REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.13.e1980
ISSN 2007-3380

Original article / Artículo original

Increased linoleic and oleic acid production in the mycelium of the edible mushroom *Lentinula edodes* produced by submerged fermentation

Aumento de la producción de ácido linoleico y oleico en el micelio del hongo comestible *Lentinula edodes* producido por fermentación sumergida

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ABSTRACT

Edible mushrooms contain linoleic, α-linolenic, and oleic acid (ω-6, ω-3, ω-9), essential fatty acids for human metabolism. Consuming them in balanced proportions (1:1 or 2:1; ω-6/ω-3) can help prevent obesity, while an unbalanced proportion is associated with adipogenesis. They are traditionally extracted from fruiting bodies, which can take 60-90 days to develop. In this regard, we evaluated the effect of the culture medium on the kinetics of mycelium production and fruiting body development of *Lentinula edodes* (shiitake), as well as the characterization of fatty acids and their comparison with those of commercial shiitake. This way, the most significant amount of mycelium was obtained from the wheat bran (WB) culture medium during 15 days of incubation through submerged fermentation (SmF). Likewise, among the cultivated mycelium, the WB presented a higher percentage of linoleic acid (ω-6), and the oleic acid content (ω-9) exceeded that of commercial shiitake by 7 times, thereby benefiting the ratio of unsaturated to saturated fatty acids (MUFA-PUFA/SFA). Additionally, this proportion was higher in the fruiting bodies obtained from the PD medium, followed by the WB, compared to commercial shiitake. In this way, SmF decreases the production time of the mycelium and increases the production of shiitake fatty acids, preserving its nutraceutical components. These mycelia are also rich in nutrients and bioactive components, which are used as dietary supplements or healthcare products in the food industry. However, toxicological studies of *L. edodes* mycelium are required for it to be considered a safe functional food.

KEY WORDS: Mycelium; submerged fermentation; shiitake; Fruiting bodies; omega fatty acid.

Please cite this article as/Como citar este artículo: Mendoza-López, M.R., Navarro-Ramírez C., Ramírez, K., Landa-Cadena, M.G., Couttolenc, A., Espinoza, C. (2025). Increased linoleic and oleic acid production in the mycelium of the edible mushroom Lentinula edodes produced by submerged fermentation. Revista Bio Ciencias, 13, e1980. https://doi.org/10.15741/revbio.13.e1980

Article Info/Información del artículo
Received/Recibido: May 26th 2025.
Accepted/Aceptado: October 29th 2025.
Available on line/Publicado: November 14th 2025.

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Revista Biociencias
ISSN 2007-3380

RESUMEN

Los hongos comestibles contienen ácido linoleico, α-linolénico y oleico (ω-6, ω-3, ω-9), ácidos grasos esenciales para el metabolismo humano. Consumirlos en proporciones equilibradas (1:1 o 2:1; ω-6/ω-3) ayuda a prevenir obesidad, mientras que una proporción desequilibrada promueve adipogénesis. Tradicionalmente se extraen de cuerpos fructíferos, que pueden tardar entre 60-90 días en desarrollarse. En este sentido, se evaluó el efecto del medio de cultivo sobre la cinética de producción de micelio, cuerpos fructíferos de *Lentinula edodes* (shiitake), así como la caracterización de ácidos grasos y su comparación con shiitake comercial. La mayor cantidad de micelio se obtuvo a partir del medio de cultivo salvado de trigo (WB) durante 15 días de incubación mediante fermentación sumergida (SmF). Asimismo, entre los micelios cultivados, el WB presentó un mayor porcentaje de ácido linoleico (ω-6); y el contenido de ácido oleico (ω-9) supera 7 veces el del shiitake comercial, beneficiando la proporción de ácidos grasos insaturados/saturados (MUFA-PUFA/SFA). Además, esta proporción fue mayor en los cuerpos fructíferos obtenidos a partir del medio PD, seguido del WB, en comparación con el shiitake comercial. De esta forma, SmF disminuye el tiempo de producción del micelio y aumenta la producción de ácidos grasos de shiitake, preservando sus componentes nutracéuticos. Estos micelios también son ricos en nutrientes y componentes bioactivos que se utilizan como suplementos dietéticos o productos para el cuidado de la salud en la industria alimentaria. Sin embargo, se requieren estudios toxicológicos del micelio de *L. edodes* para que se considere un alimento funcional seguro.

PALABLAS CLAVE: Micelio, fermentación sumergida, shiitake, cuerpos fructíferos, ácidos grasos omega.

Introduction

Lentinula edodes (Berk.), better known as "shiitake", is an edible mushroom considered in Asian culture as a medicinal mushroom due to the nutraceutical properties that have been attributed to it; among the main bioactivities reported for *L. edodes* are anticancer, hypoglycemic, anti-inflammatory, antitumor, antibacterial, antioxidant, immunomodulatory, renoprotective effects, and reduction in the incidence of hypercholesterolemia (Ahmad *et al.,* 2023; Chen *et al.,* 2022; El-Ramady *et al.,* 2022; Lysakowska *et al.,* 2023). These effects of *L. edodes* are related to its content of several kinds of bioactive compounds, such as carbohydrates (58-60 %), protein (20-23 %), fiber (9-10 %), lipids (3-4 %), vitamins, folates, niacin, and minerals (Sheng *et al.,* 2021). Among the lipids these mushrooms contain are saturated (SFA), monounsaturated-polyunsaturated (MUFA-

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PUFA) fatty acids that constitute the central energy reserve of the body and perform important physiological, immunological, and structural functions (Garcia *et al.,* 2023; Kapoor *et al.,* 2021; Radzikowska *et al.,* 2019).

Although the fatty acid concentration in mushrooms is usually low compared to other foods, mushrooms contain fatty acids that are not produced by the human body, such as linoleic and α-linolenic acid, which are essential for human metabolism and, due to their structural characteristics, are classified as omega-6 and 3 fatty acids (ω-6 and ω-3), respectively. Consuming these essential fatty acids in balanced ratios (1:1 or 2:1 ω-6/ω-3) may help prevent obesity, while an unbalanced ω-6/ω-3 ratio has been linked to adipogenesis. They also participate in the formation of high-density lipoproteins, reducing the risk of cardiovascular diseases (Alagawany *et al.,* 2019; Martínez-Ramírez *et al.,* 2023; Saini *et al.,* 2021). Other ω-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), help improve health, particularly, they are usually effective in the prevention of cardiovascular and neurodegenerative diseases, cancer, inflammatory bowel disease, and rheumatoid arthritis, among others (Zhang *et al.,* 2021).

Concerning the bioactive compounds of edible mushrooms, between 80-85 % are extracted from fruiting bodies produced on blocks of wood substrates; this can take between 60-90 days. This has required alternative methods to reduce production times, such as submerged fermentation (SmF) (Bakratsas *et al.,* 2021; Yan *et al.,* 2023). This fermentation system is an alternative to obtaining mycelium that produces bioactive compounds. It has several advantages, including maintaining a uniform distribution of the substrate, reducing environmental impact, and decreasing incubation time. It is a more precise and faster method compared to cultivating fruiting bodies. Furthermore, it has been shown that some basidiomycete fungi can grow in SmF and produce bioactive compounds beneficial in the fields of nutrition, medicine, and pharmaceuticals (Dudekula *et al.,* 2020). These mycelia are also rich in nutrients and bioactive components, which are used as dietary supplements or healthcare products in the food industry (Xv W *et al.,* 2024).

The composition of culture media includes carbon sources such as glucose and sucrose, as well as more complex media (corn liquor, molasses, starches, potato dextrose broth, agricultural waste, and sterile liquid). The most commonly used nitrogen sources are peptone and yeast extract. In this way, it has been reported that the proportion and type of carbon and nitrogen sources (rC/N) influence the performance, morphology, exopolysaccharide production, protein content, and bioactivity of the *L. edodes* mycelium produced by SmF (Bakratsas *et al.,* 2021; Colla *et al.,* 2023; Krupodorova *et al.,* 2021). In the composition of lipids, in the fruiting body of *L. edodes*, there were high levels of C16:0 and C16:1 fatty acids, a high content of monoglycerides and free fatty acids, and a low level of triglycerides and phospholipids (phosphatidylethanolamine and cardiolipin). Likewise, the mycelium of *L. edodes* produced in SmF showed triglycerides and free fatty acids as its main neutral lipids, as well as phosphatidylcholine and cardiolipin as phospholipids (Yu *et al.,* 2023).

Given this background, we evaluated the effect of the culture medium on the production of mycelium and fruiting bodies of *L. edodes* in three different culture media. In addition, the fatty acid profile was characterized both in the mycelium obtained by SmF and in the fruiting bodies obtained from cultivation in blocks of wood substrate.

Materials and Methods

Strain isolation

The mycelium used in this study was isolated from fruiting bodies of shiitake (Monte blanco) obtained from a market. The mycelial culture was obtained by making cuts approximately 0.5 cm wide from the internal and external parts of the hymenium, which were washed with drinking water and disinfected by washing with 4 % sodium hypochlorite for one minute. Subsequently, two washes were performed with sterile distilled water, from which three 0.5 cm fragments were placed in 5 cm diameter Petri dishes containing Potato Dextrose Agar (PDA) and incubated for 21 days at 25 ± 2 °C.

Culture media

Three different culture media were used for biomass production through SmF. The culture media to be studied were: Potato Dextrose (PD), Wheat Bran (WB), and Compost (CM). The amount of soluble carbon was determined by the total phenol-sulfuric acid method, and total nitrogen by the Kjeldahl method.

Submerged fermentation (SmF)

Before performing the SmF, a pre-inoculum was prepared by placing small circles of PDA with *L. edodes* mycelium in 250 mL Erlenmeyer flasks containing 50 mL of potato dextrose broth. The flasks were incubated for 21 days at 25 ± 2 °C with constant stirring at 120 rpm and in the absence of light. Subsequently, 500 mL Erlenmeyer flasks containing 100 mL of PD, WB, and CM media were inoculated with 1 mL of a mycelium suspension from the preinoculum.

The incubation period was 25 ± 2 °C with shaking at 100 rpm under dark conditions. Biomass production was evaluated every three days, separated from the culture broth by vacuum filtration, and then dried at 60 °C to determine the dry weight. The data obtained from biomass production were analyzed using a logistic model, which allowed for the determination of the growth speed, maximum biomass production rate, and correlation factor for each treatment.

Solid-state fermentation (SSF)

To compare mycelium and fruiting bodies using the culture media described in SmF, traditional solid cultures enriched with these same culture media were also performed. Obtaining preinoculum: Grain (millet) was washed and hydrated with distilled water for 12 h in plastic trays, after which the excess distilled water was removed and mixed with calcium carbonate and calcium sulfate (3.5 g/kg of grain, each). Subsequently, polypaper bags were prepared with 200 g of millet and sterilized at 121 °C for 1 h. Then, each bag was inoculated with 20 fragments of 1 cm^2 of *L. edodes* mycelium developed on PDA and incubated for 30 days at 25 ± 2 °C in the darkness.

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Cultivation in blocks: Three polypaper bags were used per treatment, each containing 300 g of oak, which were hydrated for 24 h with each of the evaluated culture media. They were subsequently sterilized at 121°C for 1 hour. After that time, each bag was inoculated with 30 g of millet that had been previously inoculated with *L. edodes* mycelium and incubated in culture chambers for 60-90 days at a temperature of 30 ± 2 °C, with a relative humidity of 60-80 %, and in dark conditions.

Total fat extraction

From 5 g of mycelium produced by each of the culture media used in the SmF, 5 g of the fruiting bodies obtained in each block of the SSF, and 5 g of commercial shiitake mushroom, it was lyophilized and extracted with hexane (EMSURE® ACS, Reag. Ph Eur). The total fat content was determined using a Soxhlet system for 24 h. After that, the excess solvent was evaporated to obtain a residue with an oily consistency. Which was transferred to an amber vial with an airtight lid and stored under refrigeration conditions (-4 °C) until subsequent analysis.

Chromatographic characterization of fatty acids

Once the total fat extracts of the mycelia obtained by SmF, SSF fruiting bodies, and the commercial fungus were obtained, the fatty acid content was analyzed through gas chromatography equipment coupled with mass spectrometry. To achieve this, the methyl esters were prepared using the technique proposed by Egan *et al.* (1981), which involved the use of BF3 in 14 % methanol. The extraction of the esters was performed using 1.0 mL of HPLC-grade hexane. The extract was then dried over anhydrous sodium sulfate (Na_2SO_4) and filtered for subsequent injection. For the analysis, an Agilent Technologies gas chromatograph, model 6890N (Net Work GC system), equipped with a DB-5, 5 %-phenyl-methylpolysiloxane (Agilent Technologies) column of 60 meters in length, 0.25 mm in diameter, with an internal and 0.25 µm film thickness. The starting temperature was 150 °C, which was maintained for 5 min; subsequently, the temperature was raised to 210 °C using a heating ramp of 30 °C/min. From 210 °C, it went to 213 °C at a speed of 1 °C/min. Finally, the temperature was increased to 225 °C at a rate of 20 °C/min for 40 min, resulting in a total of 50.6 min per run for each sample. Helium was used as carrier gas at a flow of 1 mL/min; the injector temperature was 250 °C, and split injection, with a split ratio of 50:1.

Once the chromatogram was obtained, the identification of each of the peaks was carried out by mass spectrometry using an Agilent Technologies model 5975 inert XL mass spectrometer. The mass spectra were obtained by electron impact ionization at an electron energy of 70 eV. For identification, the mass spectra obtained for each compound were compared with a database (HP Chemstation-NIST 05 Mass Spectral search program, version 2.0d). In addition to the comparison with a standard (FAME mix, C8:C22, catalog no. 18920-1AMP, Sigma-Aldrich), the sample was analyzed under the same conditions.

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Statistical analysis

Biomass production was modeled using SigmaPlot software, and the results were fitted using the logistic model. Fatty acid content was analyzed using Minitab® 19 statistical software, using ANOVA. Differences between means were compared using the Tukey post hoc test with a significance level of $p < 0.05$.

Results and discussion

Culture media

The C and N contents of the tested media were determined using the total sugars method, which employed both the phenol-sulfuric acid and Kjeldahl methods, and the C/N ratios of each medium were calculated accordingly.

The amount of C total was 22.9, 29.2, and 36.3 g/L, and the total N was 4.6, 49.0, and 72.0 g/L for the PD, WB, and CM culture media, respectively. In this way, the C/N ratio obtained from each culture medium was 4.98, 0.6, and 0.5, respectively.

Biomass production of *L. edodes* by submerged fermentation

The biomass production during the incubation time and its adjustment with the Logistic model of the PD, WB, and CM culture media using SmF is shown in Figure 1. Thus, for PD, a maximum biomass production of (Xmax = 2.2 g/L) was observed at 30 days of incubation, observing that the exponential phase of growth did not cease. On the other hand, for CM, the maximum biomass production was observed (Xmax = 2.6 g/L), and the stationary phase was reached after 12 days of incubation. Likewise, the highest biomass production was observed in the WB culture medium (Xmax = 9.8 g/L), compared to the other two culture media used, and it reached the stationary phase after 15 days of incubation.

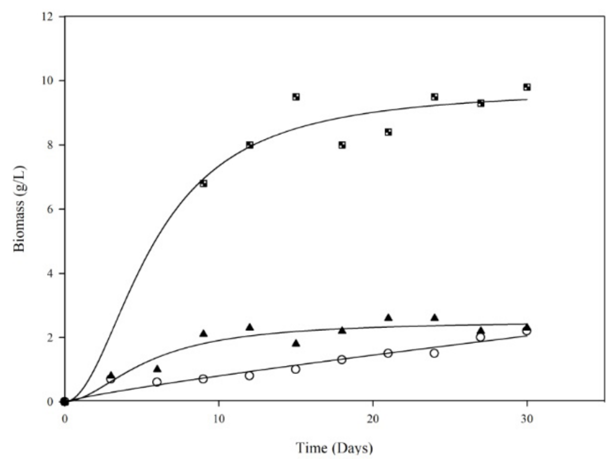

Figure 1. Growth kinetics of *L. edodes* using SmF with culture media: Potato Dextrose, Compost, and Wheat Bran. The solid line is the logistic fit.

From the logistic model, different values of specific growth rate (μ) and correlation coefficient (R^2) were obtained for each of the evaluated media, where it was observed that the WB culture medium presented the highest specific growth rate, μ = 0.8 day^{-1} and R^2 = 0.9, followed by CM culture medium (μ = 0.4 day^{-1} and R^2 = 0.9) and PD (μ = 0.1 day^{-1} and R^2 = 0.9). The data obtained for the PD and CM culture media were similar to those reported in other studies (Adil *et al.,* 2020), where they showed a biomass production of 3 g/L for the control medium, on the other hand, the WB medium presented a higher biomass production compared to that reported, where the maximum biomass production reported corresponds at 8.6 g/L for a synthetic medium supplemented with Ca^{+2}. Based on the previous results, we can observe that the proportion of C/N = 0.6 of the WB medium reached its Xmax approximately after 15 days of incubation, therefore, these conditions are considered adequated to scale the production of the edible mushroom *L. edodes*, which coincides with what has been reported in other studies, where it is reported that an ideal amount of nitrogen directly influences the production of mycelium, since if a greater amount is used of nitrogen than carbon, inhibition of the mycelial growth of the fungus occurs (Bellettini *et al.,* 2019).

Fatty acid profile from the mycelium and fruiting bodies of *L. edodes*

The total fat content was found to be 0.125 g for PD, 0.115 g for CM, and 0.149 g for WB. Regarding the content of fatty acids present in each of the analyzed samples, a wide variety of saturated, monounsaturated, and polyunsaturated fatty acids was found. Table 1 shows the fatty acid profile of the mycelium produced by SmF in the different culture media evaluated, as well as the comparison of the fatty acid content of the commercial fruiting bodies. In this sense, fatty acids containing 10 to 22 carbon atoms are presented, among them is linoleic acid (ω-6),

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which is found in all culture media, highlighting the one with the highest percentage (WB = 51.12 %), approaching the values obtained in the commercial fruiting bodies (68.17 %). Likewise, the production of oleic acid (ω-9) stands out with values of 13.35, 22.14, and 21.53 % for CM, WB, and PD, respectively, which is up to 7 times that detected in the commercial fruiting bodies (3.25 %). As for α-linolenic acid (ω-3), it was not detected in the culture media evaluated; however, in commercial fruiting bodies, it is found at around 1 %. Finally, in the commercial fruiting bodies, the following compounds were not detected: trans-9-hexadecenoic acid, cis-9-hexadecenoic acid, heptadecanoic acid, and dodecanoic acid. In contrast, in the CM medium, these compounds reached values between 1.42 and 2.65 %.

Table 1. Profile of fatty acids present in the mycelium of *L. edodes* obtained by SmF with different culture media and from the commercial fruiting bodies.

	Abundance percentage by g/dry sample			
	Commercial fruiting bodies	SmF mycelium of culture media		
Saturated fatty acids (SFA)		PD	WB	CM
C10:0 Decanoic acid (capric acid)	0.11c	1.06a	Nd	0.22b
C12:0 Dodecanoic acid (lauric acid)	1.34b	1.90a	0.18d	0.78c
C14:0 Tetradecanoic acid (myristic acid)	0.76c	8.46a	0.45d	1.69b
C15:0 Pentadecanoic acid	4.40a	1.37c	0.71d	2.40b
C16:0 Hexadecanoic acid (palmitic acid)	18.42b	27.38a	15.26c	27.34a
C17:0 Heptadecanoic acid	Nd	Nd	0.59b	1.42a
C18:0 Octadecanoic acid (stearic acid)	2.56d	8.65a	5.19c	5.50b
C22:0 Docosanoic acid (behenic acid)	Nd	0.48c	2.45b	2.51a
Monounsaturated and polyunsaturated fatty acids (MUFA and PUFA)				
C16:1 *Trans*-9-hexadecenoic acid	Nd	1.38b	0.86c	2.65a
C16:1 *Cis*-9-hexadecenoic acid (palmitoleic acid)	Nd	0.28c	1.05b	2.17a
C18:1 *Cis*-9-octadecenoic acid (oleic acid)	3.25d	21.53b	22.14a	13.35c
C18:2 *Cis*-9,12-octadecadienoic acid (linoleic acid)	68.17a	27.51d	51.12b	39.97c
C18:3 *Cis*-9,12,15-octadecatrienoic acid (α-linolenic acid)	0.99	Nd	Nd	Nd

Legend: Nd – Not detected; different letters indicate a significant statistical difference between samples according to Tukey's test (p = 0.05).

Regarding the comparison of fatty acids in the fruiting bodies produced by SSF (Table 2), we found that myristic acid in the commercial fruiting bodies is about six times higher in the fruiting bodies obtained from blocks enriched with WB and CM medium. The same trend is observed

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with stearic acid, which is up to 5.7 times higher in the block enriched with CM medium. For palmitic acid, the values are slightly higher than those in the commercial fruiting bodies (18.42 %), with the fruiting bodies from the PD, WB, and CM blocks showing 19.20 %, 19.06 %, and 24.89 %, respectively. Similarly, for linoleic acid (ω-6), the fruiting bodies from the PD-enriched block had 75.82 %, followed by WB with 73.10 %, and the commercial fruiting bodies with 68.17 %. Palmitoleic acid was not detected in the commercial fruiting bodies, but values of 0.15 %, 0.2 %, and 1.06% were found in the fruiting bodies from the PD, WB, and CM blocks, respectively. Edible mushrooms are low in fat, cholesterol-free, rich in unsaturated fatty acids, mainly linoleic acid, and represent a healthy source of essential fatty acids (Martínez-Ramírez *et al.,* 2023; Wang & Zhao, 2023). It has been reported that these acids are present in different edible mushroom species at levels ranging from 0-81 % for linoleic acid and 1-61 % for oleic acid (Bulam *et al*, 2021; Dimitrijevic *et al.,* 2019). Additionally, studies report (Chung *et al.,* 2020) that linoleic acid content varies from 77-81 % in different shiitake samples evaluated from various harvesting cycles and growing substrates. α-linolenic and oleic acids (ω-3 and ω-9) were not detected in the fruiting bodies from the blocks but were present in the commercial fruiting bodies at 0.99 % and 3.25 %, respectively.

Table 2. Profile of fatty acids present in the fruiting bodies of *L. edodes* grown in blocks enriched with different culture media and in the commercial specimen.

Saturated fatty acids (SFA)	Abundance percentage by g/dry sample			
	Commercial	PD	WB	CM
C10:0 Decanoic acid (capric acid)	0.11[b]	0.19[a]	0.14[ab]	0.05[c]
C12:0 Dodecanoic acid (lauric acid)	1.34[b]	0.42[d]	1.17[c]	3.85[a]
C14:0 Tetradecanoic acid (myristic acid)	0.76[d]	2.65[c]	4.85[a]	4.55[b]
C15:0 Pentadecanoic acid	4.40[a]	1.57[c]	1.48[d]	1.76[b]
C16:0 Hexadecanoic acid (palmitic acid)	18.42[d]	19.20[b]	19.06[c]	24.89[a]
C18:0 Octadecanoic acid (stearic acid)	2.56[b]	Nd	Nd	14.69[a]
C22:0 Docosanoic acid (behenic acid)	Nd	Nd	Nd	0.64
Monounsaturated and polyunsaturated fatty acids (MUFA and PUFA)				
C16:1 *Cis*-9-hexadecenoic acid (palmitoleic acid)	Nd	0.15[c]	0.2[b]	1.06[a]
C18:1 *Cis*-9-octadecenoic acid (oleic acid)	3.25	Nd	Nd	Nd
C18:2 *Cis*-9,12-octadecadienoic acid (linoleic acid)	68.17[c]	75.82[a]	73.10[b]	48.51[d]
C18:3 *Cis*-9,12,15-octadecatrienoic acid (α-linolenic acid)	0.99	Nd	Nd	Nd

Legend: Nd – Not detected; different letters indicate a significant statistical difference between samples according to Tukey's test (*p* = 0.05).

In Figure 2, we can observe the percentages of SFA and MUFA-PUFA in the commercial fruiting bodies and fruiting bodies with mycelium obtained using the different culture media. In this way, the commercial fruiting body (FB-C) has a MUFA-PUFA/SFA ratio of 2.6, equal to the fruiting body obtained from the block enriched with WB medium (FB-WB = 2.7). However, the highest proportion occurred in the fruiting body of the block enriched with PD (FB-PD = 3.2), followed by the mycelium obtained in the WB medium (M-WB = 3.0).

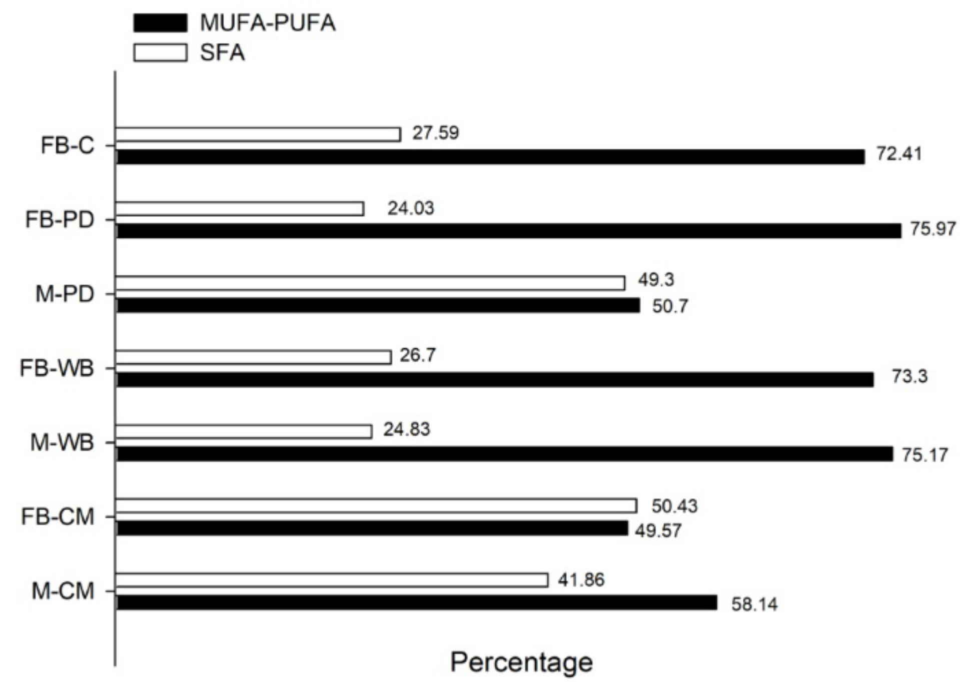

Figure 2. Percentage abundance of MUFA-PUFA and SFA present in shiitake mycelium (M) and fruiting bodies (FB) by g/dry sample.

Based on the data obtained, it is observed that FB-C contains omega-essential fatty acids such as omega-3 and omega-6, which belong to the PUFA group (Christi & Harwoo, 2020; Watanabe & Tatsuno, 2020). Regarding linoleic acid content, it was found in higher amounts in the FB-PD medium, followed by FB-WB. For oleic acid, it was only detected in the mycelium produced by liquid fermentation.

The characterization of the fatty acid profile of the commercial shiitake mushroom showed that it contains three types of omega acids (ω-3, ω-6, ω-9), and the most abundant was linoleic acid (ω-6). Of the culture media evaluated, the WB mycelium was the one that presented the highest percentage of linoleic acid (ω-6) and oleic acid (ω-9), with the latter showing a 7-fold increase compared to the commercial carpophore. The ratio of MUFA-PUFA/SFA was higher in the fruiting bodies obtained from the PD medium, followed by WB, and both were higher than in commercial fruiting bodies. Similarly, in the mycelium, the MUFA-PUFA/SFA ratio was higher in the WB medium compared to that in the commercial fruiting bodies.

Submerged mycelium contains up to 20 % lipids by dry mass, whereas fruiting bodies contain only 3-4 %. The lipids of *L. edodes* fruiting bodies primarily contain high levels of palmitic acid (C16:0) and linoleic acid, mainly. On the other hand, work carried out on other edible mushrooms, such as *Pleurotus pulmonarius* and *Morchella crassipes*, has demonstrated differences in fatty acid composition and abundance depending on the carbon source used. Additionally, it has been noted that mushroom lipid fractions play a crucial role in enhancing organoleptic properties. Unsaturated and essential fatty acids improve the nutritional value of the biomass. At the same time, flavor and aroma are influenced by certain autoxidation products of unsaturated fatty acids, such as palmitoleic, oleic, linoleic, and linolenic acids (Smiderle *et al.,* 2012; Sun *et al.,* 2020). It has also been shown that oleic and linoleic acid help reduce serum cholesterol, and oleic acid is a precursor of 1-octen-3-ol, a key aromatic compound responsible for mushroom flavor (Feng *et al.,* 2023).

Mushrooms can serve as an excellent source of bioactive compounds and nutraceutical agents that exhibit a wide range of effects, including immunomodulatory, anti-inflammatory, antioxidant, antiproliferative, antimicrobial, and hypoglycemic activities. Additionally, it has been demonstrated that lipids from edible mushrooms, unlike those from other sources, exhibit greater bioavailability, thereby enhancing these properties (Martínez-Ramírez *et al.,* 2023). Despite significant progress in SmF and SSF technologies for producing bioactive metabolites, a major challenge remains in producing basidiomes and extracting their bioactive compounds without lengthy processes that take months or risk contamination. In this context, SmF is a favorable alternative for obtaining bioactive compounds under controlled culture conditions (Zhang *et al.,* 2019). It is well established that the bioactive metabolites of edible mushrooms provide a solid foundation for incorporating mushrooms as functional foods and potential adjuvants in clinical therapies (Venturella *et al.,* 2021). In this regard, edible mushrooms are a promising option for fighting malnutrition, especially in underdeveloped and developing countries, as they contain beneficial nutraceutical metabolites (Dudekula *et al.,* 2020).

Conclusions

SmF proved to be a suitable alternative for producing mycelium and fatty acids from *L. edodes.* Thus, the mycelium from the WB culture medium presented the highest percentage of linoleic acid (ω-6) and oleic acid (ω-9), the latter showing a 7-fold increase compared to commercial fruiting bodies. Regarding the MUFA-PUFA/SFA ratio in the mycelium, only the WB treatment showed a higher ratio compared to that observed in commercial fruiting bodies. Likewise, the MUFA-PUFA/SFA ratio was highest in carpophores obtained using PD medium, followed by WB, both of which had a higher ratio compared to commercial fruiting bodies. The above supports the

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notion that SmF is an adequate cultivation alternative for producing edible mushroom mycelium with nutraceutical properties. Added by some other advantages, like shorter time and more controllable environmental conditions needed for the growth of *L. edodes* mycelium than for fruiting bodies. These mycelia are also rich in nutrients and bioactive components, which are used as dietary supplements or healthcare products in the food industry. However, toxicological studies of *L. edodes* mycelium are required for it to be considered a safe functional food.

Author contributions

Ma Remedios Mendoza-López-Formal analysis, Investigation, Validation, Writing–review & editing; Cindy Navarro-Ramírez, Karina Ramírez- Methodology, Validation, Visualization, Writing–original draft. Mahatma G. Landa-Cadena- Methodology, Investigation, Visualization. Alan Couttolenc- Formal Analysis, Investigation, Writing–review & editing. César Espinoza-Project administration, Conceptualization, Formal Analysis, Investigation, Writing–original draft.

Funding

This research received funding from the SECIHTI under the Basic and Frontier Science project (CBF-2025-I-1519).

Acknowledgments

Thanks to the SIREI-UV project (31506202571) and the UV-CA-354 Fungal Chemistry and Biotechnology research group.

Conflict of Interest

The authors declare that there is no conflict of interest.

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