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Nutritional Value Assessment of *Marasmiellus inoderma* Sporophores Cultivated on Maize Cobs and Coffee Grounds

Mbumbu Luswamu Gertrude[1], Tshisuaka Nkongolo Alain[1], Dibaluka Nlandu Carmelle[1], Monizi Mawunu[1,2], Iteku Bekomo Jeff[1], Lumande Kasali Joseph[1], Mundele Mukongo Justin[1], Koto-Te-Nyiwa Ngbolua[1]*, Dibaluka Mpulusu Simon[1], Lukoki Luyeye Félicien[1]

[1]Departement de Biologie, Faculté des Sciences et Technologies de l'Université de Kinshasa, Republique Democratique du Congo.
[2]Department of Agronomy and Botanical Garden, Polytechnic Institute of Kimpa Vita University, Angola.

Corresponding author
jpngbolua@unikin.ac.cd

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Abstract

The Democratic Republic of Congo possesses significant agricultural potential, yet its population faces food insecurity, malnutrition, and limited access to essential foodstuffs, resulting in famines in several provinces. This study conducted a comparative analysis of yields and protein content in *Marasmiellus inoderma* sporophores cultivated on maize cobs and coffee grounds, as well as those collected from the wild. Experiments were carried out at four sites: the Luki Biosphere Reserve (Kongo Central), the experimental garden, the soil laboratory, and the myciculture laboratory. Average yields after four flushes were 31.7 % on maize cobs and 28.23 % on coffee grounds. Protein content was higher in sporophores cultivated on maize cobs (29.37 %) than on coffee grounds (24.98 %), with laboratory-grown samples consistently exhibiting greater protein levels than wild-collected sporophores. These results indicate that cultivating *M. inoderma* on locally available agro-industrial substrates offers a promising strategy to enhance food security and reduce malnutrition in the DRC, with strong potential for dissemination among both urban and rural producers.

Keywords: Red ginger; metastatic; cytotoxic; soxhlet, time of extraction.

INTRODUCTION

Global population growth drives a rising demand for meat, leading to intensified livestock production and increased requirements for animal-derived proteins. It is widely recognized that an increase in plant-based protein production alone will be insufficient to meet this demand, highlighting the potential of proteins from fungi and certain microorganisms, such as bacteria and yeasts (Alexander et al., 2002). Global meat consumption reached 328 million tons in 2021, with a projected increase of 70 % by 2050 (Onyeaka, 2022). This trend intensifies pressure on traditional livestock systems, making plant-based sources inadequate for meeting protein needs. Microbial proteins, or single-cell proteins, provide an efficient, sustainable source of essential amino acids (Li, 2024). Mycoproteins, in particular, offer a meat-like texture while requiring less land, water, and CO_2 emissions than conventional meat production (Bakratsas, 2023). Advanced techniques, such as precision fermentation, enable the production of specific proteins from microorganisms, thereby further enhancing the sustainability of the food system (Zhuang, 2024).

Historically considered low-nutrient foods, edible mushrooms are now recognized for their health benefits. Local populations often incorporate them into diets that promote cardiovascular health (Pedneult, 2007). Since antiquity, wild mushrooms have supplied essential proteins, vitamins, minerals, and medicinal compounds (Boa, 2006). Certain species offer high nutritional value, comprising proteins, vitamins, minerals, and unique sugars such as trehalose and mannitol, which are found exclusively in fungi (Bokassa et al., 2012). Nutritionally, mushrooms are rich in unsaturated fatty acids but low in lipids, and their off-soil cultivation does not require arable land or fertilizers, relying instead on agro-industrial waste (Nedelec, 1993). Controlled cultivation is not seasonal, allows multiple flushes, and rapidly produces sporophores—e.g., *Pleurotus* spp. begin fruiting within 2–3 weeks (Dreprès, 2012). Mushrooms valorize agricultural residues, converting them into high-nitrogen products and contributing proteins, vitamins, and minerals to human diets (Antunes et al., 2020; FAO, 2007; Guo et al., 2022; Raman et al., 2020).

Mushroom cultivation represents only one component of a comprehensive agricultural system. It enables the valorization of agricultural, and agro-industrial waste into products with higher nitrogen content than conventional crops. Various mushroom species are

increasingly utilized for both their nutritional and medicinal properties, serving as excellent dietary supplements and forming part of the human diet for centuries due to their high nutritional value and palatability. The cultivation and harvesting of *Marasmiellus inoderma* sporophores provide an accessible source of nutrient-dense food capable of addressing protein-energy malnutrition in developing countries such as the Democratic Republic of Congo (DRC).

MATERIALS AND METHODS

Biological Material

In this study, two types of biological material were used for the production of edible *Marasmiellus inoderma* sporophores and their subsequent analyses: fungal material and plant material.

Fungal Material

The fungal material employed consisted of the *Marasmiellus inoderma* strain 201114/Kin, kindly provided by the Kin-Champignon Mushroom Production Unit, operating within the Myciculture Laboratory of the Department of Biology, Faculty of Science and Technology.

Plant Material

For plant material, maize cobs and coffee grounds were used.

Methods

The study was conducted in three sequential phases: first, laboratory production of sporophores; second, field collection of sporophores in Luki; and third, laboratory analyses of the collected sporophores.

Mushroom Cultivation Methodology

The methodology for cultivating edible mushrooms is generally standard and typically involves four essential steps:

Preparation of Agar Medium

The mother culture of *Marasmiellus inoderma* strain 201114 was prepared by the Myciculture Laboratory of the Faculty of Sciences.

Preparation of Inoculum Substrate and Spawn Production

The inoculum was obtained from the Myciculture Laboratory of the Faculty of Science and Technology, University of Kinshasa, DRC. In this study, the spawn of strain 201114 of *Marasmiellus inoderma* (Fig. 1) was produced on maize grains and subsequently used to prepare the sowing substrate composed of sawdust.

Preparation of Fruiting Substrates and Fruiting Culture

Maize cobs and coffee grounds were employed as fruiting substrates.

Maize cobs preparation

The maize cobs used in this study were obtained from local maize growers after grain removal. A substantial quantity of cobs was collected and served as one of the primary substrates for cultivating *Marasmiellus inoderma*.

a) **Substrate Treatment**

The cobs were ground into relatively fine particles and transferred into a plastic bucket, soaked with tap water, covered with a bag, and allowed to ferment for 24 hours. Two substrate treatments with different ingredient proportions were subsequently prepared:

- **Treatment 1 (T1):** 4,400 g of maize cobs (60% of total substrate) were mixed with 2,053.3 g of sawdust (28%), 733.3 g of wheat bran (10%), and 146.6 g of hydrated lime (2%). The resulting substrate was placed into 29 × 18 cm heat-resistant bags, doubled, and sealed with a plastic ring containing a piece of foam.
- **Treatment 2 (T2):** Equal amounts of maize cobs and sawdust were used. Specifically, 4,800 g of cobs (45%) were combined with 4,800 g of sawdust (45%), 853.3 g of wheat bran (8%), and 213.3 g of hydrated lime (2%). The substrate was packed into 29 × 18 cm heat-resistant bags and prepared as in T1.

Both substrate treatments were sterilized in an autoclave at 1 atm for 1 hour. Inoculation was performed in an inoculation chamber beside the flame of an alcohol lamp, which served to sterilize the sampling tools. Incubation was carried out in complete darkness in a ventilated cabinet for approximately 28 days, until the substrate was fully colonized by mycelium.

Table 1. Proportions of Ingredients in Substrates T1 and T2.

Treatments	Ingredients	Mass (g)	Proportion (%)	Water content (%)
T_1	Corncobs	4400	60	56
	Sawdust	2053.3	28	
	Wheat bran	733.3	10	
	Slaked lime	146.6	2	
T_2	Corncobs	4800	45	59
	Sawdust	4800	45	
	Wheat bran	853.3	8	
	Slaked lime	213.3	2	

Legend:
T1: Treatment based on 60% maize cobs.
T2: Treatment based on equal proportions (45%) of maize cobs and sawdust.

b) **Coffee Grounds-Based Substrate Treatment**
The coffee grounds were ground into fine particles, transferred into a plastic bucket, sprayed with water, covered with a bag, and allowed to ferment for 24 hours.

Two substrate treatments were then prepared:

- **Treatment 1 (T1):** 4,400 g of maize cobs (60% of total substrate) were mixed sequentially with 2,053.3 g of sawdust (28%), 733.3 g of wheat bran (10%), and 146.6 g of hydrated lime (2%). The resulting substrate was packed into 29 × 18 cm heat-resistant bags, doubled, and sealed with a plastic ring containing a piece of foam.
- **Treatment 2 (T2):** Equal quantities of coffee grounds and sawdust were used. Specifically, 4,800 g of coffee grounds (45%) were mixed with 4,800 g of sawdust (45%), 853.3 g of wheat bran (8%), and 213.3 g of hydrated lime (2%). The substrate was packed into 29 × 18 cm heat-resistant bags and treated as in T1.

Both substrate treatments were sterilized in an autoclave at 1 atm for 1 hour. Inoculation was performed in an inoculation chamber beside the flame of an alcohol lamp, which was used to sterilize the sampling tools. Incubation was carried out in complete darkness in a ventilated cabinet for approximately 28 days, until the substrate was fully colonized by mycelium (Table 5).

Biochemical Analysis Methodology of *Marasmiellus inoderma* Sporophores
The nutritional value of *Marasmiellus inoderma* sporophores obtained from cultivation was assessed through three types of analyses: determination of moisture content, ash content, and protein content.

Determination of Moisture and Volatile Matter
Moisture and volatile matter content were determined using the weight-loss method described by (Vervack, 1982).

Determination of Total Ash
Sample preparation was performed using direct incineration in a muffle furnace, following the method described by (Vervack, 1982; Mawunu et al., 2021).

Determination of Crude Protein Content
Crude protein content was determined using the Kjeldahl method. Total nitrogen and protein contents were calculated according to the Kjeldahl method, with digestible protein (%) = nitrogen (%) × 4.38. This factor (4.38) is used for converting nitrogen to protein in mushrooms (Mbemba, 2013; Mawunu et al., 2020).

RESULTS

Mycelial Growth on Agar Medium
Following the subculture of *Marasmiellus inoderma* strain 201114 onto agar medium, mycelial growth was first observed 24 hours after inoculation and fully colonised the medium by day 14.

Table 2. Mycelial Growth Characteristics of ***Marasmiellus inoderma*** on Agar Medium.

Strain	Duration of invasion of the medium by the mycelium	Nature of the mycelium		
		Color	Aspect	Density
201114	14 days	White	Rhizomorphe	Very dense

Analysis of Table 2 indicates that the mycelium of *Marasmiellus inoderma* developed normally, exhibiting traits suitable for use in seedling substrate production, including white coloration, a rhizomorphic appearance, and high density.

Mycelial Growth on Seedling Substrate

Complete colonization of the seedling substrate was achieved by the second week, corresponding to the 13th day of incubation in darkness.

Mycelial Growth on Final Substrate

By the 32nd day of incubation, most bags containing maize cob-based substrate were fully colonized by the mycelium of *Marasmiellus inoderma* (13 replicates), all of which produced sporophores harvested in four flushes. In contrast, the coffee grounds-based substrate bags (10 replicates) were only fully colonized by the 34th day of incubation.

Sporophore Harvesting

The final substrate bags, both maize cob and coffee grounds-based, produced sporophores harvested in four flushes, four days after exposure in the fruiting chamber. Table 3 presents the sporophore yields of *Marasmiellus inoderma* obtained from the two substrate types.

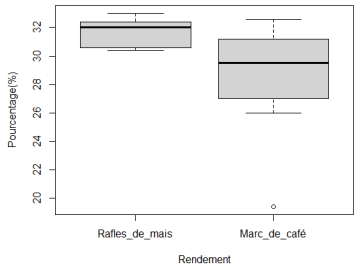
Table 3. Yields of *M. inoderma* sporophores from maize cob- and coffee ground-based substrates.

Substrate	NS	Ps	PSF					Yield (%)
			L_1	L_2	L_3	L_4	PTS	
Corn cobs	10	500	44.6±**1.07**	41.4±**1.51**	37.9±**1.20**	34.6±**1.58**	158.5±**4.53**	31.70±**0.91**
Coffee grounds	10	500	40.8±4.34	37.6±4.45	34.2±5.20	30.8±5.41	143.4±19.24	28.68±**3.85**

Legend:

N.S. : Number of bags
P.S. : Substrate weight per bag (g)
P.S.F. : Fresh sporophore weight (g)
L : Flush number
Rdt : Mean yield (%) per replicate
R.M. : Overall mean yield (%)

Table 3 shows that the mean sporophore yield of *Marasmiellus inoderma* cultivated on maize cob-based substrate was slightly higher (31.70 ± 0.91%) compared to that obtained from coffee grounds with 28.68 ± 3.85% (Fig. 1). According to the Student's t-test (p = 0.036), this difference is statistically significant. Furthermore, for both maize cob and coffee grounds substrates, the first flush produced the highest weights, whereas the fourth flush yielded the lowest values.



p-value = 0.03631

Figure 1 Comparison of *M. inoderma* yield (%) on maize cob and coffee ground substrates.

Nutritional Value of *Marasmiellus inoderma*

Moisture and Volatile Matter Content

After drying two sample masses in a Memmert oven, the moisture and volatile matter contents of the *Marasmiellus inoderma* strain F201114, collected from the wild and cultivated under laboratory conditions, are presented in Table 4.

Table 4. Moisture and volatile matter of *M. inoderma.*

M. inoderma	1st attempt		2nd attempt		Humidity (%)	Overall Average (%)
	P_1 (g)	P_2 (g)	P_1 (g)	P_2 (g)		
In nature	2	1.812	2	1.781	20.35	10.2
In the laboratory	2	1.744	2	1.761	24.75	12.4

Table 4 shows that the moisture and volatile matter contents of *Marasmiellus inoderma* sporophores, collected from the wild and cultivated under laboratory conditions, were 10.2% and 12.4%, respectively. These values are relatively close, reflecting the extended drying time in the oven prior to moisture determination. The Student's t-test indicated no significant difference between the two values (p = 0.820).

Crude Ash Content

After drying in a muffle furnace for 7 hours at 600 °C, the crude ash contents of the *Marasmiellus inoderma* (Mbema, 2013) strain F201114 cultivated in the laboratory and of Sporophores collected from the wild are presented in Table 5.

Table 5. Crude ash content of *M. inoderma.*

M. inoderma	1st attempt		2nd attempt		Total Ash (%)	Overall Average (%)
	M_1 (g)	M_2 (g)	M_1 (g)	M_2 (g)		
In nature	2	0.252	2	0.238	24.5	12.25
In the laboratory	2	0.209	2	0.200	20.45	10.22

Legend: M1: Mass of the porcelain crucible with the sample (g); M2: Mass of the crucible with the ash (g)

Analysis of Table 5 indicates that, after two replicates per sample, the ash contents of the *Marasmiellus inoderma* strain F201114 cultivated in the laboratory and of wild-collected sporophores were 10.22% and 12.25%, respectively, showing relatively similar values. The Student's t-test confirmed that the difference is not statistically significant ($p = 0.978$).

Protein Content of Marasmiellus inoderma

The total nitrogen and crude digestible protein contents of the *Marasmiellus inoderma* strain F201114, cultivated under laboratory conditions, and of wild-collected sporophores are presented in Table 6.

Table 6. Crude protein content of ***Marasmiellus inoderma*** from wild and laboratory cultivation.

Sampling Measurements	In nature		In the laboratory	
	E1	E2	E1	E2
V/ml	37.5	44	47.3	48.5
m_{at} N/1000	0.14	0.14	0.14	0.14
P (g)	1.00	1.00	1.00	1.00
Nt (%)	5.25	6.16	6.60	6.79
Digestible True Protein (%) Overall Average (%)	22.99	26.98	29.00	29.74
Digestible True Protein (%) Overall Average (%)	**24.98**		**29.37**	

Legend:
V : Volume of sodium hydroxide solution used in the blank test (mL)
0.14 : 14.007/1000 or N/P, i.e., atomic mass of nitrogen divided by sample weight in milligrams
P : Sample weight (g)
% Nt : Total nitrogen (%)

Analysis of Table 6 shows that wild-collected *Marasmiellus inoderma* sporophores contained an average protein content of 24.98%, which is lower than the 29.37% recorded for laboratory-cultivated strain F201114 sporophores.

Overall, analysis of variance indicated that each parameter of the biological evaluation of *M. inoderma* varied significantly between groups, allowing clear differentiation between cultivated and wild-collected sporophores. These parameters included moisture, ash, and protein content.

The observed differences in physicochemical composition of the sporophores may partly reflect the substrates on which they grew: cultivated sporophores developed on maize cobs, whereas wild sporophores were found on the decaying trunk of *Tola*.

In conclusion, the high protein content of both cultivated and wild sporophores of *M. inoderma* highlights their potential nutritional contribution and supports their traditional use in managing cardiovascular disorders, as reported by local communities.

DISCUSSION

Cultivation of *Marasmiellus inoderma*

After 14 days of incubation on agar medium, the *Marasmiellus inoderma* strain F201114 exhibited a white, rhizomorphic, and dense mycelium, traits typically observed in macroscopic species of the Marasmiaceae family (Oei, 1993). A similar growth period has been reported for other *Marasmiellus* species, such as *Marasmiellus palmivorus*, where significant mycelial development occurs after 14 days of incubation (Al-Janabi, 2025). Furthermore, studies on *Marasmiellus* spp. indicate substantial mycelial growth after 14 days, supporting the consistency of the findings in the present study (Thruchchelvan et al., 2012).

Marasmiellus inoderma is known to be challenging to cultivate artificially. In this study, two agricultural waste substrates—maize cobs and coffee grounds—were evaluated for their potential to support fructification. These substrates, commonly used in the cultivation of various fungal species, enable mushrooms, as natural bioconverters, to transform organic residues into essential nutrients, thereby enhancing the nutritional value of the sporophores (Chang and Milas, 2004; Bellettini et al., 2019).

Following fruiting induction, strain F201114 produced four flushes of sporophores on both tested substrates. Maize cobs yielded a mean production of 31.7%, slightly higher than that obtained with coffee grounds (28.23%). Statistical analysis revealed a significant difference between the two substrates ($p = 0.01604$), confirming the critical influence of substrate type on fungal productivity. These results align with previous observations highlighting the impact of substrate composition on both the growth and nutritional profile of edible mushrooms (Royse, 2014; Kalec, 2016).

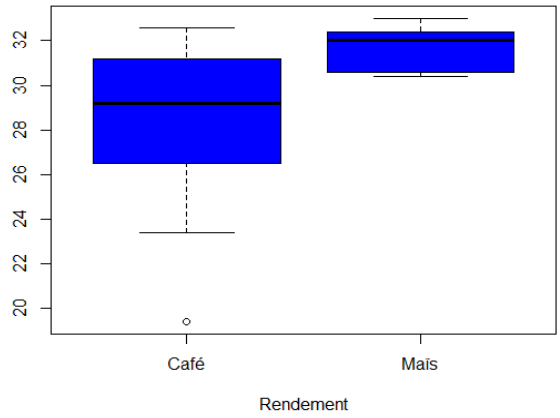


Figure 2. Comparison of yields on maize cob- and coffee ground-based substrates.

Figure 2 shows that the mean yield obtained on maize cobs (31.7%) was significantly higher than that observed on coffee grounds (28.23%), a difference likely related to the chemical composition and treatment of the substrates. These results confirm the adaptability of the *Marasmiellus inoderma* strain F201114 to both substrates, both of which exceed the 20% minimum threshold proposed by (Oei, 1993) for assessing substrate suitability in fungal cultivation.

Consistent with previous studies, the nature and nutritional richness of the substrate strongly influence the growth, productivity, and biochemical composition of edible mushrooms (Chang and Milas, 2004; Bellettini et al., 2029; Royse, 2014; Kalec, 2016). Therefore, maize cobs and coffee grounds represent suitable substrates for mass production of *M. inoderma* sporophores, with maize cobs providing superior yields and total biomass.

Figure 3 illustrates the differences in total sporophore weight obtained on maize cob and coffee ground substrates.

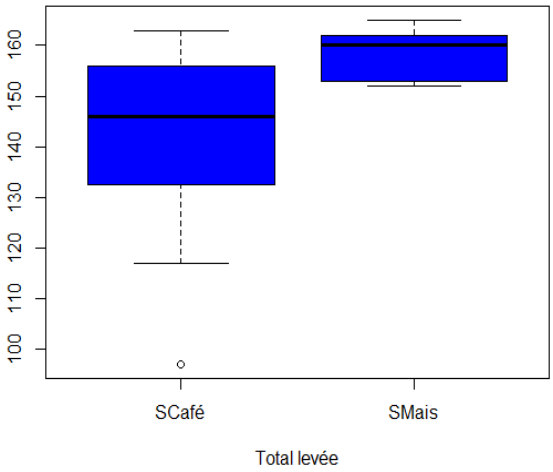


Figure 3. Comparison of total sporophore weights on maize cob- and coffee ground-based substrates.

The comparative evaluation of substrates shows that maize cobs allowed a total sporophore yield of approximately 160 g, markedly higher than that obtained on coffee grounds. This performance underscores the significance of substrate biochemical composition, particularly the availability of lignocellulose and nitrogen, which influence mycelial growth and fruiting. Previous studies have similarly emphasized that agricultural residues, such as cobs or husks, provide a favourable environment for fungal production (Chang and Milas, 2004; Kalec, 2016).

The results obtained differ from those reported by (De Kesel et al., 2008) for *Marasmiellus inoderma* cultivated on oil palm cobs, where productivity remained limited (10–20%). The advantage observed with maize cobs and coffee grounds may be related to greater bioavailability of sugars and organic compounds required for sporophore development (Bellettini et al., 2019; Kalec, 2016). These observations confirm the potential of local agro-industrial residues as optimal substrates for sustainable edible mushroom production.

Evaluation of the Nutritional Value of *Marasmiellus inoderma*

The biochemical composition of sporophores from strain F201114, both cultivated in the laboratory and collected from the wild, was analyzed to assess their nutritional significance. This study focused on the contents of moisture, total ash, and digestible protein.

The moisture contents of wild-collected and laboratory-cultivated sporophores were 10.2% and 12.4%, respectively, values comparable to the 14.09% reported by (Kouamé et al., 2018) for three edible wild mushrooms (*Psathyrella tuberculata*, *Termitomyces letestui*, and *Volvariella volvacea*). These values are

relatively low compared to the 80–90% moisture and volatile matter content reported for many fungal species. For example, Oei (1993) reported 90.1% for *Pleurotus sajor-caju*, and N'zué et al. (2020) found values of 90.1 – 90.5% for *Pleurotus geesteranus* from stipe waste over different harvest periods.

The ash content observed in this study corroborates findings by (Chapon et al., 2005), who noted that mushrooms are rich in minerals such as phosphorus, potassium, magnesium, iron, copper, zinc, iodine, fluorine, cobalt, chromium, chlorine, sulphur, and selenium, explaining their high ash content and contribution to health.

Despite the observed differences in biological evaluation, *M. inoderma* remains a good protein source. Protein contents ranged from 24.98% for wild-collected sporophores to 29.37% for laboratory-cultivated samples. Edible wild mushrooms have previously been identified as important protein sources for rural populations in developing countries (Crisan & Sand, 1978).

The higher protein content observed in laboratory-cultivated sporophores is likely due to the nutrient-rich substrates used (maize cobs and coffee grounds), which provided essential compounds that *M. inoderma* converted into protein. These values exceed those reported by (Kouamé et al., 2018) for *Psathyrella tuberculata*, *Termitomyces letestui*, and *Volvariella volvacea* (15.733 – 15.977%), and are comparable to the 24.8% reported by N'zué et al. (2020) for *Pleurotus geesteranus*. The protein content obtained in this study also surpasses that of *Lentinus* (17.5% dry weight) reported by (Diallo et al., 2017). Differences in protein content may result from variations in substrate composition, type of substrate, and cultivation conditions employed.

Previous studies indicate that edible wild mushrooms are rich in both protein and fibre. According to Malaisse et al. (2008) and, Malaisse (2010) mushroom proteins provide an important source of essential amino acids, with high bioavailability, making them a valuable dietary protein source, particularly for children in developing countries.

Thus, the high protein content of *Marasmiellus inoderma* sporophores — 29.37% for laboratory-cultivated and 24.98% for wild-collected—supports their dietary relevance and potential role in mitigating protein deficiency and improving intestinal health. Accordingly, consumption of *M. inoderma* is recommended for its nutritional benefits, particularly its high protein content.

CONCLUSION

In light of the findings of this study on the biological evaluation of cultivated and wild-harvested sporophores of ***Marasmiellus inoderma***, it is evident that maize cobs and coffee grounds represent suitable and effective substrates for the production of this species. These substrates yielded average biological efficiencies of 31.7% and 28.23%**,** respectively, after four successive flushes, with maize cobs showing a slightly higher productivity.

Biochemical analyses further confirmed the nutritional potential of *M. inoderma*. The crude protein contents were 29.37% in cultivated sporophores compared with 24.98% in those collected from the wild**,** while total ash contents reached 12.25% and 10.22%, respectively. These results highlight the richness of the species in proteins and mineral elements, with laboratory-produced sporophores recording markedly higher values than their wild counterparts.

Consequently, *M. inoderma* emerges as a promising edible mushroom that could contribute to reducing protein and mineral deficiencies, particularly in populations vulnerable to protein–energy malnutrition. Nonetheless, optimizing cultivation conditions could further enhance both yield and nutritional quality of the sporophores.

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