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Synergistic anti-glycation and antioxidant interaction among different mushroom extract combinations

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Abstract:
Although the nutrient compositions of edible mushrooms are well-studied, the effect of combining different mushrooms on their anti-glycation and antioxidant activities remains unknown. This study therefore aimed to identify mushroom combinations that exhibit synergistic anti-glycation and antioxidant activities.

Five edible mushroom species, namely *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus ostreatus*, *Pleurotus eryngii*, and *Flammulina velutipes*, were evaluated both individually and in pairwise combinations. Their bioactive profile (phenolics, tannins, flavonoids, and polysaccharides), as well as antioxidant and anti-glycation activities were analyzed to determine the types of activity interaction: synergism, addition, or antagonism.

A. bisporus (7.5 mg/mL) showed the highest reducing capacity and tannin content. *L. edodes* demonstrated the strongest radical scavenging potential, while *F. velutipes* displayed the highest anti-glycation activity and phenolic content. Despite its high polysaccharide level, *P. eryngii* showed low antioxidant activity. Pairwise combinations revealed synergistic anti-glycation and antioxidant effects at low sample concentrations, while antagonistic anti-glycation and antioxidant effects were observed at high sample concentrations. The mushrooms' polyphenols, tannins, and flavonoids were positively correlated with their antioxidant activity (r = 0.325 to 0.825, $p < 0.05$). However, they showed an inverse relationship (r = –0.349 to –0.644, $p < 0.05$) with polysaccharides and anti-glycation activity. The principal component analysis revealed that the types of bioactive content and mushroom combinations contributed to respective 53 and 23% of total activity variances.

The best-performing mushroom combinations with synergistic anti-glycation and antioxidant activities were the mixtures of 7.5 mg/mL *A. bisporus* + 15 mg/mL *F. velutipes*, 7.5 mg/mL *L. edodes* + 7.5 mg/mL *F. velutipes*, and 7.5 mg/mL L. edodes + 15 mg/mL *F. velutipes*.

Keywords: Flavonoid, fungi, phytochemical, phenolic, polysaccharide, tannin

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INTRODUCTION

Oxidation is an essential process in biological systems that facilitates the production of energy [1]. However, numerous reactive nitrogen species and reactive oxygen species are generated as by-products during the oxidation process [1]. The formation of excessive reactive radicals is further accelerated by the production of advanced glycation end-products (AGEs) under pathological conditions. AGEs are a chemically heterogenous group of compounds, including crossline, pentosidine (Pent), Nε-(carboxymethyl)lysine (CML), and pyrroline [2]. Various reactive radicals, such as hydrogen peroxide, hydroxyl, and superoxide anion, as well as AGEs, interact with biological systems in a cytotoxic manner [2]. This leads to cell death and other degenerative processes associated with ageing [3]. Excessive deposition of AGEs and free radicals in a human body is a catalyst for a multitude of detrimental diseases such as cancer, atherosclerosis, diabetes, neurological disorders, and cardiovascular diseases [4]. However, a significant proportion of these chronic diseases could be prevented with the use of antioxidants [5] and AGE inhibitors [2].

Foods with a natural abundance of antioxidants and AGE inhibitors hold the potential to mitigate the detrimental effects of oxidative damage [2, 5]. These exogenous antioxidants and AGE inhibitors effectively hinder the generation of AGEs by scavenging free radicals produced during glycation, while inhibiting advanced oxidation protein products [2]. Epidemiological studies have revealed a strong positive association between plant-based foods and a decreased risk of chronic illnesses [6]. Bioactive constituents within food can lower the risk of chronic diseases and thus improve human well-being by reducing blood cholesterol or neutralizing reactive species, as well as through anti-carcinogenic, anti-hypertensive, or anti-glycemic responses [7]. Food scientists are increasingly exploring natural sources that are rich in bioactive compounds and have antioxidant and anti-glycation effects. These compounds contribute to lesser adverse side effects and a minimized likelihood of developing resistance to them [8].

In recent years, the incorporation of mushrooms as dietary supplements has garnered considerable attention within the realm of food nutrition [7]. Mushrooms have been consumed by humans since ancient times due to their ideal nutritional compositions, appealing sensory characteristics, and amenable cultivate conditions [7]. They have been recognized for their crucial role in mitigating and preventing an array of health complications [7, 9, 10]. Mushrooms are rich in bioactive compounds such as flavonoids, lycopene, phenolic acids, ascorbic acid, tocopherols, carboxylic acids, β-carotene, and various dietary fibers [11]. Based on their intended applications, mushroom species can be classified into edible and medicinal categories [7]. Despite the growing interest in medicinal varieties, edible cultivated species such as *Lentinula edodes* and *Agariscus bisporus* continue to dominate the market [7]. *Lentinula edodes* (shiitake), *Agaricus bisporus* (button mushroom), *Pleurotus ostreatus* (grey oyster), *Flammulina velutipes* (golden needle), and *Pleurotus eryngii* (king oyster) represent some of the most prominent commercially cultivated mushrooms [12]. Among the major types of edible mushrooms, shiitake leads in production volume, followed by oyster mushroom (*Pleurotus* spp.) and button mushroom [13]. According to numerous studies, these mushroom species display various bioactivities, including anti-mutagenic, antioxidant, anti-tumor, anti-dementia, hypoglycemic, acetylcholinesterase-inhibitory, anti-microbial, and anti-inflammatory potentials [14–17].

Phytochemical extracts derived from plant-based foods have been reported to exhibit potent anti-proliferative and antioxidant activities. Antioxidant activity is significantly due to the interaction of phytochemicals, leading to synergistic, additive, or antagonistic effects [18]. This may explain why isolated antioxidants cannot supplant combinations of natural phytochemicals in foods and replicate their health advantages [9]. The interaction among phytochemicals, such as flavonoids, within a given plant extract can substantially contribute to human health by alleviating disease-related cellular damage, as the bioactive compounds involved rarely function independently [18]. Wang *et al.* [18] defined an additive effect as a food combination that yields the cumulative impact of individual components. A synergistic effect arises when the outcome surpasses the sum of individual components, whereas antagonism takes place when the combined effect is less than the anticipated mathematical sum of individual components. Although multiple studies have reported that many plant-based foods possessed numerous bioactive compounds with functional activities [19, 20], only few have investigated the synergism related to the use of fungi species. For instance, Wang *et al.* [18] did not observe any synergistic effect when *A. bisporus* was combined with other fruits, vegetables, and legumes. However, Mallard *et al.* [21] showed that a combination of β-glucans from *Lentinula edodes*, *Grifola frondosa*, and *Ganoderma lucidum* exhibited a synergistic immunomodulatory effect in human macrophages.

To the best of our knowledge, there has been no research into the effects of *L. edodes*, *A. bisporus*, *F. velutipes*, *P. eryngii,* and *P. ostreatus* combinations on antioxidant and anti-glycation activities. Assessing synergistic effect is a challenging endeavor, since various available bioassays are based on distinct chemical mechanisms [18]. Given the diversity of phytochemical classes and types of mushrooms, a multitude of bioassays are required to assess their overall antioxidant and anti-glycation capacity [15]. The sensitivity and specificity of a singular method cannot ensure a comprehensive evaluation of all dietary bioactive compounds. Therefore, a combination of multiple tests can be a more precise indicator of antioxidant and anti-glycation activities [18].

In this study, we aimed to explore the effects of *L. edodes*, *A. bisporus*, *F. velutipes*, *P. eryngii,* and *P. ostreatus* combinations. For this, we adopted seven distinct bioassays, namely ferric reducing antioxidant power, total flavonoid content, total tannins, total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity, AGE inhibition analysis, and polysaccharide assays. These assays were employed to discern the types of interactions between the phytochemicals, antioxidant and anti-glycation activities in the selected mushroom species, as well as to identify the best mushroom combinations that yield synergistic antioxidant and anti-glycation effects.

STUDY OBJECTS AND METHODS

Chemicals. All the reagents used in this study were of analytical grade. Iron(III) chloride hexahydrate, 2,2-diphenyl-1-picrylhydrazyl, sodium azide, iron(II) sulphate heptahydrate, 3,4,5-trihydroxybenzoic acid (gallic acid), sodium acetate, bovine serum albumin, sodium carbonate, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin), 2,4,6-tripyridyl-s-triazine, trichloroacetic acid, Folin-Ciocalteu's reagent, tannic acid, D-glucose, phenol, and starch were obtained from Sigma-Aldrich (USA). Aminoguanidine hydrochloride was sourced from Tokyo Chemical Industry

(Japan). Potassium acetate ($C_2H_3KO_2$), aluminum chloride ($AlCl_3$), and polyvinylpolypyrrolidone were purchased from Thermo Fisher-Scientific (USA). Absolute ethanol, glacial acetic acid, hydrochloric acid, methanol, and concentrated sulphuric acid were obtained from Merck (Germany). L-ascorbic acid was purchased from HmbG Chemicals (Germany).

Equipment. The assays were performed on the following equipment: a hot air oven (Memmert GmbH & Co. KG, Germany), a vortex mixer (IKA-Werke GmbH & Co. KG, Germany), an Alpha 1-2 LSC basic laboratory freeze dryer (Germany), an Epoch microplate spectrophotometer (Biotek Instrument Inc., USA), a centrifuge (Eppendorf AG, Germany), an electrical blender (Philips, Netherlands), a hotplate (Harmony, Japan), a water bath (Memmert GmbH & Co. KG, Germany), and a digital analytical balance (Sartorius Corporate Administration GmbH, Germany).

Sample collection. Five fresh commercial cultivated mushrooms, namely *Agaricus bisporus, Flammulina velutipes, Lentinula edodes, Pleutorus eryngii,* and *Pleurotus ostreatus,* were purchased from a local grocery store in Selangor, Malaysia. Mushroom samples with complete fruiting bodies (gills, caps, stipe, and tubes) in various sizes were randomly selected from the store's shelves. Prior to processing, all the samples were washed with distilled water and dried.

Sample processing. The mushroom samples were extracted according to Midoh *et al.* [22] with slight modifications. Briefly, clean mushrooms were blended with 80°C distilled water in a solid-to-solvent ratio of 1:5 for 15 min. The extract was subsequently filtered using cheesecloth, and the filtrates were centrifuged at 16,000 × g for 10 min at room temperature. The collected supernatant was further filtered through Whatman filter paper, and the remaining filtrates were frozen before being subjected to a 72-hour freeze-drying process. The resulting lyophilized powders were stored in airtight containers at –20°C until analysis. For experimental analysis, lyophilized powders of an individual mushroom were reconstituted in 1 mL of distilled water to achieve concentrations of 7.5, 15.0, and 30.0 mg/mL for *A. bisporus, F. velutipes,* and *L. edodes,* and 25.0, 50.0, and 100.0 mg/mL for *P. eryngii* and *P. ostreatus*. These sample concentrations were determined based on a previous pilot study finding (unpublished data). The mushroom extracts were then combined in pairs at a 1:1 (v/v) ratio to produce 90 distinct combinations (Table 2) to evaluate their antioxidant and anti-glycation interactions.

Water content. The water content in the selected mushroom samples was determined according to Li *et al*. [23]. Each mushroom sample (100 g) was cut into smaller pieces and spread in a single layer on trays in triplicates. The samples were dried in a hot air oven at 50 ± 2°C until constant weight was obtained. To monitor water loss, the sample trays were removed from the hot air oven every 12 h and weighed using a digital analytical balance. The water content, %, was calculated with the following formula:

$$\text{Water content} = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

where W_0 is the fresh weight of mushroom sample, g; W_1 is the dry weight of mushroom sample, g.

Extraction yield. The extraction yield, %, of each mushroom sample was determined according to Tepsongkroh *et al*. [24] via the following formula:

$$\text{Yield} = \frac{W_3}{W_0} \times 100 \quad (2)$$

where W_3 is the weight of lyophilized sample powder.

Antioxidant activities. ***Ferric-reducing antioxidant power.*** A sample's ferricreducing antioxidant power (FRAP) was measured based on Ranneh *et al.* [25]. Briefly, the FRAP reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine, and 20 mM iron(III) chloride hexahydrate in a 10:1:1 (v/v/v) ratio. Following the addition of the FRAP reagent to the mushroom sample, the sample's absorbance was measured at 593 nm after 4 min of incubation at room temperature. The FRAP values were expressed as millimole ferrous ion equivalent per 100 g of a fresh weight sample (mmol Fe^{2+}/100g FW), using a standard curve of iron(II) sulphate (0–1000 μM).

DPPH radical scavenging activity. A sample's 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to Gerhauser *et al.* [26] with minor modifications. 100 μM of the DPPH reagent was mixed with the sample and the change in reaction absorbance was measured for 60 min, with 15 min intervals at 515 nm. The DPPH radical scavenging capacity was expressed as milligram ascorbic acid equivalent per 100 g of a fresh weight sample (mg AA/100g FW), using a standard curve of ascorbic acid (0–1000 μM).

Advanced glycation end-products inhibition activity. A sample's anti-glycation activity was determined according to Ho *et al*. [27]. The mushroom samples were mixed with 1M glucose and 10 mg/mL bovine serum albumin in 50 mM of phosphate buffer (pH 7.4). The reaction mixtures were incubated at 80°C for 7 days, while the negative control was kept at 4°C. All the reactions were then stopped with 100% trichloroacetic acid and centrifuged for 10 min at 15,000 x g. Pellets of advanced glycation end-products and bovine serum albumin were dissolved with phosphate-buffered saline (pH 10.0), and the reaction fluorescence intensity was determined at 360 nm excitation and 450 nm emission wavelength. The sample's anti-glycation activity was expressed as milligram of aminoguanidine hydrochloride per 100 g of a fresh weight sample (mg AH/100g FW), with aminoguanidine (0–10 mg/mL) used as a standard.

Bioactive compounds. ***Total phenolic content.*** A sample's total phenolic content (TPC) was measured using Folin-Ciocalteu's method, as previously described by Yong *et al.* [19]. Briefly, 10% of Folin–Ciocalteu's reagent was added to each mushroom sample and incubated for 3 min prior to the addition of 10% sodium carbonate. The reaction was incubated at room temperature

in the dark for 60 min prior to measuring the absorbance at 750 nm. Gallic acid (0–100 µg/mL) was used as the standard. The TPC value was expressed as milligram of gallic acid equivalent per 100 g of a fresh weight sample (mg GAE/100 g FW).

Total tannin content. A sample's total tannin content was measured according to Siddhuraju & Manian [28]. Tannic acid (0 to 100 µg/mL) was used as the standard. Briefly, mushroom samples were incubated with and without polyvinylpolypyrrolidone (PVPP) at 4°C for 15 min. The sample mixtures were centrifuged for 10 min at 15,000 × g. The total phenolic content (TPC) was determined in the collected sample supernatants. The total tannin content in each sample corresponded to the difference in TPC values between the PVPP-treated sample and the water-treated sample. Tannic acid (0 to 100 µg/mL) was used as the standard. The total tannin value was expressed as milligram of tannic acid equivalent per 100 g of a fresh weight sample (mg TAE/100 g FW).

Total flavonoid content. A sample's total flavonoid content (TFC) was measured with aluminum chloride as outlined by Yong *et al.* [19]. Briefly, each mushroom sample was mixed with 95% ethanol, 1M $C_2H_3KO_2$, 10% (w/v) $AlCl_3$, and distilled water and then incubated for 30 min in the dark prior to measuring the absorbance at 415 nm. Quercetin (0–300 µg/mL) was used as the standard. The TFC value was expressed as milligram of quercetin equivalent per 100 g of a fresh weight sample (mg QE/100 g FW).

Polysaccharides. A sample's polysaccharide content was measured according to Masuko *et al.* [29]. Briefly, the mushroom sample was mixed with concentrated sulphuric acid and incubated for 30 min on a plate shaker. 5% phenol was then added to the mixture and incubated for 5 min at 90°C. The reaction plate was cooled for 5 min prior to measuring the absorbance at 590 nm. Starch (0–1.2 mM) was used as the standard. The carbohydrate value was expressed as milligrams of polysaccharide equivalent per 100 g of a fresh weight sample (mg PE/100g FW).

Bioactivity interaction. The experimental sample capacity (ESC) for individual mushroom samples and combined mushroom mixtures was determined via the following equation [30]:

$$ESC = 100 - \frac{\left(Abs_{sample} - Abs_{blank}\right)}{Abs_{control}} \times 100 \quad (3)$$

where Abs_{sample} is the absorbance of the sample reaction, Abs_{blank} is the sample blank, and $Abs_{control}$ is the absorbance of the reagent control [30].

The theoretical sample capacity (TSC) was calculated for combined sample mixtures and was described as a cumulative sum of individual sample capacities of each constituent found in the mushroom mixtures. It was determined via the following equation:

$$TSC = \left(ESC_A + ESC_B\right) - \frac{\left(ESC_A \times ESC_B\right)}{100} \quad (4)$$

where ESC_A and ESC_B are the ESC values of each individual mushroom sample in the combined mushroom mixture.

For the combinational study, individual mushroom samples were combined in a 1:1 ratio. A mathematical model for deriving synergism evaluation (SE) values [30] was used to determine the types of interaction among various mushroom combinations as follows:

$$SE = \frac{ESC_{mixture}}{TSC_{mixture}} \quad (5)$$

Interactions were categorized as synergism (SE > 1), antagonism (SE < 1), or additive (SE ≈ 1) based on the SE value.

Statistical analysis. All the analyses were carried out in triplicate, and the final data were presented as mean values ± standard deviations. One-way analysis of variance and Tukey's post hoc test were used to compare the sample values, with $p \leq 0.05$ deemed as statistically significant. Pearson's correlation and regression tests were employed to evaluate the relationship among the activity variables. Data analyses were performed with XLSTAT 2021 (Addinsoft Inc., USA) and GraphPad Prism 6 Software (GraphPad Software, USA).

RESULTS AND DISCUSSION

Mushroom extraction yield and water content. The extraction yields of the selected five mushrooms varied from 1.3 to 3.4% in the following order: *Flammulina velutipes* > *Agaricus bisporus* > *Lentinula edodes* > *Pleurotus eryngii* > *Pleurotus ostreatus* (Table 1). Among the five mushroom species, *F.velutipes* demonstrated the highest extraction yield, suggesting the presence of water-soluble phytochemicals such as phenolic compounds [31], amino acids, oligosaccharides, and monosaccharides. This speculation was substantiated by the high total phenolic content (TPC) in *F. velutipes* (Table 2).

The water content in the mushrooms ranged from 87.6 to 93.6 % (Table 1). *A. bisporus* showed the highest moisture content (93.6 ± 0.6%), followed by *P. ostreatus* (89.8 ± *0.5%)*, *F. velutipes* (88.8 ± 0.3%), *L. edodes* (87.8 ± 0.4%), and *P. eryngii* (87.6 ± 0.0%) (Table 1). These values agreed with a previous report by Yong *et al.* [19] that highlighted similar moisture values for *A. bisporus* (94.8 ± 0%) and *F. velutipes* (89.8 ± 0.1%). The differences in water content for other mushrooms, when compared to

Table 1 The extraction yield and water content of the selected edible mushrooms

Samples	Extraction yield, %	Water, %
Agaricus bisporus	3.06[a]	93.60 ± 0.59[c]
Lentinula edodes	2.53[b]	87.80 ± 0.35[a]
Flammulina velutipes	3.35[c]	88.83 ± 0.25[ab]
Pleurotus ostreatus	1.25[d]	89.75 ± 0.46[b]
Pleurotus eryngii	2.24[e]	87.60 ± 0.00[a]

Values indicate mean ± standard deviations of three independent measurements. Values within the same column followed by the same superscript lower-case letters do not differ significantly ($p > 0.05$)

Table 2 Polyphenol, polysaccharide, antioxidant, and anti-glycation values in the mushroom species under study

Mushroom species	Concentration, mg/mL	Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
		FRAP, mmol Fe^{2+}/100 g	DPPH, mgAA/100 g	Anti-AGE, mgAH/100 g	TFC, mg QE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
Agaricus bisporus	7.5	3.9 ± 0.0^{b}	501.0 ± 0.0^{a}	43.3 ± 0.5^{f}	626.8 ± 0.0^{a}	246.4 ± 0.0a	36.9 ± 0.0^{a}	6576.2 ± 0.0^{a}
	15	3.9 ± 0.0^{c}	446.2 ± 0.0^{b}	20.9 ± 0.6^{a}	484.6 ± 0.0^{b}	102.0 ± 0.0^{b}	30.4 ± 0.0^{b}	4848.2 ± 0.0^{b}
	30	3.4 ± 0.0^{d}	427.3 ± 0.0^{c}	9.2 ± 0.4^{b}	352.8 ± 0.0^{c}	87.3 ± 0.0^{c}	29.5 ± 0.0^{c}	3861.8 ± 0.1^{c}
Lentinula edodes	7.5	2.7 ± 0.0^{e}	548.0 ± 0.0^{d}	53.6 ± 0.8^{g}	623.5 ± 0.0^{d}	55.8 ± 0.0^{d}	4.3 ± 0.0^{d}	10773.7 ± 0.0^{d}
	15	2.6 ± 0.0^{f}	523.2 ± 0.0^{e}	22.6 ± 0.8^{a}	520.4 ± 0.0^{e}	86.8 ± 0.0^{e}	16.3 ± 0.0e	8633.0 ± 0.0^{e}
	30	2.5 ± 0.0g	431.8 ± 0.0^{f}	10.0 ± 0.5^{b}	386.6 ± 0.0^{f}	63.6 ± 0.0^{f}	27.4 ± 0.0^{f}	7650.0 ± 0.1^{f}
Flammulina velutipes	7.5	2.0 ± 0.0h	430.5 ± 0.0g	84.2 ± 1.4^{h}	688.5 ± 0.0^{g}	115.2 ± 0.0^{g}	2.2 ± 0.0^{g}	3344.1 ± 0.0^{g}
	15	2.2 ± 0.0^{i}	383.6 ± 0.0^{h}	37.5 ± 1.1^{i}	540.3 ± 0.0^{h}	149.9 ± 0.0^{h}	23.3 ± 0.0^{h}	2119.8 ± 0.0^{h}
	30	2.2 ± 0.0^{j}	379.0 ± 0.0^{i}	19.7 ± 0.7^{a}	390.1 ± 0.0^{i}	71.3 ± 0.1^{i}	34.7 ± 0.0^{i}	2406.6 ± 0.0^{i}
Pleurotus ostreatus	25	0.6 ± 0.0^{a}	115.1 ± 0.0^{j}	12.6 ± 0.8^{c}	391.5 ± 0.0^{j}	202.1 ± 0.0^{j}	7.8 ± 0.0^{j}	21256.4 ± 0.0^{j}
	50	0.6 ± 0.0^{a}	122.5 ± 0.0^{k}	5.9 ± 0.7^{d}	247.2 ± 0.0^{k}	118.1 ± 0.0^{k}	4.9 ± 0.0^{k}	20887.0 ± 0.0^{k}
	100	0.8 ± 0.0^{k}	112.7 ± 0.0^{l}	2.7 ± 0.1^{e}	134.0 ± 0.0^{l}	57.1 ± 0.0^{l}	16.8 ± 0.0^{l}	19417.9 ± 0.0^{l}
Pleurotus eryngii	25	0.6 ± 0.0^{a}	173.0 ± 0.0^{m}	14.8 ± 0.4^{c}	328.8 ± 0.0^{m}	70.1 ± 0.0^{m}	1.6 ± 0.0^{m}	24300.0 ± 0.1^{m}
	50	0.6 ± 0.0^{a}	153.0 ± 0.0^{n}	6.7 ± 0.4^{d}	236.1 ± 0.0^{n}	88.8 ± 0.0^{n}	3.7 ± 0.0^{n}	38533.5 ± 0.0^{n}
	100	0.5 ± 0.0^{l}	144.6 ± 0.0^{o}	3.5 ± 0.1^{e}	132.3 ± 0.0^{o}	45.8 ± 0.0^{o}	7.3 ± 0.0^{o}	49479.5 ± 0.0^{o}

Values indicate mean ± standard deviation of three independent measurements. Values within the same column accompanied by the same superscript lower-case letter do not differ significantly ($p > 0.05$). FRAP – ferric-reducing antioxidant power; DPPH – 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; TPC – total phenolic content; TT – total tannins; TFC – total flavonoid content; AGE – advanced glycation end-product; AA – ascorbic acid; AH – aminoguanidine hydrochloride; GAE – gallic acid equivalent; TAE – tannic acid equivalent; QE – quercetin equivalent; PE – polysaccharide equivalent

those reported by Yong *et al.* [20], could be attributed to variations in environmental conditions, such as humidity and temperature during the cultivation period [32].

Antioxidant activity of individual mushroom extracts. A three-sample concentration model was adopted for each mushroom species to comprehensively evaluate their phytochemical, antioxidant, and anti-glycation activities. This concentration gradient approach enabled a thorough investigation into the potency and efficacy of individual mushrooms and their combinations across a range of concentrations as recommended by Hengst *et al.* [33].

The concentrations of *L. edodes, A. bisporus,* and *F. velutipes* were selected within a 7.5–30.0 mg/mL range based on the previous pilot study and literature suggesting that this concentration range could yield observable phytochemical activity pertinent to antioxidant and anti-glycation activities [34–36]. This approach aimed to identify the lowest and highest effective sample concentration that demonstrates significant functional activity. However, an exception was made for both *P. ostreatus* and *P. eryngii,* in which the concentration series were adjusted to a higher range of 25–100 mg/mL. This modification stemmed from the pilot study conducted prior to the current experimental phase, which revealed that *Pleurotus spp.* exhibited noticeable functional activity exclusively at a threshold concentration of 25 mg/mL. This finding agreed with Reis *et al.* [37] where lower antioxidant activity was observed in *Pleutorus spp.* when compared to other cultivated mushrooms at the same concentration. The observed variance could be due to the unique phytochemical composition in each mushroom species, resulting in divergent antioxidant effects and profiles of phenolic acids [37]. Therefore, the concentration range for *Pleutorus spp.* was elevated to accurately assess the potential and ensure a fair comparison among all the mushroom species. This decision was validated by subsequent assays, which demonstrated more pronounced phytochemical, antioxidant, and anti-glycation activities in *Pleutorus* spp. within the adjusted concentration range.

The mushrooms' antioxidant activity was assessed through ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The FRAP assay relies on the reduction of the ferric to ferrous ions complex by combining with 2,4,6-tripyridyl-s-triazine (TPTZ) in an acetate buffer [38]. The resulting ferrous ion-TPTZ complex produces a blue color, with intensity dependent on the quantity of reduced ferric ions [38]. The DPPH method is a widely utilized protocol for screening antioxidants, involving the scavenging of DPPH free radicals in an alcoholic solution [38]. In this process, the presence of antioxidants changes the color from purple to yellow in the DPPH solution [38]. Given the diverse nature of antioxidant compounds and numerous reaction mechanisms, it is not possible to evaluate the antioxidant properties of natural products using a single antioxidant assay [30, 38]. Thus, we employed multiple methods to provide a comprehensive evaluation of the antioxidant properties of these compounds.

Among the five mushroom species (Table 2), *A. bisporus* showed the highest reducing potential for all three concentrations investigated. At 7.5 mg/mL, *A. bisporus* displayed a FRAP value of 3.9 mmol Fe^{2+} per 100 g, which

corresponded to the relatively high total phenolic, total tannin, and total flavonoid contents (626.8 mg GAE/100 g, 246.4 mg TAE /100 g, and 36.9 QE/100 g, respectively). This suggested an association of phenolic compounds and flavonoids to the antioxidant activity. These phytochemicals possessed an ability to disrupt the free radical chain by donating an electron to stabilize and terminate radical chain reactions [39]. Our findings align with a previous study [20] where *A. bisporus* demonstrated the highest reducing power, followed by *L. edodes* and *F. velutipes*.

The DPPH radical scavenging finding revealed that, at a concentration of 7.5 mg/mL, *L. edodes* displayed the highest DPPH scavenging capacity at 548.0 mg AA/100g, followed by 15 mg/mL of *L. edodes* (523.2 mg AA/100 g) and 7.5mg/mL of *A. bisporus* (501.0 mg AA/100g). A significant difference ($p < 0.05$) in the DPPH radical scavenging capacity was observed across all the concentrations of the aqueous mushroom extracts. This finding was consistent with that of Shah and Modi [40], who reported higher radical scavenging activity in the aqueous extracts of *L. edodes* when compared to *A. bisporus*. The relatively high radical scavenging activity in *L. edodes* could be attributed to the presence of additional hydrogen-donating components such as phenolic compounds in the mushroom extract [39]. This finding also suggested that the phytochemicals in *L. edodes* extracts were effective hydrogen atom donors. The difference between the DPPH and FRAP values may be due to the distinct mechanisms of each assay, which also highlights the diversity of antioxidant activities in different mushroom species.

Anti-glycation activity of individual mushroom extracts. The formation of endogenous advanced glycation end-products (AGEs) plays a critical role in the development of pathologies related to aging and metabolic diseases [41]. A previous study has demonstrated that a daily intake of 150–300 mg of aminoguanidine could inhibit the progression of diabetic retinopathy [42]. Expanding from this notion, Ishioka *et al.* [36] conducted a study to determine the daily intake of different vegetable samples to achieve anti-glycation activity equivalent to that of aminoguanidine. This comparison not only highlights the potential of dietary intervention with potent anti-glycation activity but also paves the way for exploring other natural sources with similar health benefits. Thus, investigating individual mushrooms and their combinations with good anti-glycation activities could lead to the discovery of new therapeutic agents to prevent diabetic complications.

The anti-glycation activity of the mushroom samples in this study was evaluated with a bovine serum albumin-glucose glycation model. We found that the water extract of 7.5 mg/mL *F. velutipes* showed the strongest inhibition of protein glycation at 84.2 mg AH/100g, followed by 7.5 mg/mL *L. edodes* (53.6 mg AH/100g) and 7.5 mg/mL *A. bisporus* (43.3 mg AH/100 g) (Table 2). Interestingly, these three mushroom samples not only displayed high anti-glycation activity but also possessed the highest total phenolic contents among the five mushroom samples. This provided further evidence to support the correlation between total phenolics and anti-glycation activity in the mushrooms. The mushroom's phenolic constituents, particularly their hydroxyl groups, may play a crucial role as AGE inhibitors by intercepting the glycation process [27].

Polyphenols and polysaccharides in individual mushroom extracts. Phenolics and tannins are secondary metabolites that possess antioxidant properties in the mushrooms [43]. The selected five mushrooms revealed substantial phenolic contents in the following order: 7.5 mg/mL *F. velutipes* > 7.5 mg/mL *A. bisporus* > 7.5 mg/mL *L. edodes* (Table 2). This was in agreement with Bach *et al.* [44] who observed higher phenolic contents in *F. velutipes* and *A. bisporus* when compared to other edible mushrooms. Kozarski *et al.* [45] previously identified ten types of phenolic compounds in *F. velutipes,* namely pyrogallol, quercetin, as well as homogentisic, protocatechuic, gallic, 5-sulfosalicylic, chlogenic, p-hydroxybenzoic, caffeic, and ferulic acids. They also identified 15 types of phenolic compounds in *Agaricus* spp., including pyrogallol, catechin, myricetin, and naringin, as well as trans-cinnamic, sulfosalicylic, rosmarinic, syringic, chlorogenic, p-hydroxybenzoic, ferulic, p-coumaric, caffeic, protocatechuic, and gallic acids. Conversely, 7.5 mg/mL *A. bisporus* showed the highest total tannin content (246.6 mg TAE/100 g), followed by 25 mg/mL *P. ostreatus* (202.1 mg TAE/100 g) and 15 mg/mL *F. velutipes* (149.9 mg TAE/100 g). The variation in total phenolic and tannin contents among the mushrooms could be due to the variation in their phenolic compositions, which are generally determined by genetic, environmental, and other factors [46]. The presence of flavonoids within the biological membranes, in conjunction with their interactions at the lipid bilayer surface, may restrict oxidant access and thereby preserve the integrity and functionality of cell membranes [43]. Therefore, the flavonoid contents in the mushrooms were pertinent to assessing their antioxidant properties. The highest flavonoid content was observed in 7.5 mg/mL *A. bisporus* (36.9 mg QE/100 g), while 25 mg/mL *P. eryngii* showed the lowest flavonoid content at 1.6 mg QE/100 g. This corresponded to the previous reports of *A. bisporus* having the highest total phenolic content among the edible mushrooms [19, 20].

Plant and fungi-derived polysaccharides, such as chitin, α-glucans, and β-glucans, constitute a promising group of antioxidants that are primary sources of bioactive compounds [16]. The consumption of polysaccharides has been associated with various health benefits, including improved gastrointestinal health, management of cardiovascular diseases, and treatment of specific cancer [11]. Therefore, polysaccharides are emerging as potential candidates for dietary supplements and treatments targeting oxidative stress-mediated conditions [11]. In our study, *P. eryngii* displayed the highest total carbohydrate content at 100 mg/mL (49479.5 mg PE/100 g). However, this mushroom showed

the lowest FRAP value (0.5 mmol Fe^{2+}/100 g) and a low DPPH value (144.6 mg AA/100 g), implying that its polysaccharides may not be associated with its antioxidant capacities. Most mushroom extracts in our study showed a decreasing trend in their bioactive contents, as well as antioxidant and anti-glycation activities, with increasing sample concentrations (Table 2). However, the increased FRAP and TFC values in *F. velutipes* and *P. ostreatus* supported the association between flavonoids and the reducing potential of both mushrooms.

Hengst *et al*. [33] have previously highlighted that a food extract concentration is a major determining factor of their functional values. They showed a decrease in antioxidant capacities for tomato extract, white tea, and strawberry nectar with increasing sample concentrations [33]. Similarly, Nowak *et al.* [47] observed a decreasing trend in antioxidant activities with increasing concentrations of polyphenols using a Fe^{2+}-EGTA-H_2O_2 system. Zhou *et al*. [48] also observed a decrease in antioxidant activity with increasing concentrations of green tea and maté extracts. The above findings may be explained by the pro-oxidant properties of plants with high polyphenol concentrations [48]. Certain dietary phenolic compounds, including phenolic acids, flavonoids, and non-flavonoids, are known to exhibit pro-oxidant behavior under specific conditions [49]. Factors such as the phenolic compound structure, high pH, and the sample's concentration may contribute to the pro-oxidant activity leading to reduced antioxidant activity [49]. Another plausible reason for the reduced antioxidant activity at high sample concentrations may be the autoxidation of flavonoids and phenolic compounds by transition metals, leading to the production of superoxide anions radicals [48]. Thus, it is crucial to account for the potential influence of pro-oxidant effects when assessing the antioxidant activity in mushroom extracts.

Antioxidant interaction in different mushroom mixtures. The theoretical sample capacity (TSC) and the experimental sample capacity (ESC) for antioxidant activity and phytochemicals in 90 combined mushroom mixtures are presented in Table 3 and Table 4, respectively. The synergism evaluation (SE) values of these mixtures are displayed in Table 5. Three best-performing mushroom combinations were selected out of 90 mixtures based on their collective phytochemical assay values and synergistic performance across all seven assays. In general, 7.5 mg/mL *A. bisporus* + 15 mg/mL *F. velutipes*, 7.5 mg/mL *L. edodes* + 7.5 mg/mL *F. velutipes,*

Table 3 Theoretical sample capacity values for polyphenols, polysaccharides, antioxidant and anti-glycation activities in different mushroom combinations

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
Agaricus bisporus	*Lentinula edodes*							
7.5	7.5	6.6 ± 0.0^{a}	1049.0 ± 0.0^{a}	96.9 ± 1.0^{a}	1250.2 ± 0.0^{a}	302.2 ± 0.0^{a}	41.2 ± 0.0^{a}	17349.9 ± 0.0^{a}
7.5	15	6.6 ± 0.0^{ab}	1024.2 ± 0.0^{b}	65.9 ± 0.9^{b}	1147.1 ± 0.0^{b}	333.2 ± 0.0^{b}	53.1 ± 0.0^{b}	15209.1 ± 0.1^{b}
7.5	30	6.4 ± 0.0^{c}	932.8 ± 0.0^{c}	53.3 ± 0.7^{c}	1013.4 ± 0.0^{c}	319.0 ± 0.0^{c}	64.23± 0.0^{c}	14226.2 ± 0.1^{c}
15	7.5	6.6 ± 0.0^{ab}	994.2 ± 0.0^{d}	74.5 ± 1.1^{g}	1108.0 ± 0.0^{d}	157.9 ± 0.0^{d}	34.7 ± 0.0^{d}	15621.9 ± 0.0^{d}
15	15	6.5 ± 0.0^{b}	969.4 ± 0.0^{e}	43.5 ± 1.0^{h}	1004.9 ± 0.0^{e}	188.8 ± 0.0^{e}	46.6 ± 0.0^{e}	13481.1 ± 0.0^{e}
15	30	6.4 ± 0.0^{c}	878.0 ± 0.0^{f}	30.9 ± 0.8^{i}	871.2 ± 0.0^{f}	165.6 ± 0.0^{f}	57.7 ± 0.0^{f}	12498.2 ± 0.1^{f}
30	7.5	6.1 ± 0.0^{e}	975.2 ± 0.0^{g}	62.9 ± 0.9^{b}	976.2 ± 0.0^{g}	143.1 ± 0.0^{g}	33.9 ± 0.0^{g}	14635.5 ± 0.1^{g}
30	15	6.1 ± 0.0^{e}	950.5 ± 0.0^{h}	31.8 ± 0.9^{ik}	873.2 ± 0.0^{h}	174.0 ± 0.0^{h}	45.8 ± 0.0^{h}	12494.7 ± 0.1^{h}
30	30	5.9 ± 0.0^{d}	859.1 ± 0.0^{i}	19.3 ± 0.6^{n}	739.4 ± 0.0^{i}	150.8 ± 0.0^{i}	56.9 ± 0.0^{i}	11511.8 ± 0.1^{i}
Agaricus bisporus	*Flammulina velutipes*							
7.5	7.5	5.9 ± 0.0^{d}	931.5 ± 0.0^{j}	127.5 ± 1.5^{x}	1315.2 ± 0.0^{j}	361.6 ± 0.0^{j}	39.0 ± 0.0^{j}	9920.2 ± 0.0^{j}
7.5	15	6.1 ± 0.0^{e}	884.6 ± 0.0^{k}	80.8 ± 1.2^{w}	1167.1 ± 0.0^{k}	396.3 ± 0.1^{k}	60.2 ± 0.0^{k}	8695.9 ± 0.0^{k}
7.5	30	6.1 ± 0.0^{e}	880.0 ± 0.0^{l}	63.0 ± 0.9^{bd}	1016.9 ± 0.0^{l}	317.7 ± 0.1^{l}	71.5 ± 0.0^{l}	8982.8 ± 0.1^{l}
15	7.5	5.9 ± 0.0^{d}	876.7 ± 0.0^{m}	105.1 ± 1.6^{j}	1173.1 ± 0.0^{m}	217.2 ± 0.0^{m}	32.5 ± 0.0^{m}	8192.2 ± 0.0^{m}
15	15	6.1 ± 0.0^{e}	829.8 ± 0.0^{n}	58.4 ± 1.3^{e}	1024.9 ± 0.0^{n}	251.9 ± 0.0^{n}	53.7 ± 0.0^{n}	6967.9 ± 0.0^{n}
15	30	6.1 ± 0.0^{e}	825.2 ± 0.0^{o}	40.6 ± 0.9^{h}	874.7 ± 0.0^{o}	173.3 ± 0.1^{o}	65.0 ± 0.0^{o}	7254.8 ± 0.0^{o}
30	7.5	5.4 ± 0.0^{w}	857.8 ± 0.0^{p}	93.5 ± 1.5^{ao}	1041.3 ± 0.0^{p}	202.5 ± 0.0^{p}	31.7 ± 0.0^{p}	7205.8 ± 0.1^{p}
30	15	5.6 ± 0.0^{j}	810.9 ± 0.0^{q}	46.8 ± 1.2^{fh}	893.1 ± 0.0^{q}	237.2 ± 0.1^{q}	52.9 ± 0.0^{q}	5981.5 ± 0.1^{q}
30	30	5.7 ± 0.0^{j}	806.3 ± 0.0^{r}	28.9 ± 0.8^{il}	742.9 ± 0.0^{r}	158.6 ± 0.1^{r}	64.2 ± 0.0^{r}	6268.4 ± 0.1^{r}

Continuation of Table 3

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
Agaricus bisporus	*Pleurotus ostreatus*							
7.5	25	4.5 ± 0.0^{f}	616.1 ± 0.0^{s}	55.8 ± 0.9^{cc}	1018.2 ± 0.0^{s}	448.5 ± 0.0^{s}	44.7 ± 0.0^{s}	27832.6 ± 0.0^{s}
7.5	50	4.5 ± 0.0^{f}	623.5 ± 0.0^{t}	49.1 ± 0.9^{f}	874.0 ± 0.0^{t}	364.5 ± 0.0^{t}	41.7 ± 0.0^{t}	27463.2 ± 0.0^{t}
7.5	100	4.7 ± 0.0^{g}	613.7 ± 0.0^{u}	46.0 ± 0.5^{f}	760.7 ± 0.0^{u}	303.5 ± 0.0^{u}	53.0 ± 0.0^{u}	25994.1 ± 0.0^{u}
15	25	4.5 ± 0.0^{fh}	561.3 ± 0.0^{v}	33.4 ± 1.0^{ik}	876.1 ± 0.0^{v}	304.2 ± 0.0^{v}	38.2 ± 0.0^{v}	26104.6 ± 0.0^{v}
15	50	4.5 ± 0.0^{fh}	568.7 ± 0.0^{w}	26.7 ± 0.9^{l}	731.8 ± 0.0^{x}	220.1 ± 0.0^{w}	35.2 ± 0.0^{w}	25735.2 ± 0.0^{w}
15	100	4.7 ± 0.0^{g}	558.9 ± 0.0^{x}	23.5 ± 0.6^{m}	618.5 ± 0.0^{y}	159.2 ± 0.0^{x}	46.5 ± 0.0^{x}	24266.1 ± 0.0^{x}
30	25	4.0 ± 0.0^{k}	542.4 ± 0.0^{y}	21.8 ± 0.9^{mn}	744.3 ± 0.0^{w}	289.4 ± 0.0^{y}	37.3 ± 0.0^{y}	25118.2 ± 0.1^{y}
30	50	4.0 ± 0.0^{k}	549.8 ± 0.0^{z}	15.1 ± 0.80^{p}	600.0 ± 0.0^{z}	205.4 ± 0.0^{z}	34.4 ± 0.0^{z}	24748.8 ± 0.1^{z}
30	100	4.3 ± 0.0^{x}	540.0 ± 0.0^{A}	11.9 ± 0.4^{p}	486.7 ± 0.0^{A}	144.4 ± 0.0^{A}	45.7 ± 0.0^{A}	23279.6 ± 0.1^{A}
Agaricus bisporus	*Pleurotus eryngii*							
7.5	25	4.5 ± 0.0^{f}	674.0 ± 0.0^{B}	58.0 ± 0.7^{cc}	955.5 ± 0.0^{B}	316.5 ± 0.0^{B}	38.5 ± 0.0^{B}	30876.2 ± 0.1^{B}
7.5	50	4.5 ± 0.0^{f}	654.0 ± 0.0^{C}	49.9 ± 0.7^{f}	862.9 ± 0.0^{C}	335.2 ± 0.0^{C}	40.6 ± 0.0^{C}	45109.7 ± 0.0^{C}
7.5	100	4.5 ± 0.0^{h}	645.6 ± 0.0^{D}	46.7 ± 0.6^{f}	759.0 ± 0.0^{D}	292.2 ± 0.0^{D}	44.2 ± 0.0^{D}	56055.6 ± 0.1^{D}
15	25	4.5 ± 0.0^{i}	619.2 ± 0.0^{E}	35.6 ± 0.7^{k}	813.3 ± 0.0^{E}	172.1 ± 0.0^{E}	32.0 ± 0.0^{E}	29148.2 ± 0.1^{E}
15	50	4.5 ± 0.0^{fi}	599.2 ± 0.0^{F}	27.5 ± 0.7^{il}	720.7 ± 0.0^{F}	190.8 ± 0.0^{F}	34.1 ± 0.0^{F}	43381.7 ± 0.0^{F}
15	100	4.4 ± 0.0^{fh}	590.8 ± 0.0^{G}	24.3 ± 0.6^{lm}	616.8 ± 0.0^{G}	147.8 ± 0.0^{G}	37.7 ± 0.0^{G}	54327.6 ± 0.0^{G}
30	25	4.0 ± 0.0^{k}	600.3 ± 0.0^{H}	24.0 ± 0.5^{lm}	681.5 ± 0.0^{H}	157.4 ± 0.0^{H}	31.2 ± 0.0^{H}	28161.8 ± 0.1^{H}
30	50	4.0 ± 0.0^{k}	580.3 ± 0.0^{I}	15.9 ± 0.6^{np}	588.9 ± 0.0^{I}	176.1 ± 0.0^{I}	33.3 ± 0.0^{I}	42395.3 ± 0.1^{I}
30	100	4.0 ± 0.0^{y}	571.9 ± 0.0^{J}	12.7 ± 0.4^{p}	485.0 ± 0.0^{J}	133.1 ± 0.0^{J}	36.9 ± 0.0^{J}	53341.2 ± 0.1^{J}
Lentinula edodes	*Flammulina velutipes*							
7.5	7.5	4.7 ± 0.0^{l}	978.5 ± 0.0^{K}	137.9 ± 1.7^{v}	1311.9 ± 0.0^{K}	171.0 ± 0.0^{K}	6.5 ± 0.0^{K}	14117.8 ± 0.0^{K}
7.5	15	4.8 ± 0.0^{m}	931.5 ± 0.0^{L}	91.2 ± 1.4^{o}	1163.8 ± 0.0^{L}	205.7 ± 0.0^{L}	27.6 ± 0.0^{L}	12893.5 ± 0.0^{L}
7.5	30	4.9 ± 0.0^{m}	927.0 ± 0.0^{M}	73.3 ± 1.1^{g}	1013.6 ± 0.0^{M}	127.1 ± 0.1^{M}	39.0 ± 0.0^{M}	13180.3 ± 0.0^{M}
15	7.5	4.6 ± 0.0^{l}	953.7 ± 0.0^{N}	106.8 ± 1.6^{j}	1208.9 ± 0.0^{N}	202.0 ± 0.0^{N}	18.4 ± 0.0^{N}	11977.0 ± 0.0^{N}
15	15	4.8 ± 0.0^{m}	906.7 ± 0.0^{O}	60.1 ± 1.4^{d}	1060.7 ± 0.0^{O}	236.6 ± 0.0^{O}	39.6 ± 0.0^{O}	10752.7 ± 0.0^{O}
15	30	4.8 ± 0.0^{m}	902.2 ± 0.0^{P}	42.3 ± 1.1^{h}	910.5 ± 0.0^{P}	158.0 ± 0.1^{P}	51.0 ± 0.0^{P}	11039.6 ± 0.1^{P}
30	7.5	4.5 ± 0.0^{hi}	862.4 ± 0.0^{Q}	94.3 ± 1.5^{ao}	1075.1 ± 0.0^{Q}	178.8 ± 0.0^{Q}	29.5 ± 0.0^{Q}	10994.1 ± 0.1^{Q}
30	15	4.6 ± 0.0^{l}	815.4 ± 0.0^{R}	47.6 ± 1.2^{f}	927.0 ± 0.0^{R}	213.4 ± 0.1^{R}	50.7 ± 0.0^{R}	9769.8 ± 0.1^{R}
30	30	4.7 ± 0.0^{l}	810.9 ± 0.0^{S}	29.7 ± 0.9^{il}	776.8 ± 0.0^{S}	134.8 ± 0.1^{S}	62.1 ± 0.0^{S}	10056.6 ± 0.1^{S}
Lentinula edodes	*Pleurotus ostreatus*							
7.5	25	3.2 ± 0.0^{n}	663.0 ± 0.0^{T}	66.2 ± 1.1^{d}	1014.9 ± 0.0^{T}	258.0 ± 0.0^{T}	12.1 ± 0.0^{T}	32030.1 ± 0.0^{T}
7.5	50	3.2 ± 0.0^{n}	670.5 ± 0.0^{U}	59.5 ± 1.1^{d}	870.7 ± 0.0^{U}	173.9 ± 0.0^{U}	9.2 ± 0.0^{U}	31660.8 ± 0.0^{U}
7.5	100	3.5 ± 0.0^{o}	660.7 ± 0.0^{V}	56.3 ± 0.8^{cc}	757.4 ± 0.0^{V}	112.9 ± 0.0^{V}	20.5 ± 0.0^{V}	30191.6 ± 0.0^{V}
15	25	3.2 ± 0.0^{np}	638.2 ± 0.0^{X}	35.2 ± 1.1^{k}	911.9 ± 0.0^{W}	288.9 ± 0.0^{W}	24.1 ± 0.0^{W}	29889.4 ± 0.1^{W}
15	50	3.2 ± 0.0^{p}	645.7 ± 0.0^{Y}	28.5 ± 1.1^{il}	767.6 ± 0.0^{X}	204.8 ± 0.0^{X}	21.1 ± 0.0^{X}	29520.0 ± 0.1^{X}
15	100	3.4 ± 0.0^{o}	635.9 ± 0.0^{Z}	25.3 ± 0.8^{l}	654.3 ± 0.0^{Y}	143.9 ± 0.0^{Y}	32.4 ± 0.0^{Y}	28050.9 ± 0.1^{Y}
30	25	3.0 ± 0.0^{q}	546.9 ± 0.0^{W}	22.6 ± 0.9^{mn}	778.1 ± 0.0^{Z}	265.7 ± 0.0^{Z}	35.2 ± 0.0^{Z}	28906.5 ± 0.1^{Z}
30	50	3.0 ± 0.0^{q}	554.4 ± 0.0^{A1}	15.9 ± 0.9^{np}	633.9 ± 0.0^{A1}	181.6 ± 0.0^{A1}	32.3 ± 0.0^{A1}	28537.1 ± 0.1^{A1}
30	100	3.3 ± 0.0^{n}	544.6 ± 0.0^{B1}	12.7 ± 0.5^{p}	520.6 ± 0.0^{B1}	120.7 ± 0.0^{B1}	43.6 ± 0.0^{B1}	27067.9 ± 0.1^{B1}
Lentinula edodes	*Pleurotus eryngii*							
7.5	25	3.2 ± 0.0^{n}	721.0 ± 0.0^{C1}	68.4 ± 0.9^{b}	952.2 ± 0.0^{C1}	125.9 ± 0.0^{C1}	6.0 ± 0.00^{C1}	35073.7 ± 0.1^{C1}
7.5	50	3.3 ± 0.0^{n}	701.0 ± 0.0^{D1}	60.3 ± 0.9^{d}	859.6 ± 0.0^{D1}	144.6 ± 0.0^{D1}	8.1 ± 0.00^{D1}	49307.3 ± 0.0^{D1}
7.5	100	3.2 ± 0.0^{p}	692.6 ± 0.0^{E1}	57.1 ± 0.9^{e}	755.7 ± 0.0^{E1}	101.6 ± 0.0^{E1}	11.7 ± 0.0^{E1}	60253.2 ± 0.0^{E1}

End of Table 3

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
15	25	3.2 ± 0.0^{np}	696.2 ± 0.0^{F1}	37.4 ± 0.9^{q}	849.1 ± 0.0^{F1}	156.8 ± 0.0^{F1}	17.9 ± 0.0^{F1}	32933.0 ± 0.1^{F1}
15	50	3.2 ± 0.0^{np}	676.2 ± 0.0^{G1}	29.3 ± 0.9^{il}	756.5 ± 0.0^{G1}	175.6 ± 0.0^{G1}	20.0 ± 0.0^{G1}	47166.5 ± 0.1^{G1}
15	100	3.1 ± 0.0^{p}	667.8 ± 0.0^{H1}	26.1 ± 0.8^{lmq}	652.6 ± 0.0^{H1}	132.5 ± 0.0^{H1}	23.6 ± 0.0^{H1}	58112.4 ± 0.1^{H1}
30	25	3.0 ± 0.0^{q}	604.8 ± 0.0^{I1}	24.8 ± 0.6^{lm}	715.4 ± 0.0^{I1}	133.6 ± 0.0^{I1}	29.0 ± 0.0^{I1}	31950.1 ± 0.1^{I1}
30	50	3.1 ± 0.0^{q}	584.9 ± 0.0^{J1}	16.7 ± 0.7^{np}	622.7 ± 0.0^{J1}	152.4 ± 0.0^{J1}	31.1 ± 0.0^{J1}	46183.6 ± 0.1^{J1}
30	100	3.0 ± 0.0^{z}	576.5 ± 0.0^{K1}	13.5 ± 0.5^{p}	518.9 ± 0.0^{K1}	109.3 ± 0.0^{K1}	34.7 ± 0.0^{K1}	57129.5 ± 0.1^{K1}
Flammulina velutipes	*Pleurotus ostreatus*							
7.5	25	2.6 ± 0.0^{r}	545.6 ± 0.0^{L1}	96.8 ± 1.6^{ao}	1080.0 ± 0.0^{L1}	317.3 ± 0.0^{L1}	10.0 ± 0.0^{L1}	24600.5 ± 0.0^{L1}
7.5	50	2.6 ± 0.0^{r}	553.1 ± 0.0^{M1}	90.1 ± 1.6^{o}	936.0 ± 0.0^{M1}	233.3 ± 0.0^{M1}	7.1 ± 0.0^{M1}	24231.1 ± 0.0^{M1}
7.5	100	2.8 ± 0.0^{s}	543.3 ± 0.0^{N1}	86.9 ± 1.4^{t}	822.4 ± 0.0^{N1}	172.3 ± 0.0^{N1}	18.4 ± 0.0^{N1}	22761.9 ± 0.0^{N1}
15	25	2.7 ± 0.0^{t}	498.6 ± 0.0^{O1}	50.1 ± 1.4^{cef}	931.8 ± 0.0^{O1}	352.0 ± 0.1^{O1}	31.1 ± 0.0^{O1}	23376.2 ± 0.0^{O1}
15	50	2.7 ± 0.0^{t}	506.1 ± 0.0^{P1}	43.4 ± 1.3^{h}	787.6 ± 0.0^{P1}	268.0 ± 0.0^{P1}	28.2 ± 0.0^{P1}	23006.8 ± 0.0^{P1}
15	100	3.0 ± 0.0	496.3 ± 0.0^{Q1}	40.2 ± 1.1^{hq}	674.3 ± 0.0^{Q1}	207.0 ± 0.0^{Q1}	39.5 ± 0.0^{Q1}	21537.7 ± 0.0^{Q1}
30	25	2.8 ± 0.0st	494.1 ± 0.0^{R1}	32.3 ± 1.0^{ik}	781.6 ± 0.0^{R1}	273.4 ± 0.1^{R1}	42.5 ± 0.0^{R1}	23663.0 ± 0.1^{R1}
30	50	2.8 ± 0.0st	501.5 ± 0.0^{S1}	25.6 ± 1.0^{lm}	637.4 ± 0.0^{S1}	189.4 ± 0.1^{S1}	39.6 ± 0.0^{S1}	23293.6 ± 0.1^{S1}
30	100	3.0 ± 0.0^{q}	491.7 ± 0.0^{T1}	22.4 ± 0.7^{n}	524.1 ± 0.0^{T1}	128.4 ± 0.1^{T1}	50.9 ± 0.0^{T1}	21824.5 ± 0.0^{T1}
Flammulina velutipes	*Pleurotus eryngii*							
7.5	25	2.6 ± 0.0^{r}	603.5 ± 0.0^{U1}	99.0 ± 1.5^{a}	1017.2 ± 0.0^{U1}	185.3 ± 0.0^{U1}	3.8 ± 0.0^{U1}	27644.1 ± 0.1^{U1}
7.5	50	2.6 ± 0.0^{r}	583.6 ± 0.0^{V1}	90.9 ± 1.5^{o}	924.6 ± 0.0^{V1}	204.0 ± 0.0^{V1}	5.9 ± 0.0^{V1}	41877.6 ± 0.0^{V1}
7.5	100	2.5 ± 0.0^{A}	575.2 ± 0.0^{W1}	87.7 ± 1.4^{u}	820.7 ± 0.0^{W1}	161.0 ± 0.0^{W1}	9.5 ± 0.0^{W1}	52823.5 ± 0.0^{W1}
15	25	2.8 ± 0.0^{t}	556.6 ± 0.0^{X1}	52.3 ± 1.2^{cf}	869.1 ± 0.0^{X1}	220.0 ± 0.0^{X1}	24.9 ± 0.0^{X1}	26419.8 ± 0.1^{X1}
15	50	2.8 ± 0.0^{t}	536.6 ± 0.0^{Y1}	44.2 ± 1.2^{h}	776.4 ± 0.0^{Y1}	238.7 ± 0.1^{Y1}	27.1 ± 0.0^{Y1}	40653.3 ± 0.0^{Y1}
15	100	2.7 ± 0.0^{B}	528.2 ± 0.0^{Z1}	41.0 ± 1.1^{h}	672.6 ± 0.0^{Z1}	195.6 ± 0.0^{Z1}	30.6 ± 0.0^{Z1}	51599.2 ± 0.0^{Z1}
30	25	2.8 ± 0.0st	552.0 ± 0.0^{A2}	34.5 ± 0.8^{kq}	718.9 ± 0.0^{A2}	141.4 ± 0.1^{A2}	36.3 ± 0.0^{A2}	26706.6 ± 0.1^{A2}
30	50	2.8 ± 0.0st	532.0 ± 0.0^{B2}	26.3 ± 0.8^{lm}	626.2 ± 0.0^{B2}	160.1 ± 0.1^{B2}	38.4 ± 0.0^{B2}	40940.1 ± 0.1^{B2}
30	100	2.7 ± 0.0^{t}	523.6 ± 0.0^{C2}	23.2 ± 0.7^{m}	522.4 ± 0.0^{C2}	117.1 ± 0.1^{C2}	42.0 ± 0.0^{C2}	51886.1 ± 0.1^{C2}
Pleurotus ostreatus	*Pleurotus eryngii*							
25	25	1.2 ± 0.0^{u}	288.1 ± 0.0^{D2}	27.4 ± 0.9^{il}	720.3 ± 0.0^{D2}	272.2 ± 0.0^{D2}	9.4 ± 0.0^{D2}	45556.4 ± 0.1^{D2}
25	50	1.2 ± 0.0^{u}	268.1 ± 0.0^{E2}	19.2 ± 0.9^{n}	627.6 ± 0.0^{E2}	290.9 ± 0.0^{E2}	11.5 ± 0.0^{E2}	59790.0 ± 0.0^{E2}
25	100	1.1 ± 0.0^{C}	259.7 ± 0.0^{F2}	16.0 ± 0.8^{n}	523.7 ± 0.0^{F2}	247.9 ± 0.0^{F2}	15.1 ± 0.0^{F2}	70735.9 ± 0.0^{F2}
50	25	1.2 ± 0.0^{u}	295.5 ± 0.0^{G2}	20.6 ± 0.8^{mn}	576.0 ± 0.0^{G2}	188.2 ± 0.0^{G2}	6.5 ± 0.0^{G2}	45187.1 ± 0.1^{G2}
50	50	1.2 ± 0.0^{u}	275.6 ± 0.0^{H2}	12.5 ± 0.8^{r}	483.3 ± 0.0^{H2}	206.9 ± 0.0^{H2}	8.6 ± 0.0^{H2}	59420.6 ± 0.0^{H2}
50	100	1.1 ± 0.0^{D}	267.1 ± 0.0^{I2}	9.3 ± 0.7^{r}	379.5 ± 0.0^{I2}	163.8 ± 0.0^{I2}	12.2 ± 0.0^{I2}	70366.5 ± 0.1^{I2}
100	25	1.4 ± 0.0^{v}	285.7 ± 0.0^{J2}	17.5 ± 0.4^{n}	462.7 ± 0.0^{J2}	127.2 ± 0.0^{J2}	17.8 ± 0.0^{J2}	43717.9 ± 0.1^{J2}
100	50	1.4 ± 0.0^{v}	265.8 ± 0.0^{K2}	9.3 ± 0.4^{rs}	370.1 ± 0.0^{K2}	145.9 ± 0.0^{K2}	19.9 ± 0.0^{K2}	57951.4 ± 0.0^{K2}
100	100	1.3 ± 0.0^{E}	257.4 ± 0.0^{L2}	6.1 ± 0.2^{s}	266.2 ± 0.0^{L2}	102.9 ± 0.0^{L2}	23.5 ± 0.0^{L2}	68897.4 ± 0.0^{L2}

Values represent mean ± standard deviations of three independent measurements. Mean values in a column with the same letter do not differ significantly ($p > 0.05$). TSC – theoretical sample capacity; FRAP – ferric-reducing antioxidant power; DPPH – 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; TPC – total phenolic content; TT – total tannins; TFC – total flavonoid content; AGE – advanced glycation end-product; AA – ascorbic acid; AH – aminoguanidine hydrochloride; GAE – gallic acid equivalent; TAE – tannic acid equivalent; QE – quercetin equivalent; PE – polysaccharide equivalent

and 7.5 mg/mL *L. edodes* + 15 mg/mL *F. velutipes* were identified as three best mushroom combinations that consistently demonstrated significant synergistic effects in most bioassays, particularly in the ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and total tannins (TT) assays. In addition, these mushroom combinations also showed minimal antagonism in the total phenolic content (TPC) when compared to the others. In the polysaccharide assay, the three mushroom combinations exhibited the lowest

synergistic effect. This characteristic is deemed beneficial, considering that a lower polysaccharide level is preferable for a diabetic individual. Beyond the observed synergistic effects, the selection criteria also considered the phytochemical values obtained in each bioassay. Remarkably, the three chosen combinations displayed some of the best values in the FRAP, DPPH, TPC, TT, polysaccharide and anti-glycation assays, marking them as superior in terms of their potential health benefits.

Based on synergism evaluation in the FRAP assay, most mushroom mixtures displayed synergistic interaction at low sample concentrations (Table 5). Out of them, the three selected mushroom mixtures (7.5 mg/mL *A. bisporus* + 15 mg/mL *F. velutipes*, 7.5 mg/mL *L. edodes* + 7.5 mg/mL *F. velutipes,* and 7.5 mg/mL *L. edodes* + 15 mg/mL *F. velutipes*) demonstrated synergistic FRAP interaction. However, their interaction depended on their concentrations, with antagonism arising in the samples with higher concentrations. This was evident in the mixtures of *A. bisporus* + *L. edodes, A. bisporus* + *P. ostreatus*, *A. bisporus* + *P. eryngii, L. edodes* + *P. ostreatus,* and *L. edodes* + *P. eryngii* (Table 5). A similar transition in antioxidant activity interaction was observed by Rúa *et al.* [50], where a higher concentration of carvacrol and thymol led to increased antagonism. This finding could be attributed to the oxidation of antioxidants with higher effectiveness by free radicals when compared to those with lower effectiveness [38]. Such

Table 4 Experimental sample capacity values for polyphenols, polysaccharides, antioxidant and anti-glycation activities in different mushroom combinations

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
Agaricus bisporus	*Lentinula edodes*							
7.5	7.5	7.3 ± 0.0^{B}	1025.5 ± 0.0^{a}	16.4 ± 0.8^{a}	1039.9 ± 0.0^{y}	499.2 ± 0.0^{c}	39.0 ± 0.0^{e}	18161.4 ± 0.0^{a}
7.5	15.0	7.0 ± 0.0^{C}	970.7 ± 0.0^{b}	8.8 ± 0.2^{b}	935.3 ± 0.0^{c}	420.3 ± 0.0^{d}	60.7 ± 0.0^{a}	17779.0 ± 0.0^{b}
7.5	30.0	6.2 ± 0.0^{a}	829.8 ± 0.0^{c}	4.8 ± 0.7^{c}	682.6 ± 0.0^{d}	250.2 ± 0.0^{e}	88.9 ± 0.0^{f}	17047.7 ± 0.0^{c}
15.0	7.5	6.8 ± 0.0^{D}	925.4 ± 0.0^{d}	9.1 ± 0.9^{bd}	842.0 ± 0.0^{e}	318.3 ± 0.0^{f}	52.8 ± 0.0^{g}	14178.4 ± 0.0^{d}
15.0	15.0	6.3 ± 0.0^{a}	880.7 ± 0.0^{e}	6.0 ± 0.8^{bc}	749.6 ± 0.0^{f}	241.6 ± 0.0^{g}	69.4 ± 0.0^{h}	14271.7 ± 0.0^{e}
15.0	30.0	5.5 ± 0.0^{b}	794.1 ± 0.0^{f}	4.7 ± 0.6^{bce}	564.8 ± 0.0^{g}	132.2 ± 0.0^{h}	78.4 ± 0.0^{i}	15661.5 ± 0.1^{f}
30.0	7.5	6.0 ± 0.0^{c}	946.7 ± 0.0^{g}	9.2 ± 0.7^{bd}	622.9 ± 0.0^{h}	211.4 ± 0.0^{i}	64.2 ± 0.0^{j}	10230.8 ± 0.0^{g}
30.0	15.0	6.0 ± 0.1^{c}	831.5 ± 0.0^{h}	8.1 ± 0.9^{bd}	560.8 ± 0.0^{i}	158.3 ± 0.0^{j}	60.3 ± 0.0^{k}	11072.2 ± 0.0^{h}
30.0	30.0	5.2 ± 0.1^{E}	743.7 ± 0.0^{i}	5.7 ± 0.7^{c}	444.0 ± 0.0^{j}	90.1 ± 0.1^{k}	95.1 ± 0.0^{l}	12837.5 ± 0.1^{i}
Agaricus bisporus	*Flammulina velutipes*							
7.5	7.5	7.6 ± 0.0^{F}	884.6 ± 0.0^{j}	16.8 ± 1.0^{a}	980.3 ± 0.0^{k}	562.84 ± 0.0^{l}	37.9 ± 0.0^{m}	5876.6 ± 0.0^{j}
7.5	15.0	7.1 ± 0.0^{G}	774.1 ± 0.0^{k}	11.0 ± 0.8^{bd}	836.8 ± 0.0^{l}	488.5 ± 0.0^{m}	59.3 ± 0.0^{n}	6660.1 ± 0.0^{k}
7.5	30.0	6.3 ± 0.0^{H}	754.6 ± 0.0^{l}	5.8 ± 0.6^{c}	608.7 ± 0.0^{m}	324.7 ± 0.0^{n}	72.4 ± 0.0^{o}	5865.4 ± 0.0^{l}
15.0	7.5	6.8 ± 0.0^{I}	828.0 ± 0.0^{m}	10.7 ± 0.8^{bd}	813.2 ± 0.0^{n}	366.2 ± 0.0^{o}	57.8 ± 0.0^{p}	10055.5 ± 0.0^{m}
15.0	15.0	6.6 ± 0.0^{J}	812.8 ± 0.0^{n}	5.7 ± 0.7^{bc}	729.6 ± 0.0^{o}	321.8 ± 0.0^{p}	47.7 ± 0.0^{q}	8171.2 ± 0.0^{n}
15.0	30.0	6.00 ± 0.0^{c}	772.4 ± 0.0^{o}	3.5 ± 0.5^{ce}	557.1 ± 0.0^{p}	182.2 ± 0.0^{q}	63.6 ± 0.0^{r}	7686.2 ± 0.1^{o}
30.0	7.5	6.23 ± 0.0^{a}	774.5 ± 0.0^{p}	12.9 ± 0.7^{d}	603.1 ± 0.0^{q}	218.7 ± 0.0^{r}	78.1 ± 0.0^{s}	8439.2 ± 0.0^{p}
30.0	15.0	6.1 ± 0.0^{c}	788.0 ± 0.0^{q}	10.8 ± 2.0^{bd}	541.3 ± 0.0^{r}	93.6 ± 0.1^{s}	65.7 ± 0.0^{t}	8628.3 ± 0.0^{q}
30.0	30.0	5.7 ± 0.0^{K}	729.3 ± 0.0^{r}	6.5 ± 0.7^{bc}	431.2 ± 0.0^{s}	82.0 ± 0.0^{t}	77.5 ± 0.0^{u}	7667.5 ± 0.0^{v}
Agaricus bisporus	*Pleurotus ostreatus*							
7.5	25.0	3.1 ± 0.0^{d}	3823.0 ± 0.0^{s}	6.9 ± 0.8^{bcd}	662.3 ± 0.0^{t}	427.8 ± 0.0^{u}	44.0 ± 0.0^{v}	41717.2 ± 0.0^{w}
7.5	50.0	2.4 ± 0.0^{e}	317.2 ± 0.0^{t}	4.0 ± 0.6^{c}	435.4 ± 0.0^{u}	270.2 ± 0.0^{v}	37.6 ± 0.0^{w}	35507.7 ± 0.1^{x}
7.5	100.0	2.5 ± 0.0^{j}	262.2 ± 0.0^{u}	2.3 ± 0.2^{ce}	249.0 ± 0.0^{v}	130.7 ± 0.0^{a}	38.1 ± 0.0^{x}	44466.7 ± 0.0^{y}
15.0	25.0	3.1 ± 0.0^{d}	434.5 ± 0.0^{v}	6.4 ± 0.6^{bc}	564.0 ± 0.0^{w}	293.6 ± 0.0^{w}	36.2 ± 0.0^{y}	25752.0 ± 0.0^{z}
15.0	50.0	2.4 ± 0.0^{ef}	340.2 ± 0.0^{w}	4.0 ± 0.2^{ce}	387.8 ± 0.0^{x}	191.1 ± 0.0^{b}	42.3 ± 0.0^{z}	34458.7 ± 0.0^{A}
15.0	100.0	2.5 ± 0.0^{fh}	301.2 ± 0.0^{x}	2.1 ± 0.3^{e}	225.2 ± 0.0^{b}	86.3 ± 0.1^{x}	50.8 ± 0.0^{A}	41683.5 ± 0.0^{B}
30.0	25.0	3.6 ± 0.0^{L}	525.9 ± 0.0^{y}	11.6 ± 0.1^{b}	446.4 ± 0.0^{z}	184.9 ± 0.0^{y}	71.8 ± 0.0^{B}	21415.2 ± 0.0^{C}
30.0	50.0	2.8 ± 0.0^{k}	412.4 ± 0.0^{z}	7.7 ± 0.5^{b}	327.2 ± 0.0^{A}	128.1 ± 0.0^{z}	57.9 ± 0.0^{C}	27305.1 ± 0.0^{D}
30.0	100.0	2.5 ± 0.0^{ghj}	338.4 ± 0.0^{A}	4.2 ± 0.3^{ce}	207.7 ± 0.0^{B}	67.2 ± 0.0^{A}	67.9 ± 0.0^{D}	30777.8 ± 0.0^{E}

Continuation of Table 4

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
Agaricus bisporus	*Pleurotus eryngii*							
7.5	25.0	3.5 ± 0.0^{M}	480.5 ± 0.0^{B}	24.0 ± 0.5^{f}	588.2 ± 0.0^{C}	360.7 ± 0.0^{B}	32.5 ± 0.0^{E}	55717.7 ± 0.1^{F}
7.5	50.0	2.9 ± 0.0^{k}	388.0 ± 0.0^{C}	13.4 ± 0.2^{ad}	412.2 ± 0.0^{D}	249.2 ± 0.0^{C}	23.2 ± 0.0^{F}	54517.1 ± 0.0^{G}
7.5	100.0	2.1 ± 0.0^{n}	321.5 ± 0.0^{D}	6.6 ± 0.2^{bc}	241.2 ± 0.0^{a}	130.6 ± 0.0^{a}	19.2 ± 0.0^{G}	52151.2 ± 0.0^{H}
15.0	25.0	3.9 ± 0.0^{N}	503.9 ± 0.0^{E}	18.5 ± 0.2^{a}	529.6 ± 0.0^{E}	266.7 ± 0.0^{D}	36.6 ± 0.0^{H}	40611.3 ± 0.1^{I}
15.0	50.0	3.0 ± 0.0^{m}	414.3 ± 0.0^{F}	11.5 ± 0.2^{bd}	375.0 ± 0.0^{F}	182.7 ± 0.0^{E}	27.5 ± 0.0^{I}	50293.2 ± 0.0^{J}
15.0	100.0	2.4 ± 0.0^{o}	347.2 ± 0.0^{G}	5.1 ± 0.1^{c}	217.3 ± 0.1^{G}	85.1 ± 0.1^{F}	23.8 ± 0.0^{J}	49786.6 ± 0.2^{K}
30.0	25.0	4.0 ± 0.0^{O}	589.2 ± 0.0^{H}	14.0 ± 0.3^{ad}	443.8 ± 0.0^{H}	186.9 ± 0.0^{G}	52.6 ± 0.0^{K}	30619.3 ± 0.0^{L}
30.0	50.0	3.2 ± 0.0^{P}	469.7 ± 0.0^{I}	8.9 ± 0.6^{b}	317.6 ± 0.0^{I}	122.1 ± 0.0^{H}	48.4 ± 0.0^{L}	40716.3 ± 0.0^{M}
30.0	100.0	2.3 ± 0.0^{Q}	371.8 ± 0.0^{J}	5.2 ± 0.2^{c}	205.5 ± 0.0^{J}	69.3 ± 0.0^{I}	28.4 ± 0.0^{b}	46121.4 ± 0.1^{N}
Lentinula edodes	*Flammulina velutipes*							
7.5	7.5	5.9 ± 0.0^{R}	1476.9 ± 0.0^{K}	60.4 ± 1.9^{h}	1105.4 ± 0.0^{K}	328.2 ± 0.0^{J}	8.7 ± 0.0^{M}	5792.6 ± 0.0^{O}
7.5	15.0	5.6 ± 0.0^{S}	1252.5 ± 0.0^{L}	36.5 ± 1.7^{i}	939.9 ± 0.0^{L}	313.1 ± 0.0^{K}	23.9 ± 0.0^{N}	9290.6 ± 0.0^{P}
7.5	30.0	5.5 ± 0.0^{I}	1076.1 ± 0.0^{M}	25.7 ± 0.9^{f}	646.1 ± 0.0^{M}	304.9 ± 0.0^{L}	60.7 ± 0.0^{a}	8238.4 ± 0.0^{Q}
15.0	7.5	5.5 ± 0.0^{bl}	1238.6 ± 0.0^{N}	40.2 ± 1.5^{j}	917.7 ± 0.0^{N}	261.0 ± 0.0^{N}	21.7 ± 0.0^{O}	16566.3 ± 0.0^{R}
15.0	15.0	5.5 ± 0.0^{b}	1088.1 ± 0.0^{O}	29.4 ± 2.3^{k}	800.2 ± 0.0^{O}	188.4 ± 0.0^{O}	39.6 ± 0.0^{P}	14397.6 ± 0.1^{S}
15.0	30.0	5.4 ± 0.0^{T}	972.4 ± 0.0^{P}	15.3 ± 1.0^{a}	575.7 ± 0.0^{P}	103.7 ± 0.1^{P}	60.0 ± 0.0^{c}	10923.0 ± 0.0^{T}
30.0	7.5	4.7 ± 0.0^{U}	886.1 ± 0.0^{Q}	22.3 ± 0.7^{f}	651.4 ± 0.0^{Q}	139.7 ± 0.0^{Q}	59.4 ± 0.0^{Q}	15088.8 ± 0.1^{U}
30.0	15.0	4.9 ± 0.0^{x}	844.6 ± 0.0^{R}	18.6 ± 0.8^{a}	578.3 ± 0.0^{R}	113.8 ± 0.0^{V}	60.0 ± 0.0^{c}	14906.0 ± 0.0^{V}
30.0	30.0	4.9 ± 0.0^{x}	771.7 ± 0.0^{S}	11.6 ± 0.9^{bd}	440.3 ± 0.0^{S}	77.6 ± 0.0^{W}	99.5 ± 0.1^{R}	12564.7 ± 0.0^{W}
Lentinula edodes	*Pleurotus ostreatus*							
7.5	25.0	2.2 ± 0.0^{pq}	615.4 ± 0.0^{T}	26.2 ± 1.0^{f}	680.7 ± 0.0^{T}	124.1 ± 0.0^{X}	29.0 ± 0.0^{S}	25314.5 ± 0.1^{X}
7.5	50.0	1.8 ± 0.0^{r}	392.1 ± 0.0^{U}	11.5 ± 0.2^{bd}	426.8 ± 0.0^{U}	101.7 ± 0.0^{Y}	42.1 ± 0.0^{T}	31419.6 ± 0.0^{Y}
7.5	100.0	2.1 ± 0.0^{o}	238.9 ± 0.0^{V}	6.6 ± 0.8^{c}	241.2 ± 0.0^{a}	119.7 ± 0.0^{Z}	79.7 ± 0.0^{U}	27301.7 ± 0.0^{Z}
15.0	25.0	2.6 ± 0.0^{hij}	616.5 ± 0.0^{W}	16.6 ± 0.4^{a}	615.3 ± 0.0^{V}	184.3 ± 0.0^{R}	29.3 ± 0.0^{V}	29445.8 ± 0.0^{A1}
15.0	50.0	1.8 ± 0.0^{r}	411.9 ± 0.0^{X}	10.2 ± 0.9^{bd}	393.0 ± 0.0^{W}	108.8 ± 0.0^{S}	40.8 ± 0.0^{d}	32030.6 ± 0.0^{B1}
15.0	100.0	2.0 ± 0.0^{t}	261.7 ± 0.0^{Y}	4.7 ± 0.6^{ce}	228.3 ± 0.0^{X}	56.6 ± 0.0^{T}	71.7 ± 0.0^{W}	34354.2 ± 0.0^{C1}
30.0	25.0	2.5 ± 0.0^{fgj}	564.3 ± 0.0^{Z}	10.0 ± 0.6^{bd}	468.8 ± 0.0^{Y}	88.7 ± 0.0^{U}	52.0 ± 0.0^{X}	29581.3 ± 0.0^{D1}
30.0	50.0	2.1 ± 0.0^{n}	409.0 ± 0.0^{A1}	6.4 ± 1.0^{bc}	335.7 ± 0.0^{Z}	62.1 ± 0.0^{A1}	51.0 ± 0.0^{Y}	28522.4 ± 0.0^{E1}
30.0	100.0	1.9 ± 0.0^{tu}	271.3 ± 0.0^{B1}	3.3 ± 0.3^{ce}	225.2 ± 0.0^{b}	42.9 ± 0.0^{B1}	74.9 ± 0.0^{Z}	29021.2 ± 0.1^{F1}
Lentinula edodes	*Pleurotus eryngii*							
7.5	25.0	2.5 ± 0.0^{gij}	728.6 ± 0.0^{C1}	23.0 ± 0.7^{f}	624.5 ± 0.0^{A1}	141.8 ± 0.0^{C1}	18.5 ± 0.0^{A1}	35956.9 ± 0.0^{G1}
7.5	50.0	2.0 ± 0.0^{t}	489.4 ± 0.0^{D1}	14.5 ± 0.8^{a}	415.6 ± 0.0^{B1}	110.9 ± 0.0^{D1}	35.1 ± 0.0^{B1}	40413.3 ± 0.0^{H1}
7.5	100.0	1.5 ± 0.0^{v}	355.4 ± 0.0^{E1}	8.7 ± 0.8^{bd}	230.0 ± 0.0^{C1}	85.4 ± 0.0^{E1}	37.2 ± 0.0^{C1}	33705.4 ± 0.0^{I1}
15.0	25.0	2.6 ± 0.0^{i}	696.7 ± 0.0^{F1}	23.3 ± 1.0^{f}	578.1 ± 0.0^{D1}	175.5 ± 0.0^{F1}	27.6 ± 0.0^{D1}	16566.3 ± 0.0^{J1}
15.0	50.0	2.0 ± 0.0^{t}	496.8 ± 0.0^{G1}	15.3 ± 0.7^{a}	373.9 ± 0.0^{E1}	103.6 ± 0.0^{G1}	29.5 ± 0.0^{E1}	14397.6 ± 0.1^{K1}
15.0	100.0	1.4 ± 0.0^{w}	368.3 ± 0.0^{H1}	8.6 ± 0.5^{bd}	222.3 ± 0.0^{F1}	56.0 ± 0.0^{H1}	33.4 ± 0.0^{F1}	10923.0 ± 0.0^{L1}
30.0	25.0	3.0 ± 0.0^{m}	617.7 ± 0.0^{I1}	18.1 ± 0.9^{g}	459.3 ± 0.0^{G1}	91.4 ± 0.1^{I1}	40.8 ± 0.0^{d}	15088.8 ± 0.1^{M1}
30.0	50.0	2.4 ± 0.0^{e}	500.5 ± 0.0^{J1}	12.4 ± 0.6^{d}	330.7 ± 0.0^{H1}	64.1 ± 0.0^{J1}	35.0 ± 0.0^{G1}	14906.0 ± 0.0^{N1}
30.0	100.0	1.8 ± 0.0^{rs}	350.5 ± 0.0^{K1}	7.4 ± 0.4^{bc}	207.0 ± 0.0^{I1}	38.1 ± 0.0^{K1}	43.7 ± 0.0^{H1}	12564.7 ± 0.0^{O1}
Flammulina velutipes	*Pleurotus ostreatus*							
7.5	25.0	1.9 ± 0.0^{tu}	510.6 ± 0.0^{L1}	22.3 ± 1.2^{f}	670.1 ± 0.0^{J1}	448.3 ± 0.0^{L1}	15.5 ± 0.0^{I1}	31488.1 ± 0.0^{P1}
7.5	50.0	1.5 ± 0.0^{v}	332.2 ± 0.0^{M1}	11.6 ± 0.5^{bd}	437.6 ± 0.0^{K1}	277.2 ± 0.0^{M1}	16.4 ± 0.0^{J1}	33609.6 ± 0.0^{Q1}
7.5	100.0	2.1 ± 0.0^{n}	220.3 ± 0.0^{N1}	6.0 ± 0.4^{bc}	249.8 ± 0.0^{L1}	136.8 ± 0.0^{N1}	38.6 ± 0.0^{K1}	36017.0 ± 0.0^{R1}
15.0	25.0	2.3 ± 0.0^{q}	549.9 ± 0.0^{O1}	19.3 ± 0.0^{g}	147.7 ± 0.0^{M1}	364.4 ± 0.0^{O1}	32.9 ± 0.0^{L1}	26150.8 ± 0.0^{S1}

End of Table 4

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
15.0	50.0	1.8 ± 0.0^{r}	352.9 ± 0.0^{P1}	11.5 ± 0.4^{ag}	116.6 ± 0.0^{N1}	228.5 ± 0.0^{P1}	28.5 ± 0.0^{M1}	33864.6 ± 0.1^{T1}
15.0	100.0	2.2 ± 0.0^{op}	249.8 ± 0.0^{Q1}	6.7 ± 0.4^{bd}	140.8 ± 0.0^{O1}	115.3 ± 0.0^{Q1}	54.7 ± 0.0^{N1}	33434.4 ± 0.0^{U1}
30.0	25.0	2.8 ± 0.0^{k}	591.4 ± 0.0^{V1}	14.7 ± 0.4^{a}	459.7 ± 0.0^{P1}	191.0 ± 0.0^{b}	43.5 ± 0.0^{O1}	22850.0 ± 0.0^{V1}
30.0	50.0	2.3 ± 0.0^{q}	425.7 ± 0.0^{W1}	10.2 ± 1.0^{bd}	327.7 ± 0.0^{Q1}	121.7 ± 0.0^{R1}	37.0 ± 0.0^{P1}	27892.7 ± 0.0^{W1}
30.0	100.0	2.2 ± 0.0^{pq}	313.1 ± 0.0^{X1}	4.3 ± 0.1^{ce}	214.2 ± 0.0^{R1}	80.0 ± 0.1^{S1}	59.0 ± 0.0^{Q1}	32366.4 ± 0.0^{X1}
Flammulina velutipes	*Pleurotus eryngii*							
7.5	25.0	2.1 ± 0.0^{n}	601.0 ± 0.0^{Y1}	18.0 ± 0.8^{ag}	632.5 ± 0.0^{S1}	259.1 ± 0.0^{T1}	13.0 ± 0.0^{R1}	41045.6 ± 0.0^{Y1}
7.5	50.0	1.7 ± 0.0^{y}	432.2 ± 0.0^{Z1}	16.6 ± 1.1^{ag}	413.4 ± 0.0^{T1}	244.1 ± 0.0^{U1}	17.3 ± 0.0^{S1}	46180.4 ± 0.0^{Z1}
7.5	100.0	1.4 ± 0.0^{w}	334.6 ± 0.0^{A2}	8.4 ± 0.1^{bd}	235.5 ± 0.0^{U1}	123.1 ± 0.0^{V1}	21.2 ± 0.0^{T1}	49527.2 ± 0.0^{A2}
15.0	25.0	2.6 ± 0.0^{zij}	644.8 ± 0.0^{B2}	21.8 ± 0.6^{f}	166.1 ± 0.0^{V1}	345.9 ± 0.0^{W1}	19.1 ± 0.0^{U1}	35028.6 ± 0.0^{B2}
15.0	50.0	2.0 ± 0.0^{nt}	479.3 ± 0.0^{C2}	15.3 ± 0.8^{ag}	130.8 ± 0.0^{W1}	205.0 ± 0.0^{X1}	22.3 ± 0.0^{V1}	41898.0 ± 0.0^{C2}
15.0	100.0	1.5 ± 0.0^{v}	362.1 ± 0.0^{D2}	7.7 ± 0.1^{bc}	99.9 ± 0.0^{X1}	85.6 ± 0.0^{Y1}	25.3 ± 0.0^{W1}	45319.0 ± 0.0^{D2}
30.0	25.0	3.1 ± 0.0^{dm}	686.7 ± 0.0^{E2}	18.1 ± 0.9^{ag}	459.3 ± 0.0^{Y1}	176.8 ± 0.0^{Z1}	34.9 ± 0.0^{X1}	25383.8 ± 0.0^{E2}
30.0	500.0	2.4 ± 0.0^{e}	523.5 ± 0.0^{F2}	12.4 ± 0.6^{d}	321.8 ± 0.0^{Z1}	90.0 ± 0.0^{A2}	30.1 ± 0.0^{Y1}	34147.1 ± 0.0^{F2}
30.0	100.0	1.7 ± 0.0^{rsy}	396.8 ± 0.0^{G2}	7.4 ± 0.6^{bc}	207.5 ± 0.0^{A2}	48.3 ± 0.0^{B2}	28.4 ± 0.0^{b}	39999.5 ± 0.1^{G2}
Pleurotus ostreatus	*Pleurotus eryngii*							
25.0	25.0	0.4 ± 0.0^{V}	341.3 ± 0.0^{H2}	19.3 ± 1.1^{ag}	471.4 ± 0.0^{B2}	317.3 ± 0.0^{C2}	16.6 ± 0.0^{Z1}	80089.3 ± 0.1^{H2}
25.0	50.0	0.5 ± 0.0^{W}	296.4 ± 0.0^{I2}	11.2 ± 0.3^{bd}	332.8 ± 0.0^{C2}	181.6 ± 0.0^{D2}	16.5 ± 0.0^{A2}	88115.0 ± 0.1^{I2}
25.0	100.0	0.6 ± 0.0^{X}	263.3 ± 0.0^{J2}	7.0 ± 0.1^{bc}	204.5 ± 0.0^{D2}	90.6 ± 0.0^{E2}	25.8 ± 0.0^{B2}	57946.4 ± 0.0^{J2}
50.0	25.0	1.0 ± 0.0^{z}	260.9 ± 0.0^{K2}	13.3 ± 0.7^{ad}	337.5 ± 0.0^{E2}	211.4 ± 0.0^{F2}	21.0 ± 0.0^{C2}	35169.9 ± 0.0^{K2}
50.0	50.0	1.0 ± 0.0^{zA}	228.2 ± 0.0^{L2}	9.9 ± 0.5^{bd}	265.4 ± 0.0^{F2}	159.0 ± 0.0^{G2}	25.0 ± 0.0^{D2}	40363.7 ± 0.1^{L2}
50.0	100.0	1.0 ± 0.0^{A}	215.8 ± 0.0^{R1}	6.3 ± 0.6^{bc}	184.3 ± 0.0^{G2}	81.3 ± 0.1^{H2}	24.9 ± 0.0^{E2}	55430.1 ± 0.1^{r}
100.0	25.0	3.6 ± 0.0^{Y}	197.3 ± 0.0^{S1}	7.2 ± 0.1^{bc}	211.3 ± 0.0^{H2}	107.8 ± 0.0^{I2}	49.7 ± 0.0^{F2}	38630.9 ± 0.2^{s}
100.0	50.0	2.6 ± 0.0^{hij}	195.2 ± 0.0^{T1}	5.9 ± 0.1^{bc}	190.2 ± 0.0^{I2}	98.9 ± 0.0^{J2}	45.8 ± 0.0^{G2}	42344.9 ± 0.1^{t}
100.0	100.0	1.6 ± 0.0^{iy}	191.4 ± 0.0^{U1}	5.4 ± 0.8^{c}	157.1 ± 0.0^{J2}	79.3 ± 0.0^{K2}	35.6 ± 0.0^{H2}	52385.5 ± 0.1^{u}

Values represent mean ± standard deviations of three independent measurements. Mean values in a column with the same letter do not differ significantly ($p > 0.05$) ESC – experimental sample capacity; FRAP – ferric-reducing antioxidant power; DPPH – 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; TPC – total phenolic content; TT – total tannins; TFC – total flavonoid content; AGE – advanced glycation end-product; AA – ascorbic acid; AH – aminoguanidine hydrochloride; GAE – gallic acid equivalent; TAE – tannic acid equivalent; QE – Quercetin equivalent; PE – polysaccharide equivalent

interactions have been previously observed between the phenolic and flavonoid compounds such as anthocyanins and quercetin [38]. It is plausible that these flavonoids formed hydrogen bonds which reduced the availability of hydroxyl groups contributing to the flavonoids' antioxidant activity and ultimately led to an antagonistic effect [38].

In contrast to the FRAP, the combined mushroom mixtures predominantly showed either synergistic or additive interactions in the DPPH radical scavenging system. Different mechanisms, such as sacrificial oxidation, metal chelation, spatial distribution, regeneration, and mutual protection, may have contributed to the observed synergism [51]. Nevertheless, a transition from synergism to antagonism with increasing sample concentrations was apparent in the sample mixtures of *L. edodes* + *P. ostreatus, F. velutipes* + *P. ostreatus,* and *P. eryngii* + *P. ostreatus.* The most likely explanation for the above observation was that the phenolic antioxidants lose their activities at higher sample concentrations by acting as pro-oxidants [52]. It is noteworthy that the 7.5 mg/mL *L. edodes* +7.5 mg/mL *F. velutipes* mixture with the highest DPPH scavenging activity also possessed the highest TPC (Table 4). Further correlation analysis (Table 6) revealed a strong positive association between the TPC and the total antioxidant activity. This implies that the phenolic compounds partly contributed to the radical scavenging capacity of the edible mushrooms. The antioxidant interactions among the mushroom mixtures vary, depending on their chemical

Table 5 Synergism evaluation values of phytochemicals, polysaccharides, antioxidant and anti-glycation activities in different mushroom combinations

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
Agaricus bisporus	*Lentinula edodes*							
7.5	7.5	1.1	1.1	0.2	0.9	1.1	1.2	1.1
7.5	15.0	1.1	1.0	0.3	0.9	1.0	1.6	1.1
7.5	30.0	1.1	1.0	0.7	0.8	0.9	1.6	1.3
15.0	7.5	1.0	1.1	0.2	0.9	1.7	1.1	0.9
15.0	15.0	1.0	1.0	0.2	0.8	1.3	1.5	1.0
15.0	30.0	0.9	1.0	0.4	0.7	0.9	1.4	1.4
30.0	7.5	0.9	1.2	0.2	0.8	1.3	1.4	0.9
30.0	15.0	1.0	1.1	0.3	0.7	0.9	1.2	1.1
30.0	30.0	0.9	1.1	0.2	0.6	0.7	1.6	1.0
Agaricus bisporus	*Flammulina velutipes*							
7.5	7.5	1.3	1.1	0.2	0.8	0.9	0.6	0.7
7.5	15.0	1.3	1.0	0.2	0.8	0.9	1.3	0.8
7.5	30.0	1.2	1.0	0.4	0.8	0.8	1.0	0.8
15.0	7.5	1.0	1.0	0.2	0.8	1.6	1.5	1.2
15.0	15.0	1.1	1.1	0.3	0.8	1.4	0.8	1.2
15.0	30.0	1.1	1.1	0.2	0.7	1.1	1.1	1.1
30.0	7.5	1.0	1.0	0.5	0.8	1.2	1.5	1.0
30.0	15.0	1.0	1.1	0.6	0.7	0.3	0.9	1.2
30.0	30.0	1.0	1.1	0.8	0.6	0.5	1.3	1.1
Agaricus bisporus	*Pleurotus ostreatus*							
7.5	25.0	1.1	1.1	0.2	0.9	0.9	1.5	1.1
7.5	50.0	1.2	1.0	0.3	0.8	0.8	1.8	0.9
7.5	100.0	1.2	1.0	0.2	0.8	0.8	1.1	1.2
15.0	25.0	0.8	1.0	0.5	0.7	0.9	0.8	0.9
15.0	50.0	0.9	1.0	0.6	0.7	0.8	1.8	1.0
15.0	100.0	1.0	1.1	0.8	0.7	0.7	1.4	1.2
30.0	25.0	0.8	1.0	0.3	0.6	0.7	1.8	0.9
30.0	50.0	0.9	1.0	0.7	0.6	0.6	2.1	1.1
30.0	100.0	1.1	1.1	0.2	0.9	1.1	1.2	1.1
Agaricus bisporus	*Pleurotus eryngii*							
7.5	25.0	1.3	1.1	0.4	0.9	1.1	1.7	0.9
7.5	50.0	1.4	1.1	0.5	0.8	0.8	1.4	1.4
7.5	100.0	1.4	1.1	0.6	0.8	0.8	1.2	1.0
15.0	25.0	1.1	1.0	0.8	0.7	1.5	1.4	0.9
15.0	50.0	1.1	1.1	0.3	0.7	1.0	1.5	1.0
15.0	100.0	1.1	1.1	0.7	0.6	0.9	1.2	1.0
30.0	25.0	0.9	1.1	1.1	0.7	1.1	1.7	1.0
30.0	50.0	1.0	1.1	0.1	0.6	0.7	2.0	1.1
30.0	100.0	0.9	1.1	0.6	0.6	0.6	1.1	1.0
Lentinula edodes	*Flammulina velutipes*							
7.5	7.5	1.3	1.0	0.9	0.9	2.2	1.8	0.9
7.5	15.0	1.2	1.1	0.1	0.9	1.3	0.5	0.5
7.5	30.0	1.2	1.1	0.2	0.8	1.3	1.2	1.0

Continuation of Table 5

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity		Polyphenols		Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
15.0	7.5	1.1	1.1	0.3	0.9	1.5	0.5	1.0
15.0	15.0	1.2	1.1	0.5	0.8	0.8	1.3	1.2
15.0	30.0	1.1	1.1	0.6	0.7	0.6	1.1	1.1
30.0	7.5	1.0	1.1	0.6	0.8	0.9	1.3	1.2
30.0	15.0	1.0	1.1	0.4	0.7	0.6	1.3	1.0
30.0	30.0	1.0	1.1	0.3	0.6	0.6	1.2	1.2
Lentinula edodes	*Pleurotus ostreatus*							
7.5	25.0	1.0	1.2	0.2	0.8	1.1	1.9	1.2
7.5	50.0	1.1	1.1	0.5	0.8	1.0	4.0	0.8
7.5	100.0	1.1	0.9	0.6	0.8	0.9	2.6	1.0
15.0	25.0	1.0	1.1	0.6	0.7	0.6	1.3	0.9
15.0	50.0	0.9	1.0	0.4	0.7	0.5	3.0	1.0
15.0	100.0	0.9	0.9	0.3	0.6	0.5	2.2	1.2
30.0	25.0	0.8	1.2	0.2	0.6	0.4	1.4	1.2
30.0	50.0	0.8	1.1	0.1	0.6	0.3	2.1	1.2
30.0	100.0	0.8	1.0	03	0.6	0.4	1.9	0.9
Lentinula edodes	*Pleurotus eryngii*							
7.5	25.0	1.2	1.1	0.4	0.8	1.4	3.5	0.9
7.5	50.0	1.2	1.1	0.3	0.8	0.7	4.5	0.9
7.5	100.0	1.1	1.1	0.2	0.7	0.6	3.2	0.8
15.0	25.0	1.0	1.0	0.1	0.8	1.2	1.9	0.9
15.0	50.0	0.9	1.1	0.3	0.6	0.6	2.2	1.7
15.0	100.0	0.9	1.1	0.8	0.6	0.5	2.2	1.2
30.0	25.0	0.9	1.1	1.1	0.7	0.8	1.3	1.0
30.0	50.0	0.9	1.2	1.5	0.6	0.4	1.4	1.1
30.0	100.0	0.9	1.1	2.1	0.6	0.4	1.6	1.0
Flammulina velutipes	*Pleurotus ostreatus*							
7.5	25.0	1.0	1.2	0.3	0.8	1.2	1.2	1.0
7.5	50.0	1.0	1.1	0.5	0.8	1.2	1.6	1.2
7.5	100.0	1.2	0.9	0.6	0.8	1.1	1.3	1.2
15.0	25.0	1.0	1.1	0.5	0.7	1.0	1.5	1.0
15.0	50.0	1.0	1.0	0	0.6	0.9	1.4	1.3
15.0	100.0	1.1	0.9	0.1	0.6	0.8	1.5	1.2
30.0	25.0	1.0	1.0	0.6	0.6	0.7	1.0	1.1
30.0	50.0	1.0	1.0	0.7	0.6	0.6	1.3	1.3
30.0	100.0	1.0	1.0	1.0	0.6	0.7	1.5	1.3
Flammulina velutipes	*Pleurotus eryngii*							
7.5	25.0	1.1	1.0	0.5	0.8	2.5	4.5	1.2
7.5	50.0	1.1	1.1	0	0.7	1.3	2.2	1.1
7.5	100.0	1.1	1.1	0.1	0.7	1.2	1.9	1.2
15.0	25.0	1.1	1.1	0.6	0.7	1.6	0.8	1.3
15.0	50.0	1.1	1.1	0.7	0.6	1.0	1.2	1.1
15.0	100.0	1.1	1.1	1.0	0.6	0.7	1.1	1.2
30.0	25.0	1.0	1.0	0.3	0.7	1.3	1.1	1.1
30.0	500.0	1.0	1.1	0	0.6	0.5	1.1	1.1
30.0	100.0	1.0	1.1	0.3	0.6	0.5	0.9	1.2

End of Table 5

Mushrooms mixtures (A+B), mg/mL		Antioxidant activity		Anti-glycation activity	Polyphenols			Polysaccharides, mg PE/100 g
Mushroom A	Mushroom B	FRAP, mmol Fe^{2+}/100 g	DPPH, mg AA/100 g	Anti-AGE value, mg AH/100 g	TPC, mg GAE/100 g	TT, mg TAE/100 g	TFC, mg QE/100 g	
Pleurotus ostreatus	*Pleurotus eryngii*							
25.0	25.0	0.8	1.1	0.1	0.7	1.2	1.6	2.0
25.0	50.0	0.9	1.1	1.1	0.6	0.7	1.4	2.3
25.0	100.0	0.9	1.1	0.9	0.6	0.6	1.4	1.5
50.0	25.0	0.8	1.1	0	0.6	1.0	2.4	1.1
50.0	50.0	0.9	1.0	0.1	0.6	0.8	2.6	1.2
50.0	100.0	0.9	1.0	0.6	0.6	0.6	2.1	1.7
100.0	25.0	0.9	0.9	0.5	0.6	0.9	1.9	1.4
100.0	50.0	0.9	1.0	1.1	0.6	0.7	1.9	1.5
100.0	100.0	0.8	1.0	0.9	0.6	0.8	1.5	2.0

The synergism evaluation values of 90 different mushroom mixtures across seven assays. SE – synergistic evaluation; FRAP – ferric-reducing antioxidant power; DPPH – 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; TPC – total phenolic content; TT – total tannin; TFC – total flavonoid content

nature and on the reactivity of bioactive compounds that undergo polymerization and structural changes [9]. For instance, the radical scavenging capacity of phenolic compounds is closely linked to their hydrogen-donating ability: the more hydroxyl groups are present, the greater their free radical scavenging activity [39].

Anti-glycation interaction in different mushroom mixtures. We also found that most combined mushroom mixtures demonstrated antagonism in their anti-glycation activity (Table 5), except for several combined mixtures containing *P. ostreatus* and *P. eryngii* which displayed synergistic effects at higher sample concentrations. The synergism was evidenced by a marked reduction in the fluorescence signal associated with the formation of advanced glycation end-products. The anti-glycation activity of the mushrooms could be attributed to the presence of bioactive compounds such as t-cinnamic, ferulic, 4-hydroxybenzoic, p-coumaric, protocatechuic, and vanillic acids, as well as myricetins, in *P. ostreatus* [53, 54] and *P. eryngii* [54]. The effectiveness of these bioactive compounds (particularly, t-cinnamic, p-coumaric, protocatechuic, ferulic, and vanillic acids) in attenuating glycation has been corroborated in recent studies [53]. Our findings agree with those of Atta *et al.* [55], who reported an increase in dose-dependent synergism in the functional activities of natural polyphenolic extracts combined with cefixime.

Bioactive compound interactions in different mushroom mixtures. Due to the positive correlation between the total antioxidant capacity and the total phenolic content (TPC) in the combined mushroom mixtures (Table 6), we anticipated that increasing the proportion of an extract with a high phenolic content in a binary mixture would increase the overall TPC in the mushroom mixture. However, most mushroom mixtures showed antagonistic effects in the TPC with increasing sample concentrations (Table 5). The least antagonistic effect was displayed by three combined mushroom mixtures (7.5 mg/mL *A. bisporus* + 15 mg/mL *F. velutipes*, 7.5 mg/mL *L. edodes* + 7.5 mg/mL *F. velutipes,* and 7.5 mg/mL *L. edodes* + 15 mg/mL *F. velutipes*). This finding contradicted that of Benamar-Aissa *et al.* [52], who reported synergism in the antioxidant activity of the combined plant extracts, possibly due to different plant species. The above finding also suggested that increasing the biological diversity of a plant mixture may not always lead to synergism. A possible explanation would be the formation of complexes and adducts among the phenolic compounds, which reduces their antioxidant capacity [56].

The combination study based on the total tannin (TT) content revealed that the mixture of 7.5 mg/mL *A. bisporus* + 7.5 mg/mL *F. velutipes* had the highest synergistic antioxidant and TT values (Table 4). This mushroom mixture also recorded the highest ferric reducing antioxidant power (FRAP) value (7.6 mmol Fe^{2+}/100 g) (Table 3), partly due to the formation of new phenolic compounds [4]. Similarly, 7.5 mg/mL *A. bisporus* + 7.5 mg/mL *L. edodes* was ranked second in the TT (499.2 mg TAE/100 g) and FRAP (7.3 mmol Fe^{2+}/100 g) values (Table 4). The moderate positive correlation between total tannins and total antioxidant activity (Table 6) justified the antioxidant role of high molecular weight polyphenols in the mushroom mixtures.

Approximately 90% of the combined mushroom mixtures displayed synergism in the total flavonoid content

Table 6 Correlation and regression among bioactive compounds, antioxidant and anti-glycation variables

Assays	*r*	R^2	Significance
Between TPC and anti-oxidant activity			
TPC versus FRAP	0.753	0.567	****
TPC versus DPPH	0.825	0.680	****
Between TT and anti-oxidant activity			
TT versus FRAP	0.509	0.260	****
TT versus DPPH	0.473	0.224	****
Between TFC and anti-oxidant activity			
TFC versus FRAP	0.585	0.342	****
TFC versus DPPH	0.325	0.106	***
Between polysaccharides and anti-oxidant activity			
Polysaccharide versus FRAP	–0.644	0.414	****
Polysaccharide versus DPPH	–0.557	0.311	****
Between reducing power and radical scavenging activity			
FRAP versus DPPH	0.841	0.708	****
Between anti-oxidant activity and anti-glycation activity			
FRAP versus anti-glycation	0.048	0.002	n.s.
DPPH versus anti-glycation	0.421	0.177	****
Between polyphenols and anti-glycation activity			
TPC versus anti-glycation	0.469	0.220	****
TT versus anti-glycation	0.153	0.023	n.s.
TFC versus anti-glycation	–0.397	0.158	****
Polysaccharide versus anti-glycation	–0.154	0.024	n.s.
Between polyphenols and polysaccharide			
TPC versus TT	0.650	0.422	****
TPC versus TFC	0.129	0.017	n.s.
TPC versus polysaccharides	–0.545	0.297	****
TT versus TFC	–0.009	0.065	n.s.
TT versus polysaccharides	–0.161	0.026	n.d.
TFC versus polysaccharides	–0.349	0.122	***

The r value denotes the Pearson's correlation value and R^2 value denotes the coefficient of determination; the level of significance was expressed as either not significant – n.s., $p < 0.005$ (***), or $p < 0.001$ (****)

(TFC) (Table 5). The mushroom mixtures containing *P. ostreatus*, *P. eryngii,* or both exhibited double or quadruple synergism in the TFC. Flavonoids, particularly catechin, quercetin, and chrysin, are the primary phenolic compounds in *Pleurotus* spp. [19]. Flavonoids possess heterocyclic structures which confer antioxidant activity by enabling conjugation between the aromatic rings [57]. These aromatic rings allow delocalization of electrons within the molecules and create resonance structures [30]. Combining various aromatic compounds could delocalize the bioactive compounds via electron transfer, allowing for more efficient reactions with free radicals and thus resulting in a synergistic effect [30]. Given the inverse correlation found between total antioxidant activity and polysaccharides, we favored the mushroom mixtures with lower carbohydrate values and antagonistic effects on polysaccharides.

In general, the combined mushroom mixtures containing 7.5 mg/mL *A. bisporus* + 15 mg/mL *F. velutipes*, 7.5 mg/mL *L. edodes* + 7.5 mg/mL *F. velutipes,* and 7.5 mg/mL *L. edodes* + 15 mg/mL *F. velutipes* displayed synergism in most antioxidant variables, except for polysaccharides, TPC, and anti-glycation values. These mushroom mixtures presented relatively high TPC, antioxidant and anti-glycation properties (Table 4), as previously discussed. All the above findings collectively suggested that the antioxidant activities and interactions in the mushroom mixtures were not solely dependent on their bioactive components but also determined by their proportions within the mixture [52].

Correlation and principal component analysis. Table 6 presents the linear correlation and regression analysis of the investigated antioxidant and anti-glycation variables in individual and combined mushroom samples. To date, the relationship between antioxidant and anti-glycation activities in plant samples is yet to be studied extensively. A few studies have reported the absence of correlation between antioxidant activity and anti-glycation values [58], while others suggested that antioxidant compounds effectively inhibited advanced glycation end-products (AGEs) [27, 55]. Our study confirmed a moderate correlation between the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and anti-glycation activity ($r = 0.4211$, $p < 0.001$). However, a similar correlation between the ferric-reducing antioxidant power (FRAP) and anti-glycation activity was non-significant ($p > 0.05$). Intagliata *et al.* [58], on the other hand, reported no direct relationship between

the antioxidant and anti-glycation activities of resveratrol. The observed discrepancy may be explained by the distinct pathways involved in AGE formation, including lipid peroxidation and the Namiki and Wolff pathways, which include various precursors and intermediates not directly influenced by the antioxidant mechanisms [58].

Our study also showed that the total phenolic content (TPC) was positively correlated ($p < 0.001$) with both the DPPH radical scavenging ($r = 0.825$) and the FRAP ($r = 0.753$) variables, while a moderate correlation existed between the TPC and anti-glycation activity ($r = 0.469$). For polyphenols, total tannins (FRAP, $r = 0.509$ and DPPH, $r = 0.473$) and total flavonoids (FRAP, $r = 0.585$ and DPPH, $r = 0.325$) were positive correlated with total antioxidant activities in the mushroom samples. These findings agreed with the previous studies [59] which highlighted a similar role of phenolics and flavonoids in the mushrooms' antioxidant properties. On the contrary, the total flavonoid content was inversely correlated with anti-glycation activity ($r = –0.397$, $p < 0.001$), possibly due to the degradation of certain flavonoids (such as quercetin) by oxidation at a high sample concentration [60]. Besides, the mushrooms' polysaccharides were inversely correlated with the total antioxidant activity (FRAP, $r = –0.644$, $p < 0.001$; DPPH, $r = –0.557$, $p < 0.001$). This could be attributed to the fact that protein components and phenolic compounds, rather than carbohydrates, mainly contributed to the overall antioxidant effect in mushrooms [59]. In addition, a strong positive correlation between the FRAP and the DPPH radical scavenging activity ($r = 0.841$, $p < 0.001$) showed the mushrooms as good reducing agents and radical scavengers.

Finally, we performed the principal component analysis (PCA) to identify the key variables that influence the mushrooms' functional characteristics under study [61]. The variables under analysis included the antioxidant parameters (FRAP and DPPH radical scavenging), anti-glycation value, polyphenols (TPC, TT, TFC), and polysaccharides. As illustrated in Fig. 1, the PCA biplot provides an overview of the relationships, as well as a distinction, between the variables for both individual and combined mushroom samples. The first principal component (F1) accounts for 52.7% of the data's variability, predominantly capturing the variance in antioxidant attributes, polyphenols and polysaccharides. The second principal component (F2) explains another 23% of the data variance, aligning positively with the TFC and inversely with anti-glycation activities. F1 effectively segregates over half of the samples to its left. These samples are characterized by a higher carbohydrate composition, as can be seen in the mixtures with *P. ostreatus* and *P. eryngii*. In contrast, the right side of F1 is populated by the mixtures containing *F. velutipes, A. bisporus*, and *L. edodes*, which were associated with high antioxidant activity and phenolic content. The biplot positions polysaccharides on the left, indicating that the mushrooms with a higher carbohydrate content tend to show lower phenolic and antioxidant activity. This trend was sup-

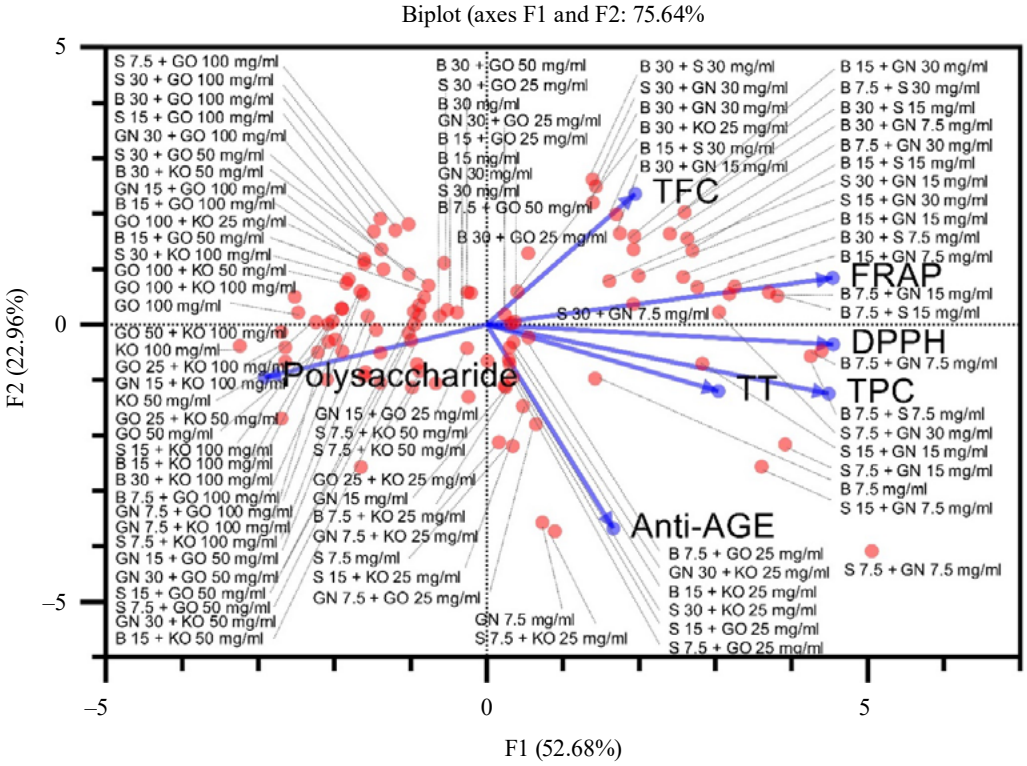

Figure 1 Biplot of different bioactive compounds with antioxidant and anti-glycation activities in the mushroom samples; FRAP – ferric-reducing antioxidant power; DPPH – 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; TPC – total phenolic content; TT – total tannin; TFC – total flavonoid content; AGE – advanced glycation end-product; B – button; S – shiitake; GN – golden needle; GO – grey oyster; KO – king oyster

ported by previous studies highlighting that polyphenols were integral to the antioxidant properties of plant-based foods [61]. The biplot further revealed that certain mushroom combinations (particularly, 7.5 mg/mL *A. bisporus* + 15 mg/mL *F. velutipes*, 7.5 mg/mL *L. edodes* + 7.5 mg/mL *F. velutipes,* and 7.5 mg/mL *L. edodes* + 15 mg/mL) displayed synergistic interaction that enhanced the observed activity variables, as suggested by their proximity to the vectors representing antioxidant activities. In addition, the inverse relationship between the TFC and anti-glycation activity suggested a need for further exploration into the stability and function of flavonoids in the combined mushroom mixtures. The biplot also showed a strong positive relationship between the FRAP and DPPH radical scavenging activities. This reinforced the premise that the selected mushroom mixtures were not only effective reducing agents but also potent radical scavengers.

CONCLUSION

Our study showed that *Agaricus bisporus* exhibited the highest reducing potential, total flavonoids, and total tannin values, while *Lentinula edodes* demonstrated the strongest radical scavenging capacity among the five selected edible mushrooms. Although *Flammulina velutipes* displayed the highest total phenolic content and anti-glycation activity, the highest polysaccharide content was observed in *Pleurotus eryngii,* which had low antioxidant activity. The combinational study evaluated all three types of interactions: synergism, addition, and antagonism. Three mushroom mixtures with the best performance in antioxidant and anti-glycation values were 7.5 mg/mL *A. bisporus* + 15 mg/mL *F. velutipes*, 7.5 mg/mL *L. edodes* + 7.5 mg/mL *F. velutipes,* and 7.5 mg/mL *L. edodes* + 15 mg/mL *F. velutipes*. These findings suggested that the bioactivity interactions in the mushroom samples could induce both positive and negative impacts on their total antioxidant capacity and anti-glycation activity.

The polyphenols (phenolics, tannins, and flavonoids) were positively associated with the mushrooms' total antioxidant activity, while an inverse association occurred between the polysaccharides and total antioxidant activity, as well as between the flavonoids and anti-glycation activity. A nutrigenomic investigation needs to be conducted into specific molecular targets to understand the underlying mechanisms behind the observed synergistic and antagonistic effects in the edible mushroom mixtures. The utilization of an *in vivo* model could further confirm the synergistic and antagonistic interactions between the mushroom phytochemicals in the biological systems. Given the widespread consumption of mushrooms, understanding the antioxidant and anti-glycation interactions among their bioactive compounds could valorize these edible fungi as a worthy functional food for developing a potential nutraceutical product.

CONTRIBUTION

Tan Weng Kuan Kimberly conducted the investigation and formal analysis, as well as wrote the original draft. Phaik Har Yong wrote, reviewed, and edited the manuscript. Zhi Xiang Ng developed the study concept and methodology, supervised the research, and was involved in writing, reviewing, and editing of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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