprise various bioactive compounds: proteins, secondary metabolites, and polysaccharide that are used for the drug development [5–7].

Ganoderma lucidum, also known as the 'Mushroom of Immortality', is a medicinal mushroom well known as Lingzhi, Reishi in Japan, and Yeongji in South Korea, and has been applied for medicinal purposes since before Common era in China [8,9]. The benefits of *Ganoderma lucidum* have drawn the attention of researchers and consumers, and they incorporate the mushroom in various food and health-supporting products [10,11]. The primary active ingredients, including polysaccharides, triterpenes, and peptidoglycans, are mainly found in mycelium, fruiting bodies, and spores [12,13]. Medicinal mushroom production utilising rural cultivation or solid state fermentation typically necessitates a longer timeframe of six months [14]. Therefore, there is a sustainable approach that requires a short period of ten days or less to grow medicinal mushroom known as mushroom urban cultivation or submerged liquid fermentation [15].

Pleurotus spp. or oyster mushroom is one of the most cultivated mushroom worldwide and utilised for culinary and commercial purposes and various therapeutic and pharmaceutical activities [16,17]. These species are also well known for their food characteristics, namely nutrition, taste, and physiological function; hence, they are highly regarded for both their sensory characteristics and excellent nutritional profile [17,18]. There are many applications of using black and grey oyster mushroom as a flavour enhancer and fat replacer [19–23]. Pink oyster mushroom or *Pleurotus djamor* is an edible mushroom and well-known as roseus mushroom, pink oyster or salmon pink oyster because of its pink sporophore, large-sized fruiting bodies, and their tasty flavour [24,25]. It is characterised as one of the rapidly growing *Pleurotus* species and requires shorter time (ten days) to form fruiting bodies compared to other *Pleurotus* species [26,27]. In recent research, *P. djamor* was applied as the fungal dye in the dye sensitised solar cells application [28].

The world's highest population growth is expected to increase food demand by 70% to 100% by 2050 in order to maintain food security for the projected 9.1 billion people [29]. Mushroom fermentation is one of the approaches that appears promising in the fight for global food security. It provides a means of producing sizable amounts of nutrient-dense food with short harvest intervals, low input needs, and small-scale land requirements [30]. Mushroom production is far less vulnerable to climate-related challenges than many conventional crops because of these qualities as well as the fact that mushrooms require minimal use of soil, water, and energy for growth [30,31]. The recent COVID-19 pandemic and the ensuing global food crisis also highlighted the rising importance of mushrooms as food [31].

According to [32,33], burgers have become increasingly prevalent among processed beef products owing to their simplicity and satisfying sensory attributes. Fast preparation time along with its appealing sensory attributes account for the majority of the high consumption [34,35]. The food industry has become interested in utilising mushrooms in meat products due to their texture and flavour characteristics, as well as their nutritional

value, antioxidant activity, and health-promoting qualities [36,37]. In the European Union, there is a constant debate on the usage of protein crops in the manufacturing of protein isolates or concentrates [38]. Numerous nations encourage farmers to explore plant-based substitutes for protein such as soybean, legumes, and mushroom.

Mushrooms are becoming recognised as a prospective food source owing to their nutraceutical and functional attributes. Functional food is defined as food that, beyond offering nutritional advantages, positively affects one or more biological activities [39,40]. Nutraceuticals are defined as food or meal ingredients that offer medical or wellness advantages, including illness prevention and treatment [41,42]. Since the beginning of time, both the mushroom itself and its extracts have been employed in traditional medicine and cuisine due to the fungus' low calorie content, pleasant flavour, and reputedly invaluable biological properties [43]. In the past, *Ganoderma lucidum* was consumed greatly as medicine. Recent molecular research has elucidated this historical history by examining the bioactive compounds now present and uncovering the health and nutritional benefits of mushrooms [44]. Liquid fermented *Ganoderma lucidum* has already been proven to be safe and non-toxic to environment [45,46]. On the other hand, *Pleurotus djamor* is categorised as a culinary mushroom and is well-known to be edible and safe [47,48].

Drying and grinding preservation techniques turn mushrooms into powder or flour, and are ideal to be utilised in snacks, bakery products, and pasta, as well as meat products [49,50]. Different mushroom powders have been used in place of wheat flour in many studies. For instance, *Lentinus edodes* powder is used in wheat flour gluten sticks [51]; extruded snacks contain 5–15% of chestnut mushroom (*Agrocybe aegerita*) [52]; 5–15% *Pleurotus sajor-caju* is used in biscuits; 10–30% of *Lentinula edodes* in cookies and steamed buns; and 4–12% *Pleurotus ostreatus*-based noodles have also proven popular was a wheat flour substitute. In addition, [20] utilised and converted the fruiting bodybase (FBB) of the oyster mushroom into mushroom flour to produce mushroomchicken patty. The flour was incorporated into chicken patties using different formulations and consumer acceptance was measured. In a previous study by [53], *Pleurotus djamor* powder were supplemented as meat replacer in beef patties' physicochemical and sensory qualities. Fruiting body biomass of *Ganoderma lucidum* were also substituted as flour replacer according to different formulation of chicken patties and was found to be low in consumer acceptance [45].

This study aims to utilise liquid-fermented *Ganoderma lucidum* and *Pleurotus djamor* as mushroom flour in developing antimicrobial and antioxidative mushroom chicken patties. Furthermore, this study also analyses the presence of beta glucan, antimicrobial and antioxidant activities of endopolysaccharide of both mushrooms which contribute to the functionality of mushroom-chicken patties.

2. Materials and Methods

2.1. Sample Preparations

2.1.1. *Ganoderma lucidum* Flour (GLF)

The Functional Omics and Bioprocess Development Laboratory, Institute of Biological Sciences, Faculty of Science, Universiti Malaya supplied the sample Malaysian *Ganoderma lucidum* strain QRS 5120 mushroom mycelium [54]. As described by [55], potato dextrose agar (PDA) powder (39 g/L) was used to grow *Ganoderma lucidum* on plate and seed culture. The liquid fermentation of *Ganoderma lucidum* was prepared according to [54] with modification using potato dextrose broth (PDB) (Oxoid no. CM0962, Oxoid Limited, Wade Road, England).

Inoculum preparation involved two seed culture phases, each of which was grown for ten and eleven days in as shaking incubator, respectively, at 30 °C and, at 100 rpm. Mycelial agar from the PDA plate was cut into three squares (1 cm × 1 cm) to initiate the

primary seed culture. They were inoculated in a 250 mL Erlenmeyer flask into 100 mL of culture medium. Subsequently, mycelium from the initial seed culture of 20% (*v*/*v*) was employed for the second seed culture, having been homogenised for 10 s in a blender to promote the formation of enhanced growing hyphal tips. The culture was inoculated within a 500 mL Erlenmeyer flask containing 100 mL of the total working volume of fresh PDB media and was allowed to incubate at 30 °C with agitation at 100 rpm. The sample was filtered using a Buchner funnel filter paper 0.45 µm (Whatman, Sigma-Aldrich, Dorset, UK), and the mycelial biomass was then washed three times with sterile distilled water. Subsequently, the mycelial biomass was desiccated using a food dehydrator at 35 °C prior to being ground into flour.

2.1.2. *Pleurotus djamor* Flour (PDF)

The fruiting bodies of *Pleurotus djamor* were obtained from the Putra Agriculture Centre, Universiti Putra Malaysia. The pretreatment of *Pleurotus djamor* was performed by cleaning of fruiting bodies and treated by soaking in 0.5 g/100 mL food grade citric acid solution for ten minutes as previously described by [20]. In addition, the treated fruiting bodies were dried in a food dehydrator at 40 °C for a 24 h duration. The dried fungal fruiting bodies were ground into flour.

2.1.3. Endopolysaccharide (ENS)

Firstly, ENS was extracted from biomass flours of the *Ganoderma lucidum* and *Pleurotus djamor*. Distilled water was added to the biomass samples (1:10 g/mL). Next, the hot-water extraction method was used by autoclaving distilled water containing biomass flours at 121 °C for 30 min [56]. For characterisation, antimicrobial, and antioxidant analysis, both endopolysaccharide of *Ganoderma lucidum* (ENS-GL) and *Pleurotus djamor* (ENS-PD) were utilised because of their solubility in water and solvents.

2.2. Functional Analysis

2.2.1. Elemental Analysis

The content of Carbon (C), Hydrogen (H), Nitrogen (N), and Sulphur (S) of ENS samples was estimated using a Thermo Scientific Flash*Smart* CHNS/O Elemental Analyzer (Waltham, Massachusetts, MA, USA) device [57].

2.2.2. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of the ENS samples were obtained using an FTIR 3000 spectrophotometer, 134 (Jasco, Japan), following the method of [57].

2.2.3. ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy

The NMR spectra of both ENS samples were determined using a DXM 500 FT-NMR spectrometer 138 (Bruker, Karlsruhe, Germany). Every spectrum was performed at a temperature of 80 °C. The chemical shifts (δ) were expressed in parts per million (ppm), and the scan number was 16. The control and reference standard were Laminarin from *Laminaria digitata* (Sigma-Aldrich, Dorset, UK).

2.2.4. Antimicrobial Activities of the ENS of *Ganoderma lucidum* and *Pleurotus djamor*

The target microorganisms were based on WHO list of critical and high priority bacteria [58] and the availability of other bacteria in the Functional Omics and Bioprocess Development laboratory. Antimicrobial activities of mushroom extract samples were performed using the disc diffusion technique described by [57]. Firstly, a Petri dish containing 20 mL of nutrient agar medium was prepared. Subsequently, all test microorganisms were calibrated to 0.5 McFarland standards utilising sterile broth medium. Approximately

200 µL of suspension containing five Gram-positive and five Gram-negative test microorganisms was applied to the prepared solidified agar. Subsequently, the standardised 11 mm sterile discs (blank) (Sigma-Aldrich, Dorset, UK) were immersed in an equivalent absorbed volume containing a precise quantity of extract and delicately placed into the agar overlay. The plates were subsequently incubated overnight at 37 °C, for 48 h at 30 °C, or two days, contingent upon the bacterium's growth requirements. Gentamicin served as the positive control, whilst ethanol (70%) functioned as the negative control. After incubation, the diameters (mm) of the inhibitory zones were measured. Inhibition zones over 7 mm were considered indicative of good antibacterial activity.

The minimum inhibitory concentration (MIC) was assessed using microdilution utilising test tubes with minimal modifications. Sterile broth medium was utilised with 0.5 McFarland standards for the modification of bacterial suspension. Both samples were solubilised in sterile water and subjected to serial dilution to concentrations of 200, 100, 20, 10, 8, 5, 3, 2, and 1 mg/mL. The final combination comprised 2.5 mL of chemicals combined with 7.5 mL of a bacterial suspension, resulting in a total working volume of 10 mL. Each test culture was transferred into the tubes and incubated for 24 h at 30 °C. Upon the conclusion of the incubation period, turbidity was regarded as an indicator of bacterial proliferation. The minimum diluted concentration at which the incubated mixture remained clear was therefore designated as the MIC. Sterile L-spreaders were utilised to guarantee consistent dispersion. Thereafter, the quantity representing the MIC, together with at least two higher dilutions, was plated and measured to determine viable colonies specifically for the assessment of minimum bactericidal concentration (MBC). The minimum bactericidal concentration (MBC) is the lowest concentration at which 99% of the bacteria are killed [59].The medium was incubated at 30 °C for 24 h to observe microbial proliferation. The lowest concentration in the medium that produced fewer than five colonies was employed for the MBC.

2.2.5. Antioxidant Assays of the ENS of *Ganoderma lucidum* and *Pleurotus djamor*

Preparation of ENS Ethanolic Extracts

The extract was made using dried ENS of *Ganoderma lucidum* and *Pleurotus djamor* and ethanol [60]. The ENS samples were stirred for 24 hr at 20 °C at 150 rpm with 1 g per 10 mL of ethanol. The broth samples were centrifuged at 3500× *g* for 10 min and filtered with Whatman No. 1 filter paper and stored at 4 °C.

DPPH Radical-Scavenging Activity Assay

The DPPH radical-scavenging activity of the ENS extract was assessed using a technique previously documented by [20,61]. The sample was diluted according to concentrations of 5, 10, 20, and 40 mg/mL. The DPPH solution was prepared by dissolving 5.9 mg of DPPH in 100 mL of ethanol. Subsequently, 3.8 mL of the ethanolic DPPH solution was incorporated into 0.2 mL of the extracts. The mixture was agitated rapidly for one minute and thereafter allowed to rest at room temperature in the absence of light for thirty minutes. The absorbance of the combination was measured at 517 nm utilising an ultraviolet–visible (UV-VIS) spectrophotometer (Genesys, Germany). All experiments were performed in triplicate. The radical-scavenging activity of the ENS samples was calculated using Equation (1).

$$\%\ \text{DPPH scavenging activity} = \frac{A_B - A_A}{A_B} \times 100\% \quad (1)$$

where

A_B = Absorbance of blank

A_A = Absorbance of sample.

Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP analysis was performed using the protocol and procedure provided by the FRAP assay kit (Catalogue Number MAK509, Sigma Aldrich). A 180 µM standard was prepared by mixing 20 µL of 1.8 mM Fe^{2+} standard with 180 µL of purified water. Next, the standards and samples were diluted and measured using a cuvette. The sample was diluted according to concentrations of 5, 10, 20, and 40 mg/mL. A UV-VIS spectrophotometer (Genesys, Germany) was used to measure the absorbance of the samples at 590 nm, and the Fe^{3+} reduction potential was obtained by using Equation (2)

$$\left(\mu M\ Fe^{3+}\ \text{reduction potential}\right) = \frac{R_S - R_B}{S} \times DF \quad (2)$$

where

R_S = OD reading of the Sample,
R_B = OD reading of Blank,
S = Slope,
DF = Sample dilution factor,
(DF = 1 for undiluted Samples).

Total Phenolic Content (TPC) Assay

TPC assay was conducted based on the protocol and procedure provided by the TPC assay kit (Catalogue Number E-BC-K354-S, Elabscience, Wuhan, China). The standard and samples were diluted according to concentrations of 5, 10, 20, and 40 mg/mL, and absorbance were read at 760 nm using an ultraviolet–visible (UV-VIS) spectrophotometer (Genesys, Germany). Equation (3) was used to obtain the total phenolic content of both samples.

$$\text{Total phenols content (mg/g tissue)} = (\Delta A760 - b) \div a \times V \div W \div 1000^* \times f \quad (3)$$

ΔA760: Absolute OD (OD Sample − OD Control).
V: the volume of added extraction solution, 2.5 mL of 60% ethanol.
W: Weight of sample, 0.1 g.
*: Unit conversion, 1000 µg = 1 mg.
f: Dilution factor of the sample before the test.

2.3. Preparation of Mushroom Chicken Patties

A total of 2 kg of chicken breast was cut manually using a cleaver and minced in a food processor. The chicken was mixed with ingredients according to Table 1 [20]. The completed chicken mixture was split into 70 g parts, and the patties were hand-formed into a consistent shape measuring 100 mm in diameter and 10 mm in thickness according to [62]. Subsequently, before food analysis, the raw chicken patties were kept in a freezer at −18 °C.

Table 1. Mushroom chicken patty formulations using both *Ganoderma lucidum* flour (GLF) and *Pleurotus djamor* flour (PDF).

Ingredients (%)	Mushroom Flour Percentages (%)			
	0	**3**	**6**	**9**
Chicken breast meat	66	66	66	66
Mushroom flour (GLF and PDF)	0	3	6	9
Fat	8	8	8	8
Iced water	15	15	15	15
Potato starch	10	7	4	1
Seasoning	1	1	1	1
Total	100	100	100	100

2.4. Food Analysis

2.4.1. Cooking Process and Cooking Loss

The patties were weighed before and after cooking for 7 to 8 min at about 176 ± 1 °C, being turned three to four times until an interior temperature of 72 ± 1 °C was attained [20] to calculate the cooking loss of chicken patties. The cooking loss which is also known as weight loss (%) of the chicken patties during the heat treatment [63] were also calculated by using Equation (4) below

$$\text{Cooking loss} = \frac{W_1 - W_2}{W_1} \quad (4)$$

where

W_1 = raw chicken patty sample weight before cooking, g.

W_2 = chicken patty sample weight after cooking, g.

2.4.2. Colour Analysis

The colorimetric analysis of the cooked mushroom chicken patties was conducted using a colorimeter (Minolta model 3500, Minolta Camera Co., Ltd., Osaka, Japan). According to [64], lightness (L*), redness (a*), and yellowness (b*) are colorimetric properties of the developed patties which will be evaluated and contrasted with those of commercially cooked chicken patties (Ramly Chicken Patty 70 g/packet, Ramly Food Processing Sdn. Bhd. Malaysia). The mushroom chicken patties are deemed acceptable if their colouration aligns with that of the commercial chicken patties.

2.4.3. Texture Analysis

The texture profile analysis (TPA) of mushroom chicken patties was performed based on [65]. The studied textural properties were hardness, springiness, gumminess, cohesiveness, and chewiness of the control and mushroom chicken patties, and they were measured using texture analyser (Stable Micro System Model TA.XT 2i/25, Surrey, UK). The measurement was carried out using central cores of each patty sample (2 cm × 2 cm × 2 cm). Each sample was compressed twice (80% of the original height and 2 mm/s crosshead speed) with the help of a compression probe (P 75).

2.4.4. Sensory Analysis

Based on sensory analysis by [20], the mushroom chicken patty samples were assigned with random 3-digit codes and cut into 1 cm × 1 cm square size before being put in labelled small polyethylene plastic containers with a lid. A total of 50 panellists were picked based on the requirements needed (18 years and above and eat chicken meat regularly) and briefed in the evaluation area after sensory evaluation forms and consent forms were distributed. The samples were thereafter allocated to each panellist, accompanied by a cup of drinking water and an unsalted cracker for palate-cleansing during sensory analysis. The mushroom

chicken patties were assessed for several sensory attributes including appearance, colour, aroma, texture, taste, aftertaste, and overall acceptability using a 9-point hedonic scale (1 = extremely poor, 2 = very poor, 3 = poor, 4 = bad, 5 = average, 6 = fair, 7 = good, 8 = very good, 9 = excellent) by 50 acceptable panellists who were 18 years old and above and consumed chicken meat regularly (at least twice a week) from the University Putra Malaysia (UPM), Selangor, Malaysia.

2.5. Statistical Analysis

All analyses were conducted in triplicate, and all findings were presented as mean ± standard deviation to ascertain significant differences among means for all tests at $\alpha = 0.05$ and *P*-value of 1, using software of GraphPad Prism 5, version 5.0. Furthermore, a radar chart displaying the results of all chicken patties for each sensory feature was prepared and created by using Microsoft Excel (Version 16.45) to tabulate the mean values of each sensory attribute of mushroom chicken patty compositions.

3. Results and Discussions

3.1. Functional Analysis

3.1.1. Elemental Analysis

Elemental analysis was conducted to determine the composition of the ENS-GL and ENS-PD in comparison with laminarin as a control or standard. ENS-GL gives the following composition of (*w*/*w*): 36.77% C, 6.06% H, 2.14% N, and 0.14% S (Table 2). ENS-PD, on the other hand, obtained 37.26% C, 5.75% H, 1.75% N, and 0.05% S. ENS-PD has a higher value for C, and lower for other elements compared to ENS-GL. This demonstrates some significant differences between different mushroom species in the elemental intake by mycelia [66].

Table 2. Elemental composition of endopolysaccharide of *Ganoderma lucidum* and *Pleurotus djamor*.

	***w*/*w* (%)**			
Sample Name	**C**	**H**	**N**	**S**
ENS-GL	36.77 ± 1.38	6.06 ± 0.29	2.14 ± 1.01	0.14 ± 0.33
ENS-PM	37.26 ± 1.38	5.75 ± 0.29	1.75 ± 1.01	0.05 ± 0.33
Laminarin-control	33.89 ± 1.38	6.21 ± 0.29	0	0.25 ± 0.33

3.1.2. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectrum of ENS-GL and ENS-PD presented in Figure 1 and Table 3 summarises the absorption peak of every sample according to vibration mode. The broad and intense absorption peaks of ENS-GL and ENS-PD at 3455 cm^{-1} and 3432 cm^{-1} were found to represent the stretching vibration of a hydroxyl group (O–H), which indicated the presence of a polyhydroxy compound. The absorption peak of ENS-PM recorded the highest, which is 2922 cm^{-1}, compared to ENS-GL and laminarin standard indicating a methylene group (CH_2) of the stretching vibration of C–H bonds. Other major absorptions by both ENS-GL and ENS-PD recorded the same readings at 1646.08 cm^{-1}, higher than laminarin.

FT-IR spectra in the wave number between 850 and 1200 cm^{-1} are considered as the "fingerprint" region for carbohydrates, which is unique to a compound in terms of polysaccharide and configuration [67]. Based on the figure, ENS-GL showed the highest absorption at 1075 cm^{-1}, followed by ENS-PM at 1054.20 cm^{-1} and laminarin at 1047.42 cm^{-1}.

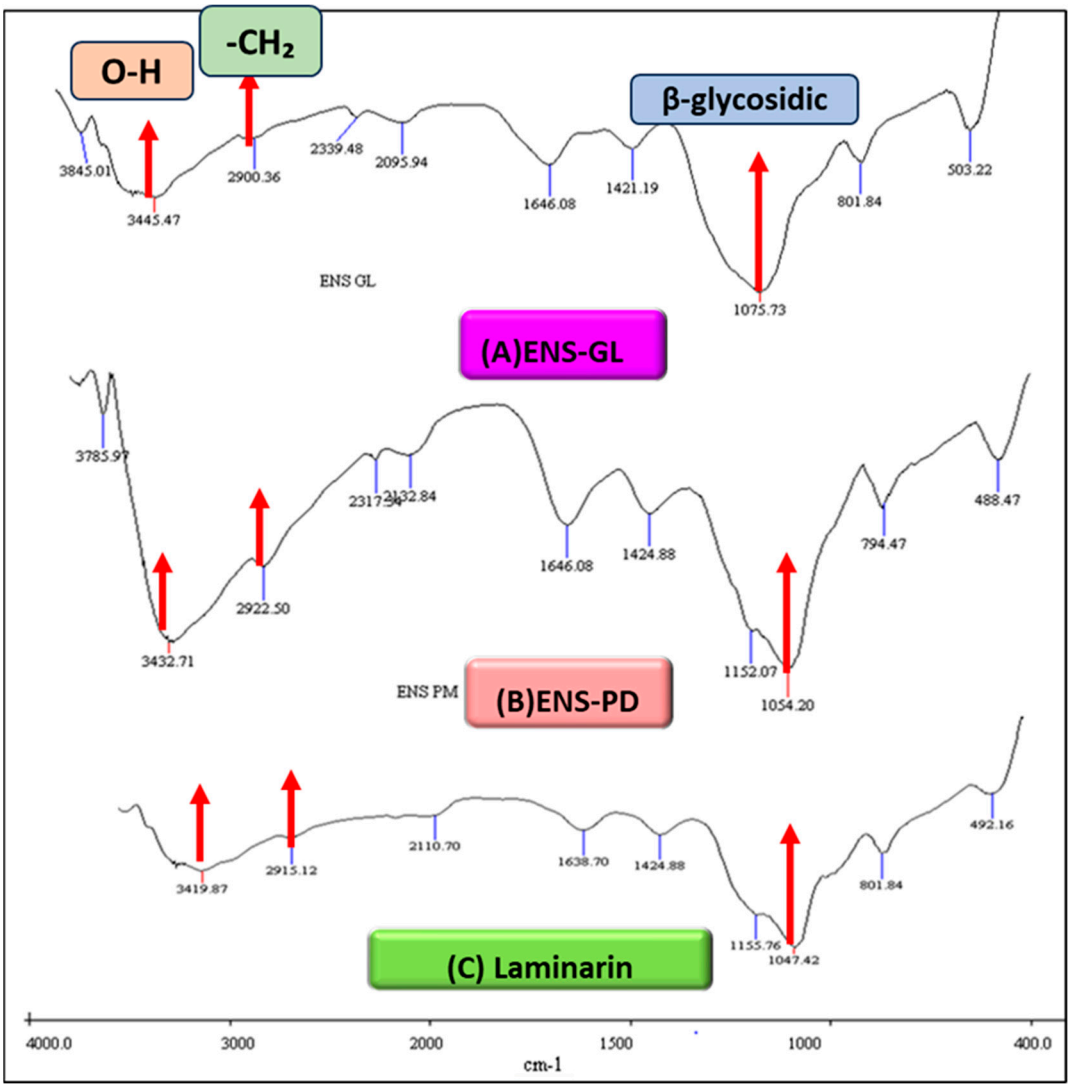

Figure 1. FT-IR spectra of (**A**) endopolysaccharide (ENS) from *Ganoderma lucidum* mycelial biomass, (**B**) endopolysaccharide (ENS) from *Pleurotus djamor* biomass, and (**C**) laminarin is a standard for (1,3)-β-D-glucan from *Laminaria digitata* (figure by author).

Table 3. The absorption peak for endopolysaccharide samples in comparison with Laminarin according to vibration mode.

	Sample (Absorption Peak, cm^{-1})			
Vibration Mode	**ENS-GL**	**ENS-PD**	**Laminarin**	**Reference Wavelength**
Hydroxyl group (O–H)	3455	3432	3419	3400–3500
Methylene group (-CH_2) = water bending	2900	2922	2915	2900–2922 [68]
Symmetric and asymmetric stretching vibration	1646	1646	1638	1400–1650 [68]
Glycosidic linkage (β-configuration)	1075	1054	1047	850–1200 [67]

3.1.3. ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy

Figure 2 illustrates the ^{1}H NMR spectroscopic analysis of ENS-GL and ENS-PD. By utilising ppm as the standardised unit for NMR investigations, the ^{1}H NMR spectra of ENS-

GL and ENS-PD were compared to the standard laminarin (β-1,3-D-glucan) obtained from *Laminaria digitata*. The ENS's chemical shifts in δ 2.99 to 4.99 ppm and δ 3.07 to 5.19 ppm in the spectrum show that both molecules are glucans. This can be noticed in Figure 2, respectively. The present study is similar to prior research conducted by [57,68], which examined the properties of laminarin and sulphated laminarin in the ^{1}H-NMR spectrum range of δ 4.49–5.5 ppm. Therefore, the spectra demonstrate that the glycosidic linkages in both ENS-GL and ENS-PM were of the β-type. The β-anomeric protons and carbons can also be observed in the 4–5 ppm range [69]. Based on the analysis of FT-IR and ^{1}H NMR, it can be inferred that the endopolysaccharide consist of (1-3)-β-D-links, resulting in a polymer structure that seems to be a 1,3-β-D-glucan.

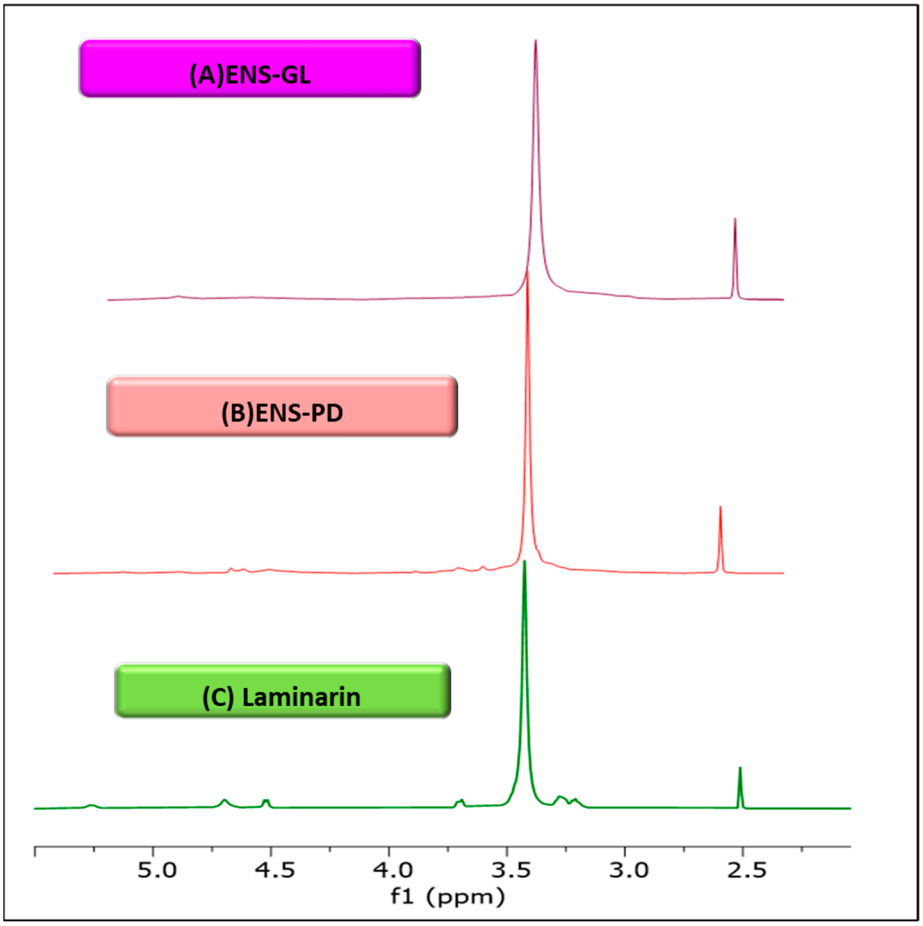

Figure 2. ^{1}H NMR spectra of (1,3)-β-D-glucan. (**A**) Glucan (G) derived from ENS-*Ganoderma lucidum*. (**B**) glucan (G) derived from ENS-*Pleurotus djamor*. (**C**) Standard glucan from laminarin (*Laminaria digitata*) (figure by author).

3.1.4. Antimicrobial Activities

Table 4 depicts the antimicrobial activity of ENS-GL and ENS-PD using the disc diffusion assay. ENS-GL shows the highest diameter of inhibition (mm) against *Actinobacteria* (15.5 mm at 300 mg/mL) and *Staphylococcus epidermis* (13 mm at 300 mg/mL), whereas the highest diameter of inhibition (mm) obtained from ENS-PD was against *Actinobacteria* (9.5 mm at 300 mg/mL). The data shows that the inhibition zone diameters increased with increasing concentration (Table 4). This conforms with the study by [57]. Table 5 records the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of ENS-GL and ENS-PD. ENS-GL effectively inhibits the growth of bacteria at 1 mg/mL against *Actinobacteria*. The sample also recorded the lowest MBC which is 5 mg/mL against

Actinobacteria. In addition, ENS-PD also inhibits bacterial growth at 5 mg/mL against *Actinobacteria*, *Staphylococcus aureus* and *Proteus*.

Table 4. Antimicrobial activity of endopolysaccharide of *Ganoderma lucidum* and *Pleurotus djamor* using disc diffusion assay.

		ENS-GL				ENS-PD			
		Diameter of Inhibition Zone (mm)				Diameter of Inhibition Zone (mm)			
No./G	**Bacteria**	**200 mg/mL**	**300 mg/mL**	**Gentamicin 30 µg**	**Distilled Water**	**200 mg/mL**	**300 mg/mL**	**Gentamicin 30 µg**	**Distilled Water**
1 (G+)	*Actinobacteria*	12 ± 2.8	15.5 ± 6.3	10 ± 0.0	8.5 ± 0.7	9 ± 2.8	9.5 ± 6.3	10 ± 0.0	7.5 ± 0.7
2 (G+)	*Bacillus ATTC*	8.5 ± 0.7	8.5 ± 0.7	10.5 ±0.7	7.5 ±2.1	7.5 ± 2.1	7.5 ± 2.1	10 ± 2.1	7.5 ±2.1
3 (G+)	*Staphylococcus epidermis*	8 ± 1.4	13 ± 0.0	11 ± 0.0	7.5 ±2.1	8 ± 1.4	9 ± 0.0	11 ± 0.0	7.5 ±2.1
4 (G+)	*Staphylococcus aureus*	8 ± 2.8	8 ± 2.8	10.5 ± 0.7	7.5 ±2.1	8 ± 2.8	8 ± 2.8	10 ± 0.7	7.5 ±2.1
5 (G+)	*Micrococcus luteus*	9 ± 0.0	9 ± 0.0	10.5 ± 0.7	7 ± 0.0	8 ± 0.0	8 ± 0.0	10 ± 0.0	7 ± 0.0
6 (G−)	*Proteus*	9 ± 0.0	11 ± 0.0	12 ± 0.7	7.5 ±2.1	8 ± 0.0	8.5 ± 0.0	10 ± 0.0	7.5 ±2.1
7 (G−)	*Ralstonia*	9.5 ± 0.7	9.5 ±2.1	10.5 ± 0.7	8 ± 0.0	8 ± 0.0	8 ± 0.0	10 ± 0.0	7 ± 0.0
8 (G−)	*Klebsiella*	9 ± 1.4	12 ± 0.0	10.5 ± 0.7	8 ± 0.0	8 ± 0.0	9 ± 1.4	10.5 ± 0.7	7 ± 0.0
9 (G−)	*Xanthomonas*	9.5 ± 0.7	10.5 ± 0.7	10.5 ± 0.7	8.5 ± 0.7	8 ± 0.0	8.5 ± 0.7	10.5 ± 0.7	7 ± 0.0
10 (G−)	*Erwinia*	9 ± 0.0	9 ± 0.0	10.5 ± 0.7	7.5 ± 0.7	8 ± 0.0	9 ± 0.0	10.5 ± 0.7	7.5 ± 0.7

Values represent mean ± S.D. G indicates Gram-positive (G+) or Gram-negative (G−) bacteria. Sterile disc size was 7 mm when indicating negative reactions and more than 7 mm for positive reactions. Distilled water was used as the negative control, while Gentamicin was used as the positive control.

Table 5. Minimum inhibitory concentrations and minimum bactericidal concentrations of endopolysaccharide of *Ganoderma lucidum* and *Pleurotus djamor.*

		ENS-GL		ENS-PD	
No./G	**Bacteria**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
1 (G+)	*Actinobacteria*	1	5	5	20
2 (G+)	*Bacillus ATTC*	3	10	8	20
3 (G+)	*Staphylococcus epidermis*	5	10	8	20
4 (G+)	*Staphylococcus aureus*	5	20	5	20
5 (G+)	*Micrococcus luteus*	5	20	8	20
6 (G−)	*Proteus*	3	10	5	10
7 (G−)	*Ralstonia*	3	10	8	20
8 (G−)	*Klebsiella*	5	20	10	20
9 (G−)	*Xanthomonas*	5	10	10	10
10 (G−)	*Erwinia*	5	20	20	100

The tested concentrations were used to see the trend of MIC were based on [57,70]. The highest tested MIC was 1000 mg/mL, followed by 250, 125, and 62.5 mg/mL. The study is to show the potential and the ability of the endopolysaccharide samples to inhibit the growth of microorganisms. The value of the inhibition diameter is supported based

on the study by [70], which produced a similar inhibition diameter of 8.67 mm at higher concentrations (500 mg/mL) compared to this study (at 300 mg/mL). Research reported that polysaccharides of *Ganoderma lucidum* have the potential to impede the growth of microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* [71]. The antimicrobial activity entails the disruption of bacterial cell walls or the interference with key bacterial enzymes [72]. The varying susceptibility of fungal strains against Gram-positive and Gram-negative bacteria may be attributed to the presence of a membrane around the peptidoglycan of Gram-negative bacteria, which hinders diffusion due to its lipopolysaccharide (LPS) coating [73]. The LPS layer is crucial for maintaining selective permeability [74].

In contrast, Gram-positive bacteria do not possess an outer membrane, but they are distinguished by a dense hydrophilic porous structure that enhances their permeability [75]. As a result, Gram-positive bacteria will exhibit greater sensitivity to mushroom extracts compared to Gram-negative bacteria according to previous studies that utilised *Ganoderma lucidum* and *Pleurotus djamor* extracts [73,76], which corresponds to the antimicrobial activity observed in this study.

3.1.5. Antioxidant Activities

DPPH Radical-Scavenging Activity

DPPH radical-scavenging activity is an antioxidant method for assessing the capacity of substances to function as scavengers of free radicals or donors of hydrogen, as well as for evaluating the antioxidant activity of food items [77]. Therefore, the DPPH assay was used to determine the antioxidant activity of ENS-GL and ENS-PD (Figure 3). It was observed that the higher the concentration of ENS samples, the higher the DPPH-scavenging activity (%). Overall, both samples showed substantial values, and ENS-PD showed greater DPPH-scavenging activity (92.6%) in comparison with ENS-GL (91.9%) at a 40 mg/mL concentration.

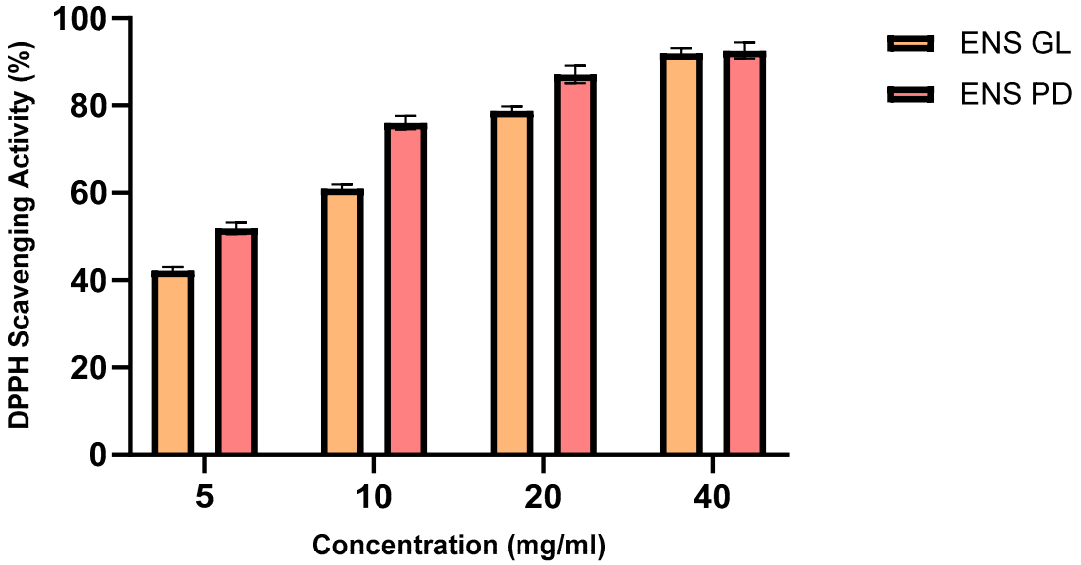

Figure 3. DPPH-scavenging capacity (%) of endopolysaccharide (ENS) from *Ganoderma lucidum* mycelial biomass and *Pleurotus djamor* biomass (Means ± SD, n = 3) (figure by author).

Ganoderma lucidum polysaccharides showed antioxidant activity, which is typically ascribed to their elevated hydrogen levels, which allow them to create stable configurations when interacting with free radicals [78,79]. Unpaired electrons in molecules make free radicals unstable and reactive. By damaging vital macromolecules, free radicals can damage

cells and tissues, disrupt homeostasis, and interfere with metabolic processes [80]. This leads to oxidative stress as a result of an overabundance of reactive oxygen species and an imbalance in the metabolism [81].

Multiple studies reported extracted polysaccharides of *Ganoderma lucidum* to show great reproducibility of DPPH-scavenging activity (%) [82,83]. On the other hand, findings indicated that the polysaccharide extracted from *Pleurotus djamor* exhibited significant DPPH radical-scavenging activity which conforms with a previous study by Raman et al. [84]. The high reproducibility of DPPH-scavenging activity by both ENS-GL and ENS-PD in mushroom chicken patty can potentially safeguard food quality by preventing oxidative degradation and allowing consumers to mitigate oxidative damage.

Ferric Reducing Antioxidant Power (FRAP)

This antioxidant analysis is conducted to show the ferric reducing ability of samples and also to measure the overall antioxidant capacity [85]. The FRAP test relies on the reduction in the ferric-tripyridyl triazine (Fe(III)-TPTZ) complex to the ferrous-tripyridyl triazine (Fe(II)-TPTZ) by a reductant under acidic conditions [86]. The compound's reduction capacity can be a valuable predictor of its potential antioxidant action.

According to Figure 4, the result showed that the greater the concentration of ENS-GL and ENS-PD, the greater the ferric reducing capability. In addition, ENS-GL also proved to produce higher reduction potential at only 5 mg/mL (0.007 µM) and increasing gradually until the highest at 40 mg/mL (0.06 µM). On the other hand, ENS-PD only revealed a reduction potential at 40 mg/mL (0.002 µM).

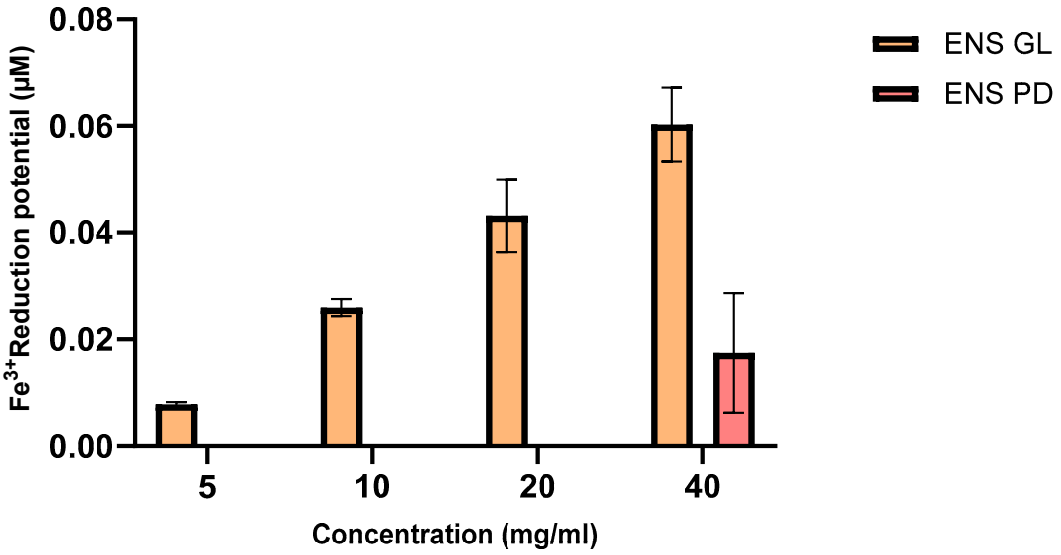

Figure 4. Fe^{3+} reduction potential (µM) of endopolysaccharide (ENS) from *Ganoderma lucidum* mycelial biomass and *Pleurotus djamor* biomass (Means ± SD, n = 3) (figure by author).

According to a study by [84], the concentration of the mushroom extracts directly correlates with an increase in their reducing power, which validates the present study. In addition, iron is a vital mineral for the correct functioning of the human body, but an excessive amount can lead to harm at the cellular level [87]. The ferrous ion is well recognised as the most potent pro-oxidant element in food systems [88]. Therefore, the chelating activity of mushrooms is highly important for mitigating oxidative stress-induced diseases by effectively removing free ions during consumption.

Total Phenolic Content (TPC)

Phenolic compounds or phenolic acids are found in mushroom extract, typically possessing redox characteristics which are responsible for their antioxidant action [89,90]. In addition, the hydroxyl groups present in most plant and fungi extracts play a crucial role in the process of scavenging free radicals [91,92].

Figure 5 depicts the total phenolic content (mg/g) obtained by both ENS-GL and ENS-PD. The results showed that with increasing concentrations of endopolysaccharide, the total phenolic content (mg/g) also increases. ENS-GL comprised the highest total phenolic content (mg/g) is at 40 mg/mL, 353.51 mg/g, whereas the highest total phenolic content (mg/g) obtained by ENS-PD is at 236.40 mg/g at 40 mg/g.

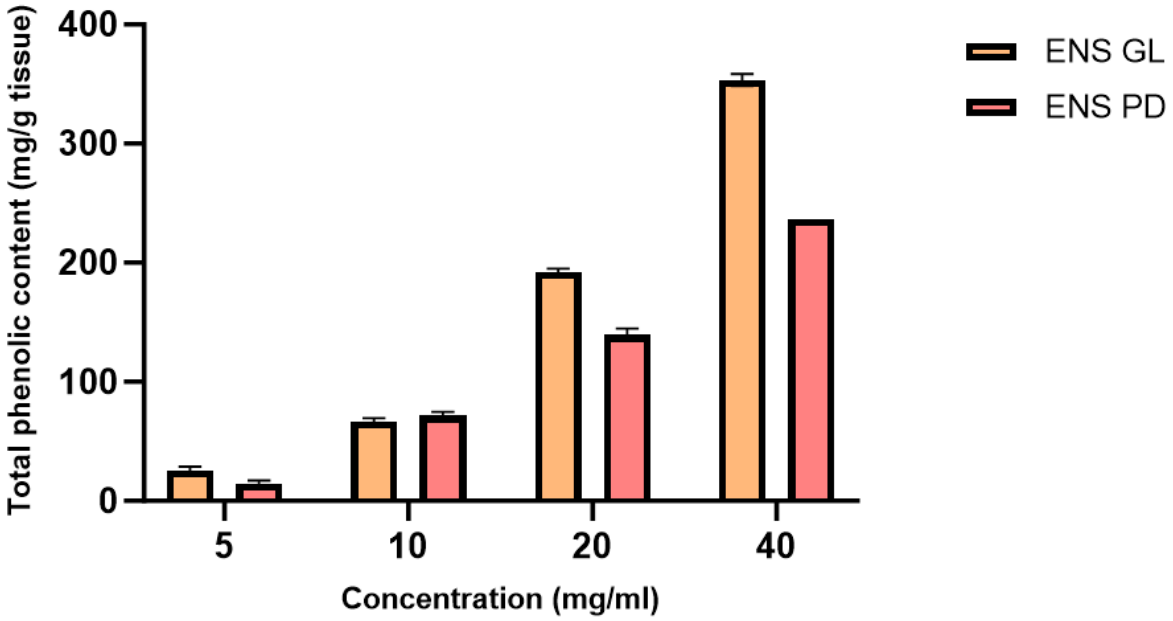

Figure 5. Total phenolic content (mg/g tissue) of endopolysaccharide (ENS) from *Ganoderma lucidum* mycelial biomass and *Pleurotus djamor* biomass (Means ± SD, n = 3) (figure by author).

Based on the study by [93], *Ganoderma lucidum* extract exhibits the highest total phenol contents (81.34 mg/g) compared to extracts of *Ganoderma tropicum* and *Cannabis indica*. In addition, [94] reported that both extracts of *Ganoderma lucidum* and *Pleurotus djamor* contain significant phenolic content, where overall, *Ganoderma lucidum* recorded the highest total phenolic content (402.75 mg/mg) compared to *Pleurotus djamor* (267.28 mg/g).

3.2. Food Analysis

3.2.1. Cooking Loss

Figure 6 indicates the cooking loss (%) of cooked mushroom chicken patties, with the cooking loss of chicken patties decreasing with the increase in GLF in chicken patties. Chicken patties with 9% GLF showed the lowest value (10.30%) compared to the positive control (26.89%), 3% GLF (23.55%), 6% (13.66%), and the negative control (31.01%). However, the cooking loss of chicken patties increases with the increase in PDF in chicken patties: 3% PDF (5.55), 6% PDF (7.78), and 9% PDF (22.34%).

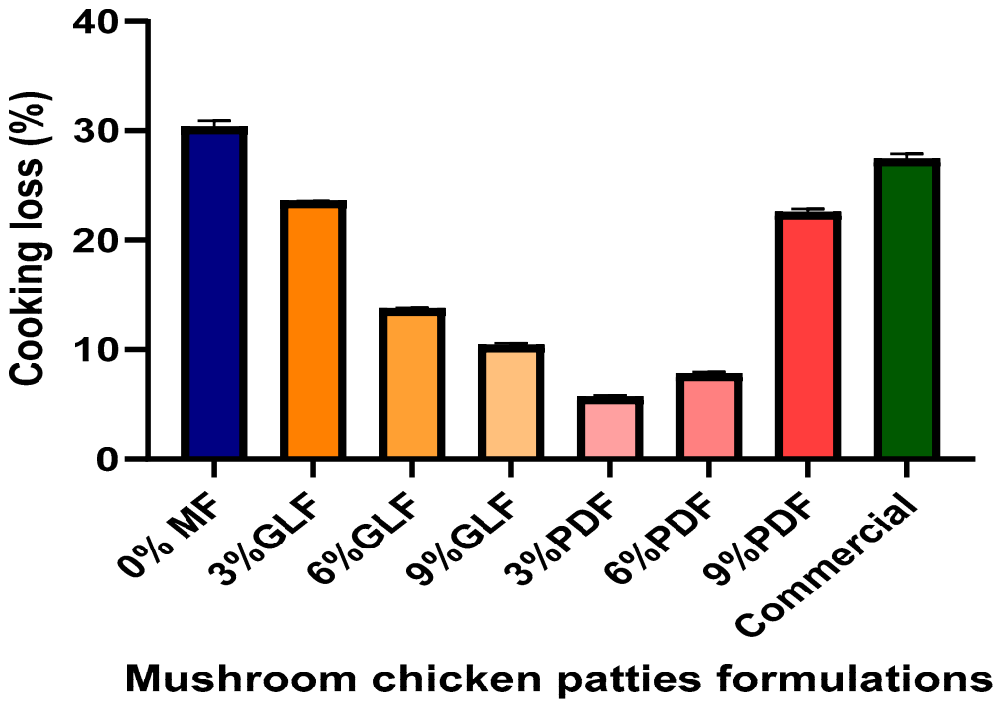

Figure 6. Cooking loss (%) of control chicken patty with 0% mushroom flour (MF), cooked *Ganoderma lucidum* flour (GLF), *Pleurotus djamor* flour (PDF) mushroom chicken patties, and commercial chicken patties. (figure by author).

The cooking loss of chicken patties drastically decreased ($p < 0.05$) with increasing levels of GLF. The moisture texture of GLF chicken patties might be due to the reduction in potato starch, whereby the binding properties of chicken patties may be compromised, resulting in a softer texture [95]. Heat-induced protein denaturation is the source of cooking loss or shrinkage in meat products. The structural proteins collagen and actomyosin complex are impacted by this denaturation, which results in undesirable alterations in the meat products' sensory qualities, including hardness and juiciness [96].

Relatively, the cooking loss occurred after cooking due to the contraction of muscle fibres and connective tissues during cooking [97]. The highest cooking loss observed in the control and commercial samples is likely due to fat being readily eliminated during cooking, potentially because of a beef protein matrix with low density and high fat instability [98,99]. In addition, a similar study reported that the high moisture content prevents *Pleurotus sajor-caju* fibre from forming a three-dimensional matrix within the patties [62,100].

3.2.2. Colour Analysis

The brightness (L*), redness (a*), and yellowness (b*) of the cooked chicken patties were assessed by colour analysis (Table 6). Chicken patties fortified with GLF and PDF exhibited significantly lower L* ($p < 0.05$) compared to the control (68.56), but all formulations of PDF chicken patty were not significantly different from the commercial (55.98) patties. In addition, 9% PDF chicken patty showed the highest reading for a* ($p < 0.05$) among formulations and controls, which was expected due to the intense and vibrant colour of the pink mushroom. As for b*, the commercial chicken patty's reading is significantly different for all formulations and the control, except for the 9% PDF chicken patty.

Table 6. Colour analysis of seven cooked chicken patties incorporated with *Ganoderma lucidum* flour (GLF) and *Pleurotus djamor* flour (PDF).

	0% (Control)	3% GLF	6% GLF	9% GLF	3% PDF	6% PDF	9% PDF	Commercial
L*	68.56 ± 1.24 ^{d}	47.43 ± 1.74 ^{bc}	44.45 ± 6.06 ^{b}	34.66 ± 3.17 ^{a}	55.47 ± 3.26 ^{c}	52.11 ± 2.40 ^{bc}	49.88 ± 1.31 ^{bc}	55.98 ± 2.73 ^{c}
a*	2.63 ± 0.16 ^{a}	12.03 ± 1.42 ^{cd}	8.94 ± 1.80 ^{bc}	9.80 ± 0.67 ^{bcd}	7.39 ± 0.36 ^{b}	7.39 ± 0.36 ^{b}	12.75 ± 1.47 ^{d}	8.15 ± 2.49 ^{b}
b*	23.38 ± 0.84 ^{bc}	25.93 ± 1.52 ^{cd}	21.66 ± 1.20 ^{b}	15.47 ± 2.49 ^{a}	24.71 ± 1.24 ^{bc}	24.71 ± 1.24 ^{bc}	29.68 ± 1.00 ^{de}	30.77 ± 1.59 ^{e}

Means with different superscript letter within the same column (attributes) indicate a significant difference ($p < 0.05$).

The significant reading might be related to the increased levels of mushroom flour in the chicken patties led to a more intense and darker look (Figure 7A). The darker look of the GLF chicken patties can be attributed to the browning process that occurs during cooking. The browning of the chicken patties is specifically produced by the polysaccharides caramelisation that occurs in mushrooms during cooking [20,101]. GLF chicken patties recorded the highest L* because of the presence of natural triterpenoid-metabolites, also known as ganoderic acid, a bioactive compound found comprised in *Ganoderma* species [11]. The disparities in readings among various formulations and controls are primarily attributed to the initial coloration of both the mushrooms and muscle foods, alongside the physical interactions and chemical reactions that may occur between them, which fundamentally influence the impact of mushrooms on meat products [102].

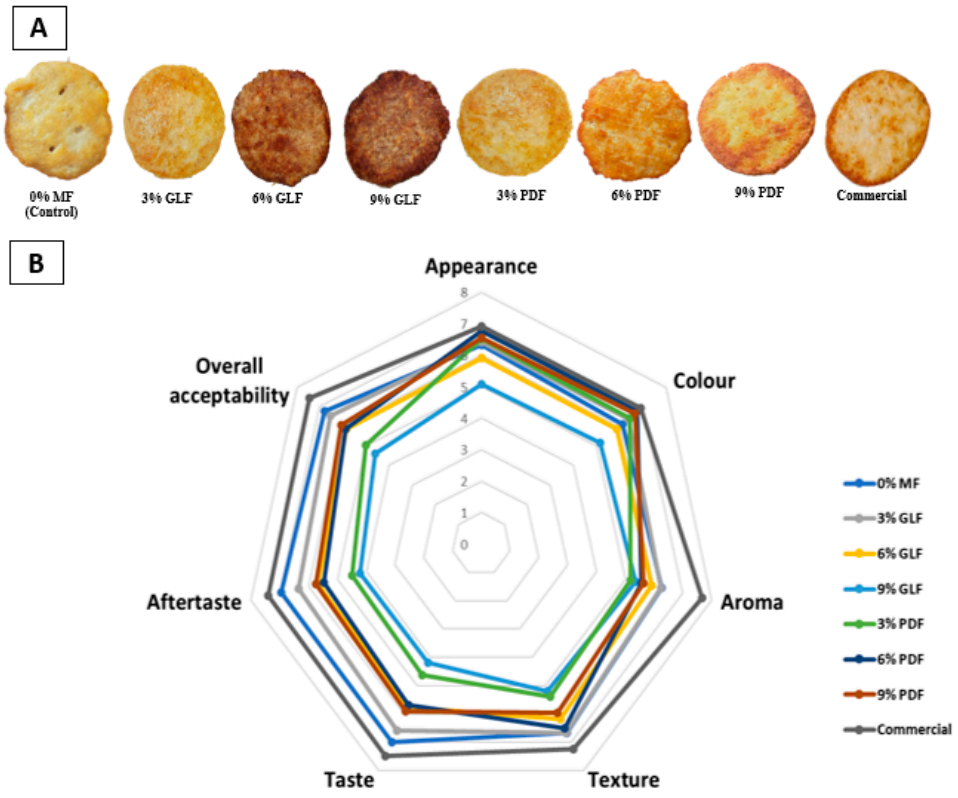

Figure 7. (**A**) Cooked control chicken patty, different formulations of mushroom chicken patties and commercial chicken patties. (**B**) Radar chart of sensory evaluation of chicken patty samples. Fifty panellists examined the sensory test to evaluate colour, odour, taste, texture, and overall acceptability using the 9-point hedonic scale (figure by author).

3.2.3. Texture Analysis

Based on Table 7, all chicken patties fortified with GLF exhibit no significant difference in hardness (1137.63–1653.62) compared to control and commercial patties. However, the chicken patties fortified with 3% and 6% PDF exhibited a considerably greater hardness ($p < 0.05$) ranging from 2003.49 to 2192.08. The decrease in hardness of mushroom chicken patties might be ascribed to the elevated moisture level in *Ganoderma lucidum* liquid-fermented and *Pleurotus djamor* mushrooms [98,102]. It can be seen that the higher the percentage of PDF mushroom flour added, the lower the hardness of PDF chicken patties. It can be seen the texture became crumbly and not well-formed compared to GLF chicken patties, which are denser.

Table 7. Texture value of cooked chicken patties incorporated with *Ganoderma lucidum* flour (GLF) and *Pleurotus djamor* flour (PDF).

	0%	3%GLF	6%GLF	9%GLF	3%PDF	6%PDF	9%PDF	Commercial
Hardness	1137.63± 468.34 ^{a}	1214.25± 1176.46 ^{a}	1453.86± 919.56 ^{a}	1653.62± 936.68 ^{ab}	2003.49± 617.93 ^{b}	2192.08± 472.11 ^{b}	919.03± 270.76 ^{c}	1520.83± 1011.47 ^{a}
Springiness	0.376± 0.13 ^{a}	0.42 ± 0.12 ^{a}	0.46± 0.18 ^{a}	0.43 ± 0.15 ^{a}	0.51 ± 0.17 ^{a}	0.57 ± 0.07 ^{a}	0.39 ± 0.046 ^{a}	0.41 ± 0.16 ^{a}
Cohesiveness	0.85± 0.01 ^{b}	0.83 ± 0.08 ^{b}	0.81± 0.09 ^{b}	0.84 ± 0.06 ^{b}	0.66 ± 0.14 ^{ab}	0.47 ± 0.12 ^{b}	0.54 ± 0.00 ^{b}	0.86 ± 0.08 ^{b}
Gumminess	963.77± 389.32 ^{ab}	948.83± 836.05 ^{ab}	1126.1± 633.50 ^{bc}	1347.23± 700.74 ^{cd}	1275.67± 205.22 ^{cd}	1058.13± 456.34 ^{b}	425.03± 117.29 ^{e}	1247.27± 759.03 ^{cd}
Chewiness	396.93± 257.99 ^{cd}	442.03± 140.67 ^{d}	588.63± 48.07 ^{a}	648.8 ± 47.36 ^{b}	645.89± 244.66 ^{b}	619.75± 115.99 ^{b}	168.26± 67.50 ^{e}	589.33± 44.25 ^{bc}

Means with different superscript letters within the same column (attributes) indicate a significant difference ($p < 0.05$).

The texture of chicken patties ranged from 425.03 (9% PDF) to 1347.23 (9% GLF) and 168.26 (9% PDF) to 648.8 (9% GLF) in terms of gumminess and chewiness, respectively. The chicken patties showed a range of cohesiveness and springiness values, with cohesiveness ranging from 0.47 (6% PDF) to 0.85 (9% GLF) and springiness ranging from 0.376 (9% PDF) to 0.57 (6% PDF). The value range of cohesiveness and springiness is similar to the one reported in a study by [20]. In addition, the 3% GLF showed texture attributes—hardness, gumminess, and chewiness—similar to those of the control. All GLF formulations also exhibited texture values comparable to the commercial sample.

The high errors are due to the different formulations of mushrooms biomass supplemented in chicken patties and compared to commercial chicken patty. Although it is challenging to correlate this engineering parameter with a sensory experience, this approach is highly beneficial for comprehending potential alterations to meat processing procedures to achieve values comparable to those previously approved by customers [103].

The structure of the patties that have been incorporated with both GLF and PDF may undergo certain changes in textural properties throughout the cooking process. The research study by [104] utilising flours derived from mushrooms of the species *Pleurotus ostreatus*, *Agaricus bisporus*, and *Agaricus brunnescen* also stated that the incorporation of non-meat components, the majority of which are derived from plants, into reformed meat products typically results in the production of finished products that are more tender in comparison to the original product.

3.2.4. Sensory Acceptance by Consumers

To elicit, quantify, examine, and comprehend the reactions to the properties of food and substances as they are perceived through the senses of vision, smell, flavour, texture, and sound, sensory evaluation uses scientific methodologies to assess these attributes [105].

Eight samples (Figure 7A), including negative control without mushroom flour (0% MF), GLF chicken patties (3%, 6% and 9%), PDF chicken patties (3%, 6% and 9%), and commercial chicken patties, were tested by 50 panellists for this study. The qualities of chicken patties were evaluated on a nine-point hedonic scale for appearance, colour, scent, texture, taste, aftertaste, and overall acceptance; the results showed above moderate acceptance.

According to Figure 7B, the commercial chicken patty from the supermarket achieved the highest scores in all sensory attributes, indicating its superior sensory profile including the presence of salt and other preservatives. The scores for each attribute include appearance, colour, aroma, texture, taste, aftertaste, and overall acceptability with respective mean scores of 6.92, 6.92, 7.69 7.22, 7.47, 7.41, and 7.45 (Table 8). Additionally, there were no significant differences ($p < 0.05$) for the majority of sensory attributes between the negative control, commercial, and other formulations of mushroom chicken patties, except for the aftertaste attributes.

Table 8. Mean scores of the attributes of chicken patties incorporated with *Ganoderma lucidum* liquid-fermented flour (GLF) and *Pleurotus djamor* (PDF) flour.

Formulation	Appearance	Colour	Aroma	Texture	Taste	Aftertaste	Overall Acceptability
0% GLF (Positive control)	6.33 ± 1.5 ^{a}	6.12 ± 1.55 ^{a}	6.27 ± 1.56 ^{a}	6.67 ± 1.37 ^{a}	6.98 ± 1.40 ^{a}	6.96 ± 1.36 ^{a}	6.78 ± 1.22 ^{a}
3% GLF	6.43 ± 1.81 ^{a}	6.37 ± 1.60 ^{a}	6.24 ± 1.67 ^{a}	6.65 ± 1.40 ^{a}	6.59 ± 1.58 ^{a}	6.35 ± 1.66 ^{a}	6.55 ± 1.65 ^{a}
6% GLF	5.9 ± 1.94 ^{a}	5.86 ± 1.71 ^{a}	5.92 ± 1.67 ^{a}	6.16 ± 1.70 ^{a}	5.75 ± 1.86 ^{a}	5.67 ± 1.93 ^{b}	5.86 ± 1.87 ^{a}
9% GLF	5.08 ± 2.10 ^{b}	5.16 ± 2.05 ^{a}	5.37 ± 1.72 ^{b}	5.18 ± 2.0 ^{b}	4.18 ± 2.04 ^{b}	4.22 ± 1.98 ^{b}	4.59 ± 1.93 ^{b}
3% PDF	6.53 ± 1.47 ^{a}	6.43 ± 1.47 ^{a}	5.18 ± 1.68 ^{b}	5.37 ± 1.67 ^{b}	4.61 ± 1.78 ^{b}	4.49 ± 1.73 ^{b}	5.02 ± 1.68 ^{b}
6% PDF	6.78 ± 1.32 ^{a}	6.76 ± 1.52 ^{a}	5.57 ± 1.75 ^{b}	6.49 ± 1.46 ^{a}	5.67 ± 1.77 ^{b}	5.47 ± 1.99 ^{b}	5.88 ± 1.73 ^{b}
9% PDF	6.55 ± 1.35 ^{a}	6.65 ± 1.20 ^{a}	5.65 ± 1.21 ^{a}	5.96 ± 1.90 ^{b}	5.9 ± 1.81 ^{b}	5.76 ± 1.85 ^{b}	6.08 ± 1.62 ^{a}
Commercial (Negative control)	* 6.92 ± 1.35 ^{a}	* 6.92 ± 1.45 ^{a}	* 7.69 ± 1.35 ^{a}	* 7.22 ± 1.45 ^{a}	* 7.47 ± 1.39 ^{a}	* 7.41 ± 1.30 ^{a}	* 7.45 ± 1.24 ^{a}

* Means with different superscript letters within the same column (attributes) indicate a significant difference ($p < 0.05$) between samples.

All formulations with GLF (3%, 6%, and 9%) did not differ significantly from the negative control (0% GLF), in terms of appearance. Chicken patty with 9% GLF even obtained the same mean score (6.37) as the negative control chicken patty. However, the positive control (Ramly burger) had the highest score (7.32), indicating a more appealing appearance. According to the visualised representation of the GLF chicken patties in Figure 7A, the addition of GLF led to darker-coloured chicken patties, which correlated with the percentage of GLF used.

In the context of industrial application and production line for a new food product, it is crucial to conduct sensory analysis to evaluate the acceptability and feasibility of manufacturing [106,107]. Therefore, the formulations of samples are chosen in this study (3%, 6%, and 9%). Additionally, the satisfaction of customers with food is determined by its quality, which encompasses sensory or organoleptic attributes [108,109]. *Ganoderma lucidum* is considered a natural dye or food colourant, and the brownish or blackish appearance observed in the GLF chicken patties was correlated to the total phenolic compounds, especially melanin, found in *Ganoderma lucidum* [110]. This justification conforms with the results in the study conducted by [10], in which the brown appearance of GL wines becomes less visible as the GL percentages in the wines decrease. Notably, the 6% GLF formulation received a more acceptable colour score (6.74) compared to the 9% GLF formulation (6.30).

The overall acceptability scores using *Ganoderma lucidum* liquid-fermented biomass as mushroom flour in chicken patty are interestingly higher compared to the preliminary study using fruiting body form and all formulations (3%, 6%, and 9%) of PDF chicken patties. The difference in concentration of ganoderic acids in different forms of mushrooms [111] showed the influence in terms of appearance, flavour, and aftertaste of chicken patties. Hence, this study has achieved the application of *Ganoderma lucidum* in food products without compromising the attributes' quality.

3.3. Comparison and Acceptability of Mushroom-Incorporated Savoury Food Products with Previous Studies

The comparison of mean scores of overall acceptability of GLF and PDF mushroom chicken patties and other food products fortified with mushroom flour is shown in Table 9. *Ganoderma lucidum* liquid-fermented biomass flour and *Pleurotus djamor* mushroom flour chicken patties indicated the highest acceptance scores compared to all studies. Currently, no study shows the usage of *Ganoderma lucidum* liquid-fermented biomass flour in food product applications. Therefore, the higher mean score of 6.55 for the 3% GLF (medicinal mushroom) chicken patty proved that the incorporation is valid, compared to a score of 6.08 for the 9% PDF (culinary mushroom) chicken patty. The lowest mean scores were was 10% and 20% for *Ganoderma lucidum* chicken patty from fruiting bodies form (3.03 and 2.10).

Table 9. Comparison of *Ganoderma lucidum* liquid-fermented biomass flour (GLF) and *Pleurotus djamor* mushroom flour (PDF) chicken patty with food products from previous studies.

Mushroom Species	Food Products	Flour Concentration	Overall Acceptability	Reference
Ganoderma lucidum liquid-fermented biomass flour *Pleurotus djamor* mushroom flour	Chicken patty	3% 9%	6.55 6.08	Current study
Ganoderma lucidum (Fruiting body powder)	Smoked Fish Sausage	0.25%	4.35	[112]
	Sausage	1%	5.73	[113]
	Chicken patty	10% and 20%	3.03 and 2.10	[45]
Pleurotus sapidus (Fruiting-body-base flour)	Chicken patty	10%,	5.73	[20]
	Meatballs	5%	3.38	[114]
Agaricus bisporus (Fruiting body powder)	Meatballs	5%	2.71	[114]

3.4. Significance of the Study

The execution of this research to develop functional mushroom biomass flour and incorporate it in food products is relevant to the current settings, which are briefly summarised in (Figure 8).

Firstly, the study is in line with the Malaysia's announced National Food Security Policy 2021–2025 (DSMN Action Plan) which encompasses the availability and sustainability of food supply in Malaysia following unexpected situations like the COVID-19 pandemic. The production of high-protein mushroom biomass flour, which can be fermented in laboratories promotes sustainability and enhances food security.

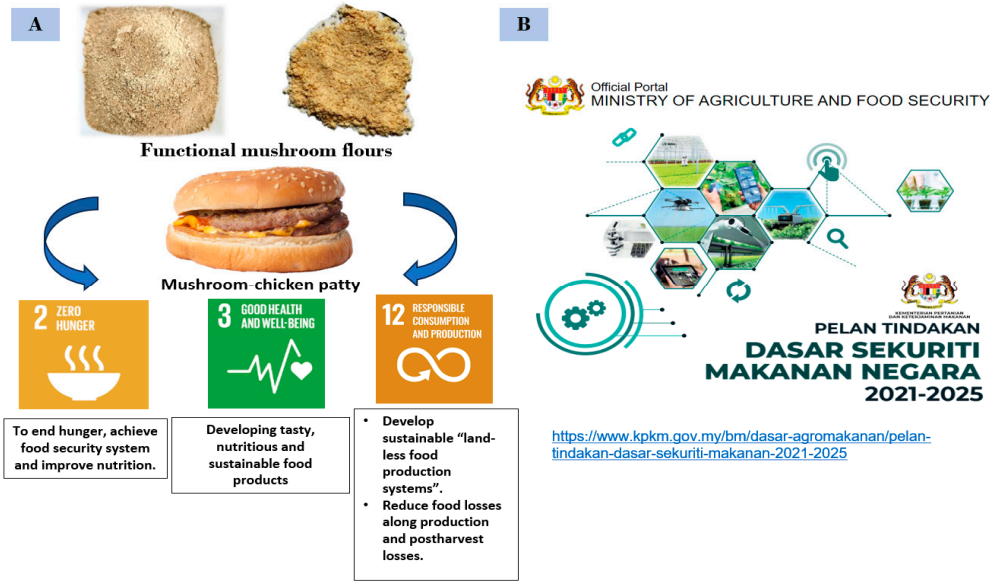

Figure 8. (**A**) Outcomes of this study in accordance to Sustainable Development Goals, (**B**) National Food Security Policy 2021-2025, Retrieved from https://www.kpkm.gov.my/bm/dasar-agromakanan/pelan-tindakan-dasar-sekuriti-makanan-2021-2025 (accessed on 18 May 2025) (figure by author).

To eradicate hunger, achieve zero hunger, and attain better nutrition and sustainable agriculture by 2030 are among the Sustainable Development Goals. The global COVID-19 pandemic worsened the hunger crisis, resulting in nutritional deficiency and malnutrition of infants. The number of undernourished people has risen from 690 million in 2019 to 720–811 million [115]. The most undernourished people are found in Asia (381 million). Africa ranks second (250 million) after the Caribbean and Latin America (48 million) [116]. Hence, mushrooms are utilised to balance mainly protein deficiency and for populations that do not eat animal proteins due to unavailability or religious belief [117]. Aligned with sustainable development goals (SDGs) [118], this food product development will help to achieve both the second and third goals which are zero hunger and good health and well-being, respectively, by 2030.

World Food Program (WFP) and Food and Agriculture Organization (FAO) and, World Health Organization (WHO) interpret hunger from many perspectives. These encompass food insecurity, food supply shortages, chronic undernourishment, reduced food intake accompanied by bodily manifestations of hunger, and persistent concern over the timing and availability of their next meal [119]. Around 702 to 828 million individuals faced hunger in 2021 [120], while over 1 billion people globally endure hunger, malnutrition, and food insecurity, with a significant proportion residing in Sub-Saharan Africa and South Asia. The index of hunger globally has been increasing due to undernutrition and child mortality [121].

The FAO [120] reported that global hunger escalated further in 2021, contrary to anticipations that the COVID-19 pandemic would have subsided and food security would begin to improve. By 2021, almost 30% of the global population, equating to around 2.3 billion individuals, experienced moderate to severe food insecurity. The global population was anticipated to reach 8 billion on 15 November 2022, is now projected to approach 9 billion by around 2037 and forecasted to attain 10 billion around 2058 [122], which would provide increases of 50%, 60%, and 55% in the demands for water, energy, and food, correspondingly [123,124]. This forecast leads to further ramifications and possibilities. Significant manufacturing waste is one of the issues. The World Bank forecasts that global garbage, projected at 2.01 billion tons annually in 2016, would rise by more than 70% by 2050 [125].

The global output of mushrooms has risen, resulting in around 53 million tons of mushroom waste, since 1 kg of mushrooms necessitates 5 kg of mushroom media substrate [126]. Currently, over 12.74 million tonnes of mushrooms are consumed globally, and projections indicate that by 2026, the global mushroom market value is expected to attain 20.84 million tonnes [4,127]. Furthermore, increasing waste mushroom substrate (WMS) arises from augmented mushroom cultivation [128].

In addition, mushrooms rapidly degrade due to browning, wilting, and liquefaction, resulting in texture, fragrance, and flavour loss, making them unmarketable [129]. The high moisture content and delicate texture result in a short post-harvest life and can be maintained only for 24 h under tropical conditions [130]. Therefore, the production of *Ganoderma lucidum* liquid-fermented biomass is in line with SDG 12, which is responsible for consumption and production using the landless food concept.

The core principle of the circular economy is to proactively enhance and acknowledge every phase of production to reduce the waste produced by industrial processes [131]. Agriculture is seen as a relevant domain for the implementation of the circular economy due to its focus on substantial waste generation, constraints in nutrient circulation, and environmental sustainability [132,133]. The growing human need for protein-rich food and the limitations of current technology have prompted the pursuit of cost-effective alternatives for generating protein-rich meals [134]. In Nigeria, where individuals turn to mushroom gardening to address malnutrition, poverty, and hunger, mushrooms exemplify a viable means of achieving food security due to their high fibre and protein content [117].

3.5. Potential Future Studies

Mushrooms have been extensively utilised as an ingredient and substitute in several food products [20,22,135,136]. This research serves as a foundation for further investigations into the uses and optimisation of different medicinal mushrooms as liquid-fermented biomass in other savoury food items. Additionally, biomass flour and mushroom chicken patties may be developed for industrial-scale manufacturing, establishing connections with primary ingredient suppliers and facilitating worldwide marketability. Further analysis for this study should include the flavouring of the patties and more research on the nutritional profile of each mushroom–chicken burger to enhance commercialisation. The utilisation of mushroom-based flour can also be commercialised as a gluten-free filler material with nutritional advantages in the production of functional food.

4. Conclusions

The study focused on the production of biomass flour from medicinal mushroom (*Ganoderma lucidum*-GL) through liquid fermentation, and the culinary mushroom (*Pleurotus djamor*-PD), to analyse their functional properties and develop mushroom chicken patties.

The ENS were extracted from both mushroom biomasses and assessed for characterisation, antimicrobial, and antioxidant properties. The structure of extracted ENS-GL and ENS-PD was successfully characterised using elemental analysis, Fourier transform infrared spectroscopy (FT-IR), and NMR spectroscopy. In addition, ENS-GL and ENS-PD also prove that they can act as antibacterial agents, by hindering the growth of bacteria and exhibiting a significant level of antioxidant activities.

Consequently, 3% GLF chicken patty was accepted overall, achieving the maximum score of 6.55, appealing to consumers' preferences due to its attractive appearance (6.43), colour (6.37), aroma (6.24), texture (6.65), taste (6.59), and aftertaste (6.35) compared to other GLF and PDF formulations. In addition, the lowest cooking loss observed in the 9% GLF and 3% PDF chicken patties suggests a more optimal eating quality. However, for

commercialisation purposes, it is recommended to incorporate a minimum of 3% GLF, as this formulation obtained the highest overall acceptability score of 6.55.

Given the present increase in demand for health food, *Ganoderma lucidum* and *Pleurotus djamor* have proven to be alternative flours containing functional properties (antimicrobial and antioxidant) in a formulated chicken patties product. The medicinal mushroom *Ganoderma lucidum* and *Pleurotus djamor* biomass, with a high protein content and a good nutritional profile, were successfully used as an alternative flour to replace potato starch in chicken patty formulations.

This study provides a blueprint for food companies to develop chicken patties from laboratory-grown biomass powder. It also highlights its relevance and importance, both domestically and globally in terms of achieving three of the Sustainable Development Goals (Goal 2: Zero Hunger, Goal 3: Good Health and Well-Being, and Goal 12: Responsible Consumption and Production) and is following Malaysia's National Food Security Policy (DSMN Action Plan) 2021–2025.

Author Contributions: Conceptualization, W.A.A.Q.I.W.-M. and N.A.Z.-A.; writing—original draft preparation, N.A.Z.-A., Z.I. and A.A.J.; writing—review and editing, A.A.J., N.A.Z.-A., C.S.Y., M.A.U.-R., Z.I., M.H.A.R., S.R. and N.H.; visualisation, W.A.A.Q.I.W.-M., N.A.Z.-A. and C.S.Y.; supervision, W.A.A.Q.I.W.-M., Z.I. and A.A.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been funded by Universiti Malaya Partnership Grant. Thank you to the Universiti Malaya Partnership Grant ''Transforming Soy Sauce Processing Liquid Waste into Essential Polyunsaturated Fatty Acids Utilising Thraustochytrids from Malaysian Mangroves (MG004-2025)" and ''Formulation of Lampam Sungai Sustainable Fish Feed Using Petronas Waste (MG002-2025)".

Institutional Review Board Statement: The study was conducted in accordance with the Universiti Malaya Research Ethics Guidelines, and approved by the Universiti Malaya Research Ethics Committee (UMREC) of Universiti Malaya (UM.TNC2/UMREC_4045) and December 2024 till December 2027).

Informed Consent Statement: Written informed consent has been obtained from the consumers to publish this paper.

Data Availability Statement: All data sets are available upon request.

Acknowledgments: Thank you to the Universiti Malaya Partnership Grant ''Transforming Soy Sauce Processing Liquid Waste Into Essential Polyunsaturated Fatty Acids Utilising Thraustochytrids From Malaysian Mangroves (MG004-2025)" and ''Formulation of Lampam Sungai Sustainable Fish Feed Using Petronas Waste (MG002-2025)".

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

Abbreviations

The following abbreviations are used in this manuscript:

GLF	*Ganoderma lucidum* flour
PDF	*Pleurotus djamor* flour
ENS-GL	Endopolysaccharide of *Ganoderma lucidum*
ENS-PD	Endopolysaccharide of *Pleurotus djamor*
FT-IR	Fourier Transform Infrared Spectroscopy
^{1}H NMR	Nuclear Magnetic Resonance with respect to hydrogen-1 nuclei

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