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RESEARCH PAPER

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BIOTECHNOLOGICAL MODULATION OF METABOLITE PROFILES IN *Ganoderma resinaceum* CULTIVATED ON AGRICULTURAL RESIDUES

Mustafa Kemal Soylu[1], Nuray Çömlekçioğlu[2], Metin Turan[3]

ABSTR ACT

Ganoderma resinaceum is a medicinal macrofungus recognised for its diverse pharmacologically active metabolites, including amino acids, organic acids, and polysaccharides with antioxidant, anti-inflammatory, and anticancer potential. This study aimed to elucidate how substrate formulation based on agricultural residues can modulate the metabolic composition of *G. resinaceum* under controlled cultivation conditions. Nine substrate mixtures were prepared using chickpea (*Cicer arietinum*), pea (*Pisum sativum*), and poppy (*Papaver somniferum*) stalks, as well as corncobs (*Zea mays*), in combination with beech sawdust and wheat bran. The fruiting bodies were analysed for amino acid, organic acid, and sugar profiles using high-performance liquid chromatography (HPLC). The results demonstrated that substrate composition markedly influenced metabolite accumulation. Chickpea- and pea-based substrates promoted the biosynthesis of key amino acids, whereas poppy stalk affected organic acid balance, and corncob formulations modified sugar metabolism. The findings indicate that metabolite production in *G. resinaceum* is strongly substrate-dependent, reflecting both nutrient availability and biochemical adaptability of the fungus. Overall, the study highlights a sustainable biotechnological approach to enhance bioactive metabolite production through tailored substrate design. The outcomes provide a foundation for future optimisation of *G. resinaceum* cultivation toward pharmaceutical and nutraceutical applications.

Keywords: *Ganoderma resinaceum*, agricultural residues, substrate optimization, metabolite profiling, amino acids, fungal biotechnology

INTRODUCTION

Ganoderma species have been used in traditional medicine for centuries, particularly across East Asia. These mushrooms contain substantial amounts of peptides, proteins, and minerals, making them an important nutritional source [Paliya et al. 2014, Rašeta et al. 2020]. Beyond their nutritional value, *Ganoderma* species are rich in bioactive compounds that have been widely investigated for their antioxidant, anti-inflammatory, and anticancer properties [Wachtel-Galor et al. 2011, Kozarski et al. 2020, Sułkowska-Ziaja et al. 2023a]. However, despite extensive research on various *Ganoderma* species, studies focusing specifically on the bioactive composition of *Ganoderma resinaceum* remain limited.

Ganoderma species are known to contain a wide spectrum of bioactive molecules, with more than 600 chemical compounds identified to date, including alkaloids, terpenoids, nucleobases, nucleosides, and polysaccharides

[1] Atatürk Horticultural Central Research Institute, Yalova, Türkiye, https://orcid.org/0000-0003-3492-0043; corresponding author: mustafakemal.soylu@tarimorman.gov.tr
[2] Eskişehir Osmangazi University, Faculty of Agriculture, Department of Horticulture, Eskişehir, Türkiye, https://orcid.org/0000-0001-7189-613X
[3] Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, İstanbul, Türkiye, https://orcid.org/0000-0002-4849-7680

[Paliya et al. 2014, Chen et al. 2018, Ren et al. 2021], proteins [Obodai et al. 2017, Sułkowska-Ziaja et al. 2023b], flavonoids and phenolic acids [Gong et al. 2019, Ahmad et al. 2021], as well as steroids and triterpenes [Niu et al. 2007, Wang et al. 2020, Galappaththi et al. 2023]. Among these, triterpenes are considered the principal pharmacologically active constituents [Gong et al. 2019, Sułkowska-Ziaja et al. 2023b]. Importantly, the chemical profile of *Ganoderma* mushrooms can vary significantly depending on species, cultivation conditions, substrate composition, developmental stage, tissue type, and storage environment [Wang et al. 2020, Amiri-Sadeghan et al. 2022].

In Türkiye, agricultural production generates substantial quantities of crop residues that may serve as valuable raw materials for fungal cultivation. According to FAO statistical data, the annual production in 2023 reached approximately 6.5 million tonnes of maize (*Zea mays*), 580 thousand tonnes of chickpea (*Cicer arietinum*), 235 thousand tonnes of pea (*Pisum sativum*), and 90 thousand tonnes of poppy (*Papaver somniferum*). These figures highlight the considerable amount of residual biomass available from chickpea stalk, pea stalk, poppy stalk, and maize cobs in Türkiye [FAO 2024]. Such residues represent an underutilised yet promising resource for developing sustainable substrate formulations in mushroom biotechnology.

Although *Ganoderma* species are widely recognized for their medicinal and economic value, their cultivation in Türkiye remains limited and is currently restricted to small-scale production by a few enterprises. Consequently, most *Ganoderma*-based products, including teas, capsules, powders, extracts, and functional formulations, are largely imported. In contrast, global production of *Ganoderma* – particularly *Ganoderma lucidum* – has increased substantially in recent years, reaching approximately 110,000–120,000 tons annually, with China accounting for the majority of production (around 70–85%) [FAO 2024, Bennett 2026]. The global market value of *Ganoderma*-based products has been estimated to exceed USD 2–4 billion, driven by increasing demand for nutraceuticals and functional foods [Andersen 2026]. Major producing countries include China, South Korea, Japan, the United States, and India, where *Ganoderma* cultivation is well established both for pharmaceutical and functional food industries. These global trends highlight the significant economic potential of *Ganoderma* cultivation and underline the importance of developing locally adapted production strategies in Türkiye.

The use of agricultural by-products as substrates for mushroom cultivation offers multiple advantages, including local availability, cost-effectiveness, and environmental sustainability [Chang and Miles 2004]. Optimising substrate composition can enhance both yield and the synthesis of bioactive metabolites in cultivated fungi [Royse et al. 2017]. Similar to other *Ganoderma* species, *G. resinaceum* produces amino acids, organic acids, and polysaccharides; however, detailed information on its specific metabolite composition remains scarce [Elisashvili 2012, Stojkovic et al. 2014, Rashad et al. 2019].

Therefore, the present study aimed to investigate the potential use of selected agricultural residues as cultivation substrates for *G. resinaceum* and to evaluate their effects on its biochemical composition. By identifying how substrate composition influences the biosynthesis of amino acids, organic acids, and sugars, this work provides novel insights into the biotechnological modulation of metabolite production in *G. resinaceum*. Furthermore, the findings contribute to the development of sustainable and value-added bioprocesses for the large-scale cultivation of medicinal fungi, supporting future applications in pharmaceutical biotechnology and functional food production.

MATERIAL AND METHODS

Fungal material

The spawn of *Ganoderma resinaceum* used in this experiment was obtained from the Atatürk Horticultural Central Research Institute (Yalova, Türkiye). The strain employed in the study was designated as isolate 44.3 (collection no. GBK035), originally collected on 30 August 2008 from a horse chestnut (*Aesculus hippocastanum*) stump in Yalova. The culture is currently maintained in the institutional fungal collection.

Substrate formulation and analysis

Substrate formulations were prepared using stalks of pea (*Pisum sativum*), poppy (*Papaver somniferum*), and chickpea (*Cicer arietinum*), as well as corn (*Zea mays*) cobs, beech (*Fagus orientalis*) sawdust, and wheat (*Triticum aestivum*) bran, resulting in a total of nine different media compositions (Table 1). Agricultural wastes were reduced to a particle size range of 1–2 cm. Substrate components were weighed, moistened, and homogenized according to the formulations (Table 1). Beech sawdust and wheat bran were selected as the control substrate formulation, as they are among the most commonly used lignocellulosic substrates in the commercial cultivation of *Ganoderma* species [Chang and Miles 2004, Amiri-Sadeghan et al. 2022].

Table 1. Composition of different substrate formulations used in the cultivation experiments of *Ganoderma resinaceum*

Substrate	Content
S1	40% beech sawdust + 40% chickpea stalk + 20% bran
S2	60% beech sawdust + 20% chickpea stalk + 20% bran
S3	40% beech sawdust + 40% pea stalk + 20% bran
S4	60% beech sawdust + 20% pea stalk + 20% bran
S5	40% beech sawdust + 40% poppy stalk + 20% bran
S6	60% beech sawdust + 20% poppy stalk + 20% bran
S7	40% beech sawdust + 40% corncob + 20% bran
S8	60% beech sawdust + 20% corncob + 20% bran
S9 (Control)	80% beech sawdust + 20% bran

Nitrogen (N) content of dried and ground substrate samples was determined using the Kjeldahl method [Kacar 1972], while moisture and ash contents were analyzed according to AOAC [2019]. Carbon (C) content was estimated as 50% of organic matter [Cormican and Staunton 1991], and the C : N ratio was calculated

Cultivation and fruiting

The substrates were moistened to approximately 60%, and pH was adjusted to 8.0–8.5 by adding lime prior to sterilisation. Each prepared substrate was packed into 1.5 kg heat-resistant polypropylene bags, into which a plastic aeration tube was inserted centrally. The bags were sealed with rings and lids, then sterilised at 121 °C and 1.2 atm for 120 min. After cooling to room temperature, 75 g of spawn was inoculated into each bag. Incubation was carried out in a controlled room at 25–26 °C and 65–70% relative humidity. Following complete mycelial colonization (18–26 days after spawning), the bags were transferred to the cultivation room and opened to initiate fruiting. Environmental conditions were maintained at 25–26 °C, 70–80% relative humidity, and a light intensity of 400 lux for 10 h day^{-1}. Fruiting bodies were harvested at maturity (51–53 days after spawning), when the margins of the basidiocarps turned reddish and reached the desired size (Figure 1). Mature fruiting bodies of suitable size were harvested and used for subsequent analyses.

Preparation of fruiting body samples

Fruiting bodies of *G. resinaceum* from each treatment were washed with tap water, followed by 0.1 N HCl and deionised water, and then blotted dry with filter paper. Samples were oven-dried at 65 °C for 48–72 h until constant weight was achieved, and subsequently ground using a laboratory mill (Spice and Herb Grinder, model IC-25B) to pass through a 20-mesh sieve. The resulting powders were used for subsequent analyses.

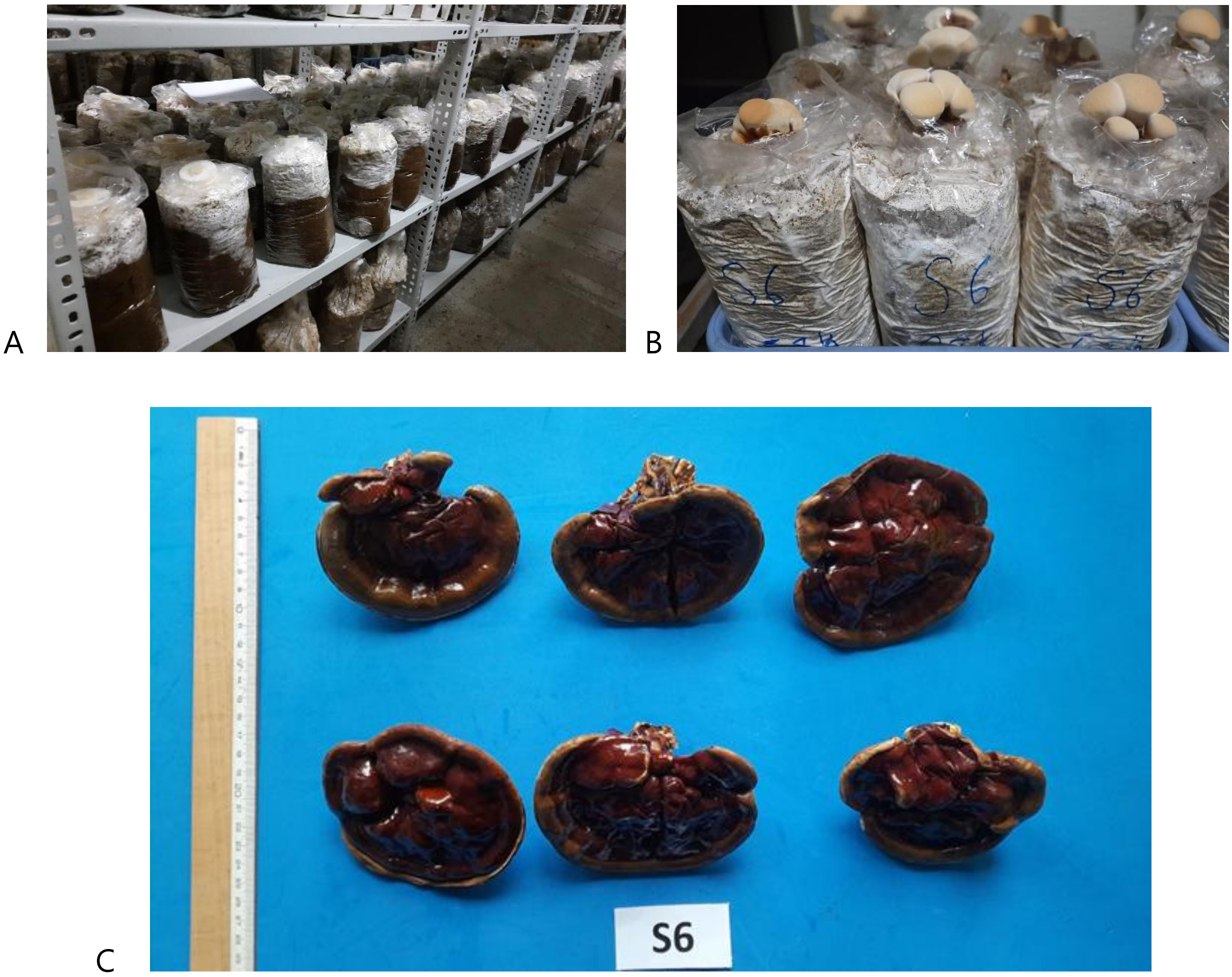
Amino acid, organic acid and sugar analysis

Amino acid and organic acid analyses of dried fruiting body samples were performed according to the methods described by Olgun et al. [2016], and Henderson et al. [1999].

For amino acid analysis, 5 mg of dried fruiting body sample was mixed with 5 mL of 0.1 N HCl. The mixtures were homogenized using an IKA Ultra Turrax D125 Basic homogenizer and incubated at 4 °C for 12 h. The homogenized samples were vortexed and centrifuged at 1200 rpm for 50 min. The resulting supernatants were filtered through 0.22 µm Millex Millipore filters and transferred to vials for high-performance liquid chromatography (Agilent, 1200 HPLC) analysis. Amino acid concentrations were determined after 26 min of HPLC derivatization and expressed as pmol µL^{-1}.

For organic acid analysis, 5 mg of homogenized fruiting body sample was extracted with 10 mL of deionized water. The extracts were centrifuged at 1200 rpm for 50 min, filtered through 0.22 µm Millex Millipore filters, and transferred to vials. Organic acids were analyzed using an Agilent 1200 HPLC system equipped with a Zorbax Eclipse-AAA column (4.6 × 250 mm, 5 µm). Detection was carried out at 220 nm using a UV detector. The flow rate was set at 1 mL min^{-1}, with the column maintained at 25 °C. Organic acid contents were determined using 25 mM potassium phosphate (pH 2.5) as the mobile phase and expressed as ng µg^{-1}.

Figure 1. Different cultivation phases of *Ganoderma resinacium*. A. Mycelium growing phase. B. Primordia phase. C. Basidiocarp



Sugar analysis of dried fruiting body samples was performed using Agilent 1200 HPLC coupled with evaporative light scattering detection (ELSD), following the method modified by Ma et al. [2014]. Results were expressed as percentages.

Statistical analysis

Experimental data were analysed using the JMP statistical software package (Version 5.0, SAS Institute Inc., Cary, NC, USA) based on a randomised complete block design in triplicate. Analysis of variance (ANOVA) was performed to assess differences among treatments, and mean comparisons were conducted using Tukey's honestly significant difference (HSD) test at a significance level of $P < 0.01$.

RESULTS

Substrate physicochemical properties

The physicochemical characteristics of the different substrate formulations are presented in Table 2. Following sterilisation, pH values ranged from 5.67 (S8) to 6.30 (S3), indicating a slightly acidic character suitable for fungal growth. Moisture content varied between 58.14% (S1) and 63.74% (S2), reflecting optimal water-holding capacity for mycelial colonisation. Carbon and nitrogen contents ranged from 45.59% (S1) to 48.13% (S8) and 0.77% (S9) to 1.52% (S3), respectively. The C/N ratio varied from 30.14 (S3) to 62.12 (S9), while ash content, representing the mineral fraction of the substrate, ranged between 3.74% (S8) and 8.82% (S1).

Table 2. Chemical composition of different substrate formulations

Substrate	pH	Moisture (%)	Carbon – C (%)	Nitrogen – N (%)	C/N	Ash (%)
S1	5.75 f	58.14 i	45.59 i	1.18 b	38.63 d	8.82 a
S2	5.68 g	63.74 a	47.17 f	1.02 c	46.24 c	5.65 d
S3	6.30 a	62.10 d	45.79 h	1.52 a	30.14 e	8.41 b
S4	5.95 d	58.76 h	47.10 g	1.21 b	38.93 d	5.80 c
S5	5.94 d	63.24 b	47.24 e	1.06 c	44.62 c	5.53 e
S6	5.88 e	61.34 e	47.73 c	0.86 d	55.32 b	4.54 g
S7	6.16 b	62.74 c	47.66 d	1.02 c	46.71 c	4.69 f
S8	5.67 g	61.26 f	48.13 a	0.83 d	58.33 b	3.74 i
S9	5.98 c	61.25 g	47.86 b	0.77 e	62.12 a	4.28 h

*Mean values marked with a different letter in each column are statistically different from each other ($p \leq 0.01$)

Amino acids profiles

The concentrations of amino acids in *G. resinaceum* cultivated on different substrates are presented in Table 3. Substrate composition significantly influenced amino acid accumulation ($p < 0.01$). Chickpea-based substrates (S1–S2) promoted the biosynthesis of aspartic acid, tyrosine, tryptophan, and phenylalanine, whereas pea stalk substrates (S3–S4) favoured the accumulation of alanine, valine, isoleucine, leucine, sarcosine, and proline. The addition of 20% poppy stalk (S6) resulted in the highest overall amino acid concentrations, while corncob substrates (S7–S8) produced moderate levels. Control substrate S9 consistently yielded the lowest amino acid contents.

Organic acid profiles

Table 4 summarises the organic acid composition of *G. resinaceum* fruiting bodies. Substrates containing chickpea stalks (S1–S2) yielded the highest tartaric acid contents, along with elevated levels of butyric, malic, lactic, citric, and succinic acids. Pea stalks substrates (S3–S4) produced moderate levels of the same acids, whereas poppy stalks substrates (S5–S6) showed reduced organic acid yields. Corncob substrates (S7–S8) enhanced the accumulation of oxalic, propionic, lactic, maleic, and succinic acids, comparable to or higher than the control (S9).

Sugar profiles

The sugar composition (sucrose, glucose, fructose, rhamnose, xylose, and arabinose) of *G. resinaceum* cultivated on various substrates is presented in Table 5. Substrate composition significantly affected sugar accumulation ($p < 0.01$). Chickpea stalk substrates (S1–S2) yielded the highest glucose (23.17%) and fructose (19.32%) levels, while S2 produced the highest sucrose concentration (12.53%). Pea stalk substrates (S3–S4) favoured the biosynthesis of rhamnose and xylose, and poppy stalk (S6) resulted in the greatest arabinose enrichment. Corncob substrates yielded relatively low sugar contents.

DISCUSSION

The results demonstrate that substrate composition exerts a decisive influence on the biochemical profile of *G. resinaceum*, affecting amino acid, organic acid, and sugar metabolism. Substrates containing chickpea, pea, and poppy stalks were particularly effective in promoting amino acid biosynthesis. Chickpea stalks, rich in protein and free amino acids such as glutamic acid and aspartic acid [Jukanti et al. 2012, Begum et al. 2023], enhanced the synthesis of aromatic amino acids (phenylalanine, tyrosine, tryptophan). Pea stalk, which contains high levels of lysine and leucine [Day 2013, Nosworthy et al. 2017], favoured the accumulation of branched-chain amino acids (valine, isoleucine, leucine) and aliphatic amino acids (alanine, proline). Moderate supplementation with poppy stalk (20%) significantly increased total amino acid content, whereas higher levels (40%) were inhibitory. This dual effect likely reflects the balance between nutrient availability and the presence of alkaloids and phenolic compounds, which may stimulate metabolism at low concentrations but hinder enzymatic activity and nutrient uptake at higher doses [Jonathan and Fasidi 2001, Chang and Miles 2004, Nastišin et al. 2025].

Table 3. Amino acid contents of *G. resinaceum* grown on different substrates (pmol μl^{-1})

Amino acid	Chickpea salk		Pea stalk		Poppy stalk		Corncob		Control
	S1 (40%)	S2 (20%)	S3 (40%)	S4 (20%)	S5 (40%)	S6 (20%)	S7 (40%)	S8 (20%)	S9
Aspartic acid	1372.63 ±177.74ab	1213.17 ±66.07 bc	948.88 ±36.64 de	696.70 ±13.03e	700.97 ±68.94 e	1516.07 ±128.15 a	1278.42 ±75.15 abc	1081.80 ±108.19 cd	125.48 ±8.36 f
Glutamic acid	644.13 ±77.34 c	466.89 ±44.93 d	704.16 ±98.25 bc	693.14 ±3.21 bd	577.47 ±18.94 cd	1017.92 ±16.11 a	824.91 ±48.49 b	675.54 ±80.11 bc	136.34 ±9.51 e
Serine	980.59 ±93.75 abc	757.89 ±93.35 c	1004.43 ±102.22 ab	754.13 ±98.59 c	767.85 ±31.11 bc	1128.04 ±95.35 a	951.22 ±55.91 abc	788.42 ±85.56 bc	474.96 ±67.43 d
Asparagine	1538.15 ±101.13 cde	1369.39 ±51.51 de	1253.14 ±26.29 e	1659.03 ±72.22 cd	1313.04 ±12.98 e	2569.60 ±217.20 a	2166.80 ±127.36 b	1834.03 ±183.37 c	402.47 ±70.16 f
Glutamine	668.52 ±93.28 d	443.45 ±18.28 de	685.97 ±64.84 d	598.23 ±25.61 d	490.26 ±46.96 de	2799.32 ±236.62 a	2360.52 ±138.75 b	1915.39 ±245.76 c	123.41 ±12.09 e
Histidine	659.21 ±84.16 de	406.31 ±22.36 e	673.49 ±72.16 d	566.61 ±26.11de	508.30 ±16.46 de	3098.85 ±142.01 a	2556.88 ±150.29 b	2050.91 ±118.65 c	150.07 ±22.83 f
Threonine	401.07 ±24.99 d	395.57 ±31.25 d	397.31 ±32.98 d	384.92 ±63.12 d	343.10 ±64.82 d	3461.98 ±292.63 a	2919.31 ±171.59 b	2326.17 ±256.25 c	90.43 ±26.99 d
Alanine	494.03 ±75.11 d	564.64 ±18.84 d	2036.88 ±150.33 a	1585.27 ±83.67 b	1468.38 ±90.55 bc	1751.52 ±148.05 b	1476.96 ±86.82 bc	1248.58 ±125.02 c	176.57 ±19.04 e
Tyrosine	1247.19 ±95.16 ab	1389.87 ±78.86 a	1139.00 ±93.41 b	824.09 ±22.82 c	765.50 ±32.02 cd	827.77 ±69.97 c	698.01 ±41.03 cd	592.80 ±59.17 d	621.35 ±35.50 d
Cystine	426.22 ±58.59 cd	310.26 ±28.44 d	520.13 ±51.44 bc	356.68 ±35.41 d	332.96 ±73.88 d	756.63 ±63.96 a	638.02 ±37.50 ab	529.87 ±56.80 bc	9.82 ±2.21 e
Valine	638.10 ±116.08 a	414.94 ±70.88 bcd	610.14 ±152.18 ab	558.14 ±57.17 abc	511.91 ±73.38 a-d	421.50 ±35.63 a-d	355.43 ±20.89 cd	294.03 ±32.31 d	14.03 ±0.32 e
Methionine	779.89 ±111.61 bc	662.35 ±22.60 c	1271.45 ±71.00 a	868.06 ±168.54 bc	768.45 ±154.43 bc	1186.69 ±100.30 a	1000.67 ±58.82 ab	839.14 ±85.77 bc	18.95 ±5.46 d
Tryptophan	975.53 ±104.18 a	858.11 ±67.92 ab	682.93 ±151.92 bc	430.12 ±15.19 de	384.66 ±26.33 e	838.99 ±70.92 ab	707.48 ±41.58 bc	598.84 ±59.88 cd	13.97 ±4.40 f
Phenylalanine	2734.03 ±563.03 a	2811.73 ±96.43 a	2287.25 ±246.28 ab	1973.52 ±158.15 b	1721.63 ±110.57 bc	1198.27 ±101.29 cd	1010.44 ±59.39 d	845.32 ±87.23 d	28.61 ±2.56 e
Isoleucine	496.31 ±84.12 cde	335.06 ±51.39 e	1376.21 ±150.18 a	1251.77 ±84.70 ab	1072.32 ±63.67 b	688.74 ±58.22 c	580.78 ±34.14 cd	464.72 ±67.69 de	9.39 ±0.65 f
Leucine	598.64 ±53.15 c	366.69 ±10.59 d	993.51 ±96.44 a	942.39 ±17.67 a	884.25 ±50.38 a	838.66 ±70.89 ab	707.20 ±41.57 bc	572.66 ±74.84 c	18.76 ±6.75 e
Lysine	586.19 ±82.62 b	412.47 ±12.04 c	500.16 ±89.17 bc	1227.31 ±59.58 a	1109.08 ±24.80 a	604.92 ±51.13 b	510.10 ±29.98 bc	425.77 ±44.40 c	8.55 ±0.82d
Hydroxyproline	353.80 ±87.49 bc	287.80 ±8.15 bc	610.40 ±26.85 a	506.44 ±28.98 a	537.23 ±27.90 a	385.00 ±32.54 b	324.65 ±19.08 bc	263.78 ±33.45 c	5.93 ±1.64 d
Sarcosine	834.55 ±45.04 c	861.03 ±90.06 c	1769.94 ±285.73 a	1441.18 ±83.67 b	1235.56 ±78.44 b	905.51 ±76.54 c	763.57 ±44.88 c	637.97 ±66.21 c	19.28 ±7.25 d
Proline	354.81 ±90.27 b	294.81 ±50.94 b	402.30 ±47.46 ab	367.88 ±35.19 ab	357.91 ±45.19 b	501.24 ±42.37 a	422.67 ±24.84 ab	346.65 ±40.61 b	22.35 ±2.02 c

*Mean values marked with a different letter in each row are statistically different from each other. Values are expressed as mean ± standard deviation (SD) of three independent replicates

Table 4. Organic acid contents of *G. resinaceum* grown on different substrates (ng μg^{-1})

Organic Acid	Chickpea stalk		Pea stalk		Poppy stalk		Corncob		Control
	S1 (40%)	S2 (20%)	S3 (40%)	S4 (20%)	S5 (40%)	S6 (20%)	S7 (40%)	S8 (20%)	S9
Oxalic acid	31.49 ±4.27 c	24.95 ±3.78 c	23.83 ±3.59 c	24.44 ±1.14 c	23.03 ±2.02 c	21.26 ±1.10 c	245.48 ±1.69 a	211.03 ±11.18 b	263.70 ±16.89 a
Propionic acid	46.29 ±9.79 bc	38.12 ±5.77 c	36.40 ±5.49 c	37.32 ±2.97 c	36.47 ±5.54 c	31.61 ±2.88 c	111.99 ±1.72 ab	155.92 ±34.84 a	152.22 ±33.53 a
Tartaric acid	701.54 ±115.37 a	544.36 ±82.41 ab	519.80 ±78.39 ab	27.75 ±4.05 c	31.68 ±4.02 c	28.94 ±4.10 c	381.53 ±44.44b	427.89 ±69.05 b	438.73 ±59,25 b
Butyric acid	370.46 ±60.92 a	287.46 ±43.52 ab	274.49 ±41.39 b	23.81 ±1.04 c	19.87±2.61 c	20.20 ±4.64 c	224.13 ±6.33 b	271.37 ±21.63 b	247.32 ±36.81 b
Malonic acid	525.38 ±86.40 a	407.66 ±61.72 ab	389.27 ±58.70 b	37.27 ±10.95 d	34.32 ±2.58 d	28.73 ±5.41 d	137.92 ±4.89 cd	184.11 ±49.81 c	144.02 ±4.38 cd
Malic acid	287.09 ±47.21 a	222.77 ±33.72 ab	212.71 ±32.08 abc	22.38 ±0.33 d	21.67 ±5.26 d	18.09 ±3.10 d	116.67 ±12.25 cd	167.09 ±36.20 bc	127.82 ±29.98 bc
Lactic acid	244.59 ±40.22 a	189.80 ±28.73 a	181.23 ±27.33 a	19.15 ±1.01 b	18.78 ±2.19 b	17.04 ±0.80 b	181.04 ±37.27 a	217.63 ±26.71 a	208.01 ±34.60 a
Citric acid	193.60 ±40.95 a	159.45 ±24.14 ab	152.26 ±22.96 ab	21.18 ±5.35 c	23.41 ±4.81 c	23.13 ±4.48 c	121.41 ±7.50 b	117.34 ±19.76 b	108.23 ±20.63 b
Maleic acid	232.22 ±38.19 a	180.19 ±27.28 ab	172.06 ±25.95 ab	16.81 ±8.53 b	10.42 ±5.22 b	11.48 ±1.31 b	181.61 ±9.30 ab	284.21 ±47.13 a	291.16 ±21.30 a
Fumaric acid	399.22 ±65.65 b	309.78 ±46.90 bc	295.80 ±44.61 c	40.52 ±3.54 def	34.28 ±4.64 f	36.42 ±5.68 ef	135.06 ±3.00 de	601.44 ±44.72 a	136.62 ±11.85 d
Succinic acid	432.43 ±71.11 a	335.55 ±50.80 a	320.41 ±48.32 a	26.22 ±1.25 b	28.15 ±5.99 b	29.64 ±2.20 b	327.31 ±12.23 a	303.77 ±39.68 a	371.90 ±54.78a

*Mean values marked with a different letter in each row are statistically different from each other. Values are expressed as mean ± standard deviation (SD) of three independent replicates

Table 5. Sugar contents (%) of *G. resinaceum* grown on different substrates

Sugar	Chickpea stalk		Pea stalk		Poppy stalk		Corncob		Control
	S1 (40%)	S2 (20%)	S3 (40%)	S4 (20%)	S5 (40%)	S6 (20%)	S7 (40%)	S8 (20%)	S9
Sucrose	10.65 ±0.04 ab	12.53 ±1.21 a	9.88 ±1.55 abc	11.43 ±2.08 ab	8.81 ±0.62 abc	7.51 ±1.80 bc	7.32 ±1.57 bc	8.92 ±1.62 abc	6.23 ±1.42 c
Glucose	23.17 ±3.67 a	12.59 ±2.07 b	10.68 ±0.48 b	12.37 ±1.40 b	9.47 ±1.56b	8.87 ±1.30 b	8.02 ±1.46 b	10.01 ±1.14 b	6.47 ±3.32 b
Fructose	19.32 ±5.89 a	11.84 ±1.92 b	10.43 ±0.71b	11.64 ±1.44 b	9.31 ±1.02 b	7.69 ±1.24 b	7.92 ±1.43 b	9.42 ±1.16 b	6.52 ±2.56 b
Rhamnose	10.22 ±3.48 ab	13.64 ±1.88 a	11.96 ±1.48 ab	13.37 ±1.58 a	11.48 ±0.87 ab	10.76 ±0.15 ab	10.29 ±1.19 ab	10.82 ±1.28 ab	8.56 ±5.09 b
Xylose	5.59 ±1.40 ab	7.32 ±1.43 a	6.42 ±0.52 ab	7.18 ±0.97 a	5.56 ±1.34 ab	4.13 ±0.81 b	4.71 ±1.20 ab	5.81 ±1.22 ab	4.93 ±0.52 ab
Arabinose	9.81 ±2.98 ab	13.68 ±2.21 ab	11.40 ±0.64 ab	13.44 ±1.51 ab	12.14 ±1.98 ab	14.88 ±2.41 a	10.21 ±1.20 ab	10.87 ±1.22 ab	9.17 ±0.52b

*Mean values marked with a different letter in each row are statistically different from each other. Values are expressed as mean ± standard deviation (SD) of three independent replicates

Organic acid profiles varied markedly among substrate types. Chickpea and pea residues enhanced the synthesis of tartaric, malic, citric, and succinic acids—key intermediates of the tricarboxylic acid (TCA) cycle [Vohra and Satyanarayana 2001, Nelson et al. 2017]. The observed enhancement suggests that nitrogen-rich legume residues stimulate fungal energy metabolism and carbohydrate oxidation. Conversely, poppy stalk reduced organic acid accumulation, potentially due to its alkaloid and lignin content, which may inhibit central carbon metabolism [Duke 2017]. Corncob substrates, rich in lignocellulosic carbon, induced increased oxalic and propionic acid formation, possibly reflecting altered carbon fluxes in response to structural carbohydrate degradation.

Sugar accumulation patterns revealed distinct substrate effects. Chickpea-based substrates enhanced glucose, fructose, and sucrose, consistent with the high starch and soluble carbohydrate content of chickpea residues [Jukanti et al. 2012]. Pea stalk substrates favoured rhamnose and xylose accumulation, likely due to hemicellulose degradation [Rehman et al. 2015]. Poppy stalk increased arabinose content, consistent with its pectic and arabinoxylan components [Kocabaş et al. 2020]. Corncob substrates, high in lignin, supported limited sugar release and metabolism [Limayem and Ricke 2012].

Despite the long-standing medicinal use of *Ganoderma* species, systematic investigations into the chemical composition and biological activities of *G. resinaceum* remain limited [Chen et al. 2017, Chen et al. 2018]. *Ganoderma* species contain a wide array of bioactive compounds, including triterpenes (e.g., ganoderic acids), polysaccharides, peptides, sterols, and alkaloids, which exhibit diverse pharmacological effects such as anticancer, antioxidant, anti-inflammatory, neuroprotective, hypoglycemic, hypotensive, antimicrobial, and antiviral activities [Stojkovic et al. 2014, Rashad et al. 2019, Kozarski et al. 2020, Sułkowska-Ziaja et al. 2023b, Rašeta et al. 2024, Ren et al. 2025]. Nevertheless, challenges remain in compound isolation, purification, and evaluation of synergistic effects, along with clinical standardization and safety testing. Notably, Peng et al. [2024] reported hepatoprotective properties of *G. resinaceum* triterpenoids, independent of antioxidant mechanisms.

Similar substrate-dependent variations in metabolite profiles have also been reported in other cultivated fungi. For instance, studies on *Pleurotus* spp. and *Lentinula edodes* have demonstrated that substrate composition significantly influences amino acid and organic acid contents, with nitrogen-rich substrates generally enhancing amino acid biosynthesis, while lignocellulosic substrates modulate carbon metabolism and organic acid production [Royse 2002, Elisashvili 2012]. In *Pleurotus ostreatus*, substrates supplemented with leguminous residues were found to increase protein and amino acid content, consistent with the results observed in the present study. Similarly, lignin-rich substrates such as corncob have been reported to alter carbohydrate metabolism and reduce soluble sugar accumulation in various basidiomycetes [Elisashvili 2012]. These findings indicate that the substrate-driven metabolic responses observed in *G. resinaceum* are in line with general physiological patterns reported in other edible and medicinal fungi.

Overall, the present results highlight how substrate formulation can be used to modulate fungal metabolism for biotechnological applications. By selecting appropriate agricultural residues, metabolite profiles in *G. resinaceum* can be tailored to enhance the yield of specific amino acids or organic acids with potential pharmaceutical relevance. For instance, amino acids such as phenylalanine and tryptophan serve as precursors of bioactive secondary metabolites with antioxidant and immunomodulatory effects. Organic acids such as succinic and malic acid play important roles as intermediates in biosynthetic and energy pathways, supporting their use in pharmaceutical and nutraceutical production. Overall, this study highlights the potential of agricultural residues as sustainable and low-cost substrates for optimising the biochemical and therapeutic potential of medicinal fungi.

CONCLUSION

The results of this study demonstrate that the metabolite composition of *Ganoderma resinaceum* is strongly influenced by substrate formulation. Substrate-dependent amino acid enrichment reflects both the intrinsic composition of agricultural residues and the fungus's capacity to assimilate available nitrogen sources, with chickpea and pea stalk significantly enhancing essential and aromatic amino acids. Organic acid production was closely associated with substrate characteristics and TCA cycle activity, while sugar profiles varied according to substrate type, with chickpea, pea, and poppy stalk promoting distinct sugar fractions. Overall, these findings indicate that substrate optimisation is an effective strategy for modulating metabolite production in *G. resinaceum*.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

AUTHORS' CONTRIBUTIONS

M.K.S.: conceptualization, methodology, investigation, mushroom cultivation, formal analysis, statistical analysis, writing – original draft preparation. N.C.: conceptualization, methodology, supervision, writing – review and editing. M.T.: sample preparation, laboratory analyses, data curation.

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