Гриби і грибоподібні організми
Fungi and Fungi-like Organisms

https://doi.org/10.15407/ukrbotj78.05.327

RESEARCH ARTICLE

Fatty acid profile of an indigenous strain of *Lentinus sajor-caju* (*Basidiomycota*)

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Abstract. The aim of the present study was to investigate the fatty acid composition of an indigenous strain of *Lentinus sajor-caju* collected in the wild and cultivated under laboratory conditions. This edible mushroom is widely consumed in different parts of the world. The study revealed the presence of 26 fatty acids, including saturated fatty acids (SFA-27.69%), monounsaturated fatty acids (MUFA-5.42%), and polyunsaturated fatty acids (PUFA-65.06%) in varying quantities ranging from 0.01% to 60.62%. Amongst the estimated fatty acids, linoleic acid (60.62%) was preponderantly present in comparison to all other fatty acids. Palmitic acid (17.6%) was found to be the second and oleic acid (3.95%) the third most abundant fatty acid in the fungus.

Keywords: Flame ionization detector (FID), gas chromatography (GC), *Lentinus sajor-caju*, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), sporophores

Article history. Submitted 2 June 2021. Revised 11 August 2021. Published 29 October 2021

Citation. Lata, Atri N.S. 2021. Fatty acid profile of an indigenous strain of *Lentinus sajor-caju* (*Basidiomycota*). *Ukrainian Botanical Journal*, 78(5): 327–334. https://doi.org/10.15407/ukrbotj78.05.327

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Introduction

The sporophores of edible mushrooms are considered to be valuable health foods that are appreciated for their texture, flavour, nutritional properties, as well as for their therapeutic properties due to the effectiveness in preventing hypercholesterolemia, coronary atherosclerosis, for antitumor activities and immunomodulation. Mushrooms possess high amounts of proteins, vitamins, minerals, essential unsaturated fatty acids, and low proportions of fat content (Crisan, Sands, 1978; Tressl et al., 1982; Grosch, Wurzenberger, 1984). Fatty acids are carboxylic acids with long hydrocarbon chains which are either saturated or unsaturated. These are the major source of energy for the human beings and are reported to play a major role in tissue development and the absorption of the fat-soluble vitamins A, D, E, K and other food components, such as carotenoids.

Lentinus sajor-caju (Fr.) Fr. is an agaricoid basidiomycetous fungus belonging to the family *Polyporaceae* (*Polyporales*, *Agaricomycetes*). The edibility and culinary relevance of *L. sajor-caju* has been documented in the earlier literature published by many investigators from different countries including India, Malaysia, Philippines, Tanzania, Vietnam, etc. (Chin, 1981; Corner, 1981; Purkayastha, Chandra, 1985; Verma et al., 1995; Puttaraju et al., 2006; Kavishree et al., 2008; De Leon et al., 2012; Singdevsachan et al., 2013; Sharma, Atri, 2014; Afiukwa et al., 2015; Dulay et al., 2015; Gaur et al., 2016; Hussein et al., 2016; Reneses et al., 2016). As far as India is concerned, not much work has been done in this regard. In view of this, detailed investigation was carried out using the sporophores of *L. sajor-caju* produced from the indigenous culture of the species collected from the wild in order to study its nutritional and nutraceutical constituents, including the fatty acid profile presented in this article.

Materials and Methods

Fatty acids were estimated using gas chromatography with a flame ionization detector (GC-FID) following standard protocol given by Ranganna (1986).

Material. The sporophores produced during laboratory cultivation from the indigenous culture of *Lentinus sajor-caju* collected from the wild were used for investigation of its fatty acid profile. The culture has been deposited in the Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Mohali, Punjab, under MTCC No. 10945.

Chemicals and reagents. All reagents and chemicals used for our analysis were of analytical grade. The fatty acid reference standard mixture of 37 fatty acids, methanol and sulphuric acid were obtained from SIGMA while n-hexane, petroleum ether, toluene and sodium sulphate were obtained from MERCK.

Standard preparation. For the calibration purpose, 37 fatty acids control reference standard was prepared.

Sample preparation. To cultivate *L. sajor-caju*, an indigenous culture raised in the laboratory through tissue culture technique was used. For cultivation, locally available ligno-cellulosic natural substrates, namely paddy straw, wheat straw, sawdust and wooden flakes, were used separately and in combination in the ratio of 1:1:1:1. For each substrate and their combinations used for cultivation, three polypropylene bags with 500 g substrate on dry weight basis were taken. Substrates of each such bag were soaked in water for at least two days. Excess of water was decanted off from the soaked substrate and filled in polythene bags followed by moist heat sterilization in an autoclave at 15 psi (pound-force per square inch) pressure, for 1 hour at least twice before use, in order to make it free from any infection. Spawning of the substrate was done aseptically with 7–8% of the spawn prepared on wheat grains. The inoculated bags were incubated in the incubator at 33±1 °C. Polythene covering was removed from the colonized substrates on complete colonization and which were then transferred to a cropping room maintained at 28 ± 1 °C temperature and high relative humidity (85%–90%). The required humidity was maintained in the cropping room with the help of a humidifier. The sporophores raised through cultivation under laboratory conditions were harvested and dried in a hot air drier at 45±1 °C (Atri et al., 2005).

For fat extraction, 100 g of mushroom powder prepared by crushing sporophores was soaked in n-hexane. The extracted fat was converted to methyl esters. For methylation, the extracted fat was mixed with a transmethylation mixture (150 mL of methanol + 70 mL of toluene + 7.5 mL concentrated sulphuric acid) which was then kept in water bath under reflux for 90 minutes. Petroleum ether and ultra-pure water were added to it on cooling and the aqueous layer was collected. This was followed by repeated washing and addition of sodium sulphate. The clear ether layer so obtained was evaporated to dryness. Residue obtained was then dissolved in the petroleum ether and used for the fatty acid profile through gas chromatography.

Instrumentation. Fatty acid profiling was performed with Thermo Scientific Model Trace GC Ultra equipped with a HP-88 column (100 mm × 0.25 mm × 0.20 μm), a flame ionization detector (FID), and a split injector. Oven temperature and injector temperature were maintained at 250 °C and 60–140 °C, respectively. Helium gas was used as the mobile phase. For analysis, 1 μl of control reference standard and the mushroom sample to be investigated were injected in the instrument. Fatty acid identification was done by comparing the relative retention time of an individual fatty acid. The peaks obtained in the sample chromatogram were compared with the standard chromatogram. Results are expressed in relative values (%).

Results

Mushroom samples of *L. sajor-caju* were analysed and quantified for the presence of 37 fatty acids, out of which 26 fatty acids were observed in *L. sajor-caju*. Fatty acid composition of the mushroom revealed the presence of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and trans-fatty acids (TFA) in varying quantities ranging from 0.01% to 60.62%. The fatty acid composition, the amount (%) of the individual component obtained and chromatographic spectral data obtained for *L. sajor-caju* are summarized in Tables 1, 2 and depicted in chromatogram Figs 1, 2.

The carbon chain length of saturated fatty acids ranged from 4 to 24 and content from 0.09% to 17.6%. Some of the documented saturated fatty acids in the mushroom which were present in high proportion include palmitic acid (17.6%), pentadecylic acid (2.73%), stearic acid (1.75%), tricosylic acid (1.72%) and margaric acid (1.49%), while those determined in smaller proportion were myristic acid (0.72%), undecylic acid (0.42%), lignoceric acid (0.42%), lauric acid (0.40%), behenic acid (0.35%) and caprylic acid (0.09%). Other fatty acids,

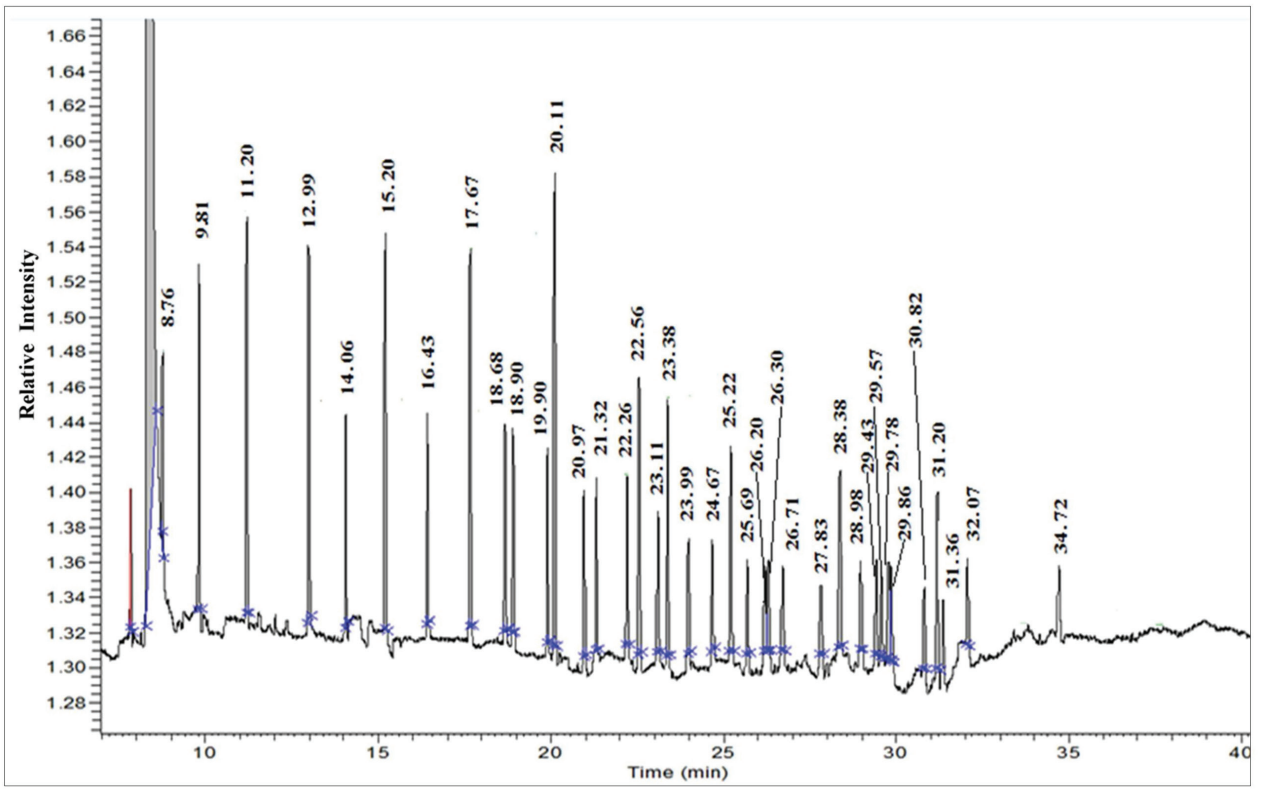

Fig. 1. Gas chromatography chromatogram of fatty acid standards

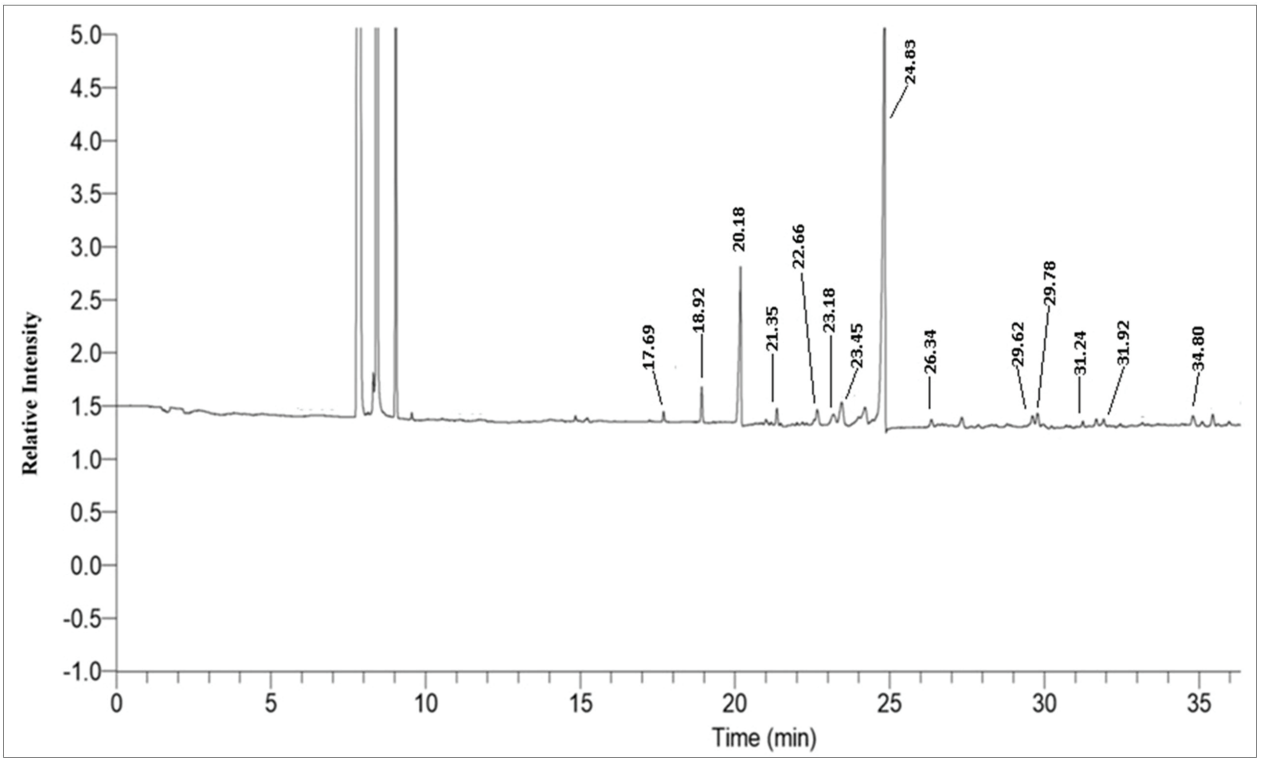

Fig. 2. Gas chromatography chromatogram of fatty acid composition of *Lentinus sajor-caju*

such as butyric acid, caproic acid, capric acid, tridecylic acid, arachidic acid and heneicosylic acid, were not detected in *L. sajor-caju*. In all, 27.69% saturated fatty acids were accounted of the total fatty acids.

For comparison, the quantity of unsaturated fatty acids ranged from 0.01% to 60.62% with the carbon chain length extending from 14 to 24. Amongst the evaluated fatty acids in the mushroom, the essential fatty acid, linoleic acid (60.62%), was recorded in substantially high proportions.

Amongst the monounsaturated fatty acids, oleic acid (3.95%) was observed in higher proportion followed by nervonic acid (0.94%) and palmitoleic acid (0.39%). Some of the monounsaturated fatty acids, including myristoleic acid, cis-10-pentadecanoic acid and cis-11-eicosenoic acid, were not detected in the sporophores. On overall basis, monounsaturated fatty acids accounted for 5.42% of the total fatty acids were present in the evaluated samples.

Lentinus sajor-caju contained high proportions of polyunsaturated fatty acid content because of the presence of the highest quantity of linoleic acid (60.62%). Polyunsaturated fatty acids accounted for 65.06% of the

Table 1. **Saturated fatty acid composition of *Lentinus sajor-caju***

S. no.	Fatty acids	Carbon number	Retention time (minutes)	Amount of fatty acids, total content (%)
1	Butyric/Butanoic acid	C4:0	-	ND
2	Caproic/Hexanoic acid	C6:0	-	ND
3	Caprylic/Octanoic acid	C8:0	11:23	0.09
4	Capric/Decanoic acid	C10:0	-	ND
5	Undecylic/Undecanoic acid	C11:0	14:04	0.42
6	Lauric/Dodecanoic acid	C12:0	15:23	0.40
7	Tridecylic/Tridecanoic acid	C13:0	-	ND
8	Myristic/Tetradecanoic acid	C14:0	17:69	0.72
9	Pentadecylic/Pentadecanoic acid	C15:0	18:92	2.73
10	Palmitic/Hexadecanoic acid	C 16:0	20:18	17.6
11	Margaric/Heptadecanoic acid	C 17:0	21:35	1.49
12	Stearic/Octadecanoic acid	C 18:0	22:66	1.75
13	Arachidic/Eicosanoic acid	C 20:0	-	ND
14	Heneicosylic/Heneicosanoic acid	C 21:0	-	ND
15	Behenic/Docosanoic acid	C 22:0	28:31	0.35
16	Tricosylic/Tricosanoic acid	C 23:0	29:78	1.72
17	Lignoceric/Tetracosanoic acid	C24:0	31:24	0.42
Total saturated fatty acids				27.69

Table 2. **Unsaturated and trans fatty acid composition of *Lentinus sajor-caju***

S. no.	Fatty acid	Carbon number	Omega (ω) type	Retention time (minutes)	Amount of fatty acids, total content (%)
Monounsaturated fatty acids					
1	Myristoleic acid	C14:1	7	-	ND
2	Cis-10-pentadecanoic acid	C15:1	7	-	ND
3	Palmitoleic acid	C16:1	7	21:00	0.39
4	Cis-10-heptadecanoic acid	C17:1	7	22:18	0.13
5	Oleic acid	C18:1	9	23:45	3.95
6	Cis-11-eicosenoic acid	C20:1	9	-	ND
7	Erucic acid	C22:1	9	29:35	0.01
8	Nervonic acid	C24:1	9	31:92	0.94
Total Monounsaturated Fatty Acids					5.42
Polyunsaturated fatty acids					
1	α-Linolenic acid	C18:3	3	26:34	0.87
2	Cis-11,14,17-eicosatrienoic acid	C20:3	3	29:62	1.68
3	Cis-5,8,11,14,17-eicosapentaeoic acid	C20:5	3	31:44	0.12
4	Cis-4,7,10,13,16,19-docosahexaenoic acid	C22:6	3	34:80	1.05
5	Linoleic acid	C18:2	6	24:83	60.62
6	γ-Linolenic acid	C18:3	6	-	ND
7	Cis-11,14-eicosadienoic acid	C20:2	6	27:87	0.30
8	Cis-8,11,14-eicosatrienoic acid	C20:3	6	28:89	0.17
9	Arachidonic acid	C20:4	6	-	ND
10	Cis-13,16-docosadienoic acid	C22:2	6	30:72	0.25
Total Polyunsaturated fatty acids					65.06
Trans fatty acids					
1	Elaidic acid	C18:1	9	23:18	1.17
2	Linoelaidic acid	C18:2	6	23:99	0.66
Total Trans fatty acids					1.83

total fatty acids were found in the evaluated mushroom samples. In addition to linoleic acid, cis-11,14,17-eicosatrienoic acid (1.68%), cis-4,7,10,13,16,19-docosahexaenoic acid (1.05%) and α-linolenic acid (0.87%) were the other polyunsaturated fatty acids documented in the sporophores. As compared, cis-11,14-eicosadienoic acid (0.30%); cis-13,16-docosadienoic acid (0.25%), cis-8,11,14-eicosatrienoic acid (0.17%) and cis-5,8,11,14,17-eicosapentaeoic acid (0.12%) were recorded to be present in traces while γ-linolenic acid and arachidonic acid were not detected in the studied mushroom. Elaidic acid (1.17%) and linoelaidic acid (0.66%) are the trans fatty acids which were also recorded during the estimation of fatty acids of *L. sajor-caju.*

Discussion

In the cultivated sporophores of *L. sajor-caju*, saturated fatty acids accounted for 27.69% of the total fatty acids content. Kavishree et al. (2008), while working on 23 wild mushroom fruiting bodies, documented 20% total saturated fatty acid content in *L. sajor-caju*, 30.6% in *L. squarrosulus* Mont., 25.1% in *Pleurotus djamor* (Rumph. ex Fr.) Boedijn and 27.1% in *P. sajor-caju* which is almost comparable to the amount of saturated fatty acids documented in the presently evaluated samples. In cultivated sporophores of *Lentinus squarrosulus* SQW (27.43%), *L. squarrosulus* LSF (25.78%), *Pleurotus ostreatus* EM-I (21.87%), *P. sajor-caju* (26.62%), and naturally growing *P. tuber-regium* (Fr.) Singer (42.19%), excepting *P. tuber-regium*, comparable proportion of total saturated fatty acid are reported by Obodai et al. (2014). While investigated the wild fruiting bodies of *Lentinus connatus* Berk. and *L. cladopus* Lév., Sharma and Atri (2014) recorded 27.05% and 27.76% of saturated fatty acid in these species, respectively. However, much higher amounts of total saturated fatty acid were reported by Sharma and Atri (2014) in the naturally growing *L. sajor-caju* (53.89%), *L. torulosus* (Pers.) Lloyd (56.8%) and *L. squarrosulus* (57.36%) as compared to the presently analysed specimens of *L. sajor-caju*.

The palmitic acid content in the investigated specimens of *L. sajor-caju* (17.6%) is almost comparable to the proportion of this fatty acid reported in *L. sajor-caju* (15.4%), *L. squarrosulus* (16.6%), *Pleurotus djamor* (15.8%), and *P. sajor-caju* (13.9%) by Kavishree et al. (2008). Obodai et al. (2014) also reported 18.04% palmitic acid in *L. squarrosulus* LSF, 19.62% in *L. squarrosulus* SQW, 17.84% in *P. sajor-caju*, 14.31% in *P. ostreatus* EM-I and 21.19% in *P. tuber-regium*, which is also almost comparable to the amount of this fatty acid that we documented in the evaluated samples of *L. sajor-caju*. Palmitic acid has been reported as the integral part of wild sporophores of *L. sajor-caju* (41.29%), *L. torulosus* (41.83%), *L. squarrosulus* (45.13%), *L. cladopus* (22.79%) and *L. connatus* (14.25%) by Sharma and Atri (2014). From this it is apparent that in comparison to the wild samples (41.29%), the amount of palmitic acid in the cultivated samples (17.6%) evaluated presently is much lower than half of the amount in the wild samples. Ravikrishnan et al. (2015) reported 20% palmitic acid in naturally growing *L. polychrous* Lév. While working with wild *L. squarrosulus*, slightly higher amount of palmitic acid (18.89 mg/100 g) was reported by Ghate and Sridhar (2019) and much less percentage (4.55%) of the same fatty acid was reported by Manjunathan et al. (2017) in *L. tuber-regium* cultivated in the laboratory. Roy et al. (2020) documented 10.74% palmitic acid in naturally growing *L. squarrosulus.* Palmitic acid is reported to possess significant atherogenic and thrombogenic potential (Tvrzicka et al., 2011).

Substantially higher percentage of monounsaturated fatty acid has been determined by Kavishree et al. (2008) in *Lentinus sajor-caju* (25.1%), *Pleurotus djamor* (29.4%) and *P. sajor-caju* (19.1%) in comparison to the presently investigated sample of *L. sajor-caju*; however, Obodai et al. (2014) reported almost comparable amount of MUFA in *L. squarrosulus* (6.8%). Total monounsaturated fatty acid content in the presently investigated mushroom is on the lower (5.42%) side in comparison to the proportion of monounsaturated fatty acid revealed in *L. squarrosulus* SQW (8.75%), *L. squarrosulus* LSF (9.36%), *Pleurotus tuber-regium* (24.07%), *P. ostreatus* EM-I (18.92%) and *P. sajor-caju* (24.21%) by Obodai et al. (2014). Sharma and Atri (2014) also detected high amount of total monounsaturated fatty acid content in the wild samples of *L. sajor-caju* (16.27%), *L. connatus* (32.72%), *L. torulosus* (17.93%), *L. cladopus* (67.35%) and *L. squarrosulus* (27.1%) in comparison to the presently evaluated sample of *L. sajor-caju* cultivated in the laboratory.

Oleic acid (3.95%) was found to be the most abundant monounsaturated fatty acid in the presently investigated mushroom specimens; it is reported to have therapeutic importance in decreasing the concentration of triacylglycerols (TAG), LDL-cholesterol and increasing the concentration of HDL-cholesterol and regulating

insulin sensitivity. Oleic acid was also found to influence the anti-inflammatory response and reported to possess a protective role in carcinogenesis (Tvrzicka et al., 2011). However, its total proportion in the presently evaluated sample is on the lower side in comparison to its amount documented in *Lentinus sajor-caju* (23.5%), *L. squarrosulus* (5.8%), *Pleurotus djamor* (29.4%) and *P. sajor-caju* (19.1%) by Kavishree et al. (2008). Oleic acid was reported to occur in high amount in *L. squarrosulus* SQW (7.89%), *L. squarrosulus* LSF (8.67%), *Pleurotus tuber-regium* (21.21%), *P. ostreatus* EM-I (18.30%) and *P. sajor-caju* (22.62%) by Obodai et al. (2014). While working with different wild sporophores of *Lentinus* species, much higher percentage of oleic acid has been reported in *L. sajor-caju* (13.9%), *L. connatus* (23.38%), *L. torulosus* (13.56%), *L. cladopus* (47.87%) and *L. squarrosulus* (23.38%) by Sharma and Atri (2014). Ravikrishnan et al. (2015) also recorded high percentage (18.50%) of oleic acid in *L. polychrous*. Oleic acid was documented in slightly high amount in *L. tuber-regium* (5.98%) and *L. squarrosulus* (5.27%) by Manjunathan et al. (2017) and Adeoye-Isijola et al. (2018), respectively.

Nervonic acid is the second most abundant (0.94%) monounsaturated fatty acid presently recorded in *L. sajor-caju*. This is an important monounsaturated fatty acid reported to be involved in the biosynthesis of nerve cell myelin and preventing disorders such as adrenoleukodystrophy and multiple sclerosis in elderly people (Sargent et al., 1994; Nakalembe, Kabasa, 2013).

Lentinus sajor-caju has been evaluated to contain substantially high (65.06%) proportion of polyunsaturated fatty acid in comparison to the other edible species, including *L. sajor-caju* (54.9%), *L. squarrosulus* (62.9%), *Pleurotus djamor* (45.5%) and *P. sajor-caju* (53.8%) by Kavishree et al., (2008). In the presently investigated mushroom samples, the net amount of polyunsaturated fatty acid (65.06%) is much on the higher side in comparison to proportion of saturated fatty acid (27.69%) and monounsaturated fatty acid (5.42%) evaluated. Similar trend has been documented by Obodai et al. (2014) for *Lentinus squarrosulus* SQW (63.62%), *L. squarrosulus* LSF (64.87%), *Pleurotus ostreatus* EM-I (59.21%), *P. sajor-caju* (49.17%) and *P. tuber-regium* (33.75%). Sharma and Atri (2014) reported very low proportion of polyunsaturated fatty acid in the wild samples of *Lentinus sajor-caju* (1.31%), *L. connatus* (0.70%), *L. torulosus* (1.3%), *L. cladopus* (0.76%) and *L. squarrosulus* (1.45%) in comparison to the presently investigated mushroom cultivated under laboratory conditions. From the therapeutic point of view, n-3 and n-6 polyunsaturated fatty acid, mainly linoleic acid, linolenic acid, cis-5,8,11,14,17-eicosapentaeoic acid and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA), are known to modify membrane fluidity and thickness, regulate enzymes involved in lipid metabolism, exert anti-inflammatory effects on asthma, inflammatory bowel disease, rheumatoid arthritis, etc. (FAO, 2010). One of the polyunsaturated fatty acid, cis-4,7,10,13,16,19-docosahexaenoic acid (DHA), has been reported to prevent cardiovascular disease and play critical role in neurogenesis, neurite growth, neuronal integrity, gene expression in the brain, synapse formation and function, glucose transport, cognitive development and learning ability (Norris et al., 2013).

The proportion of linoleic acid observed during the present study in *L. sajor-caju* is much on the higher side (60.62%) in comparison to its amount reported in *L. squarrosulus* (37.29%) by Ghate and Sridhar (2019) and in *L. sajor-caju* (54.9%), *Pleurotus djamor* (45.5%) and *P. sajor-caju* (53.8%) by Kavishree et al. (2008). Obodai et al. (2014), while investigating some of the edible species of *Pleurotus* and *Lentinus*, detected high amount of linoleic acid in *L. squarrosulus* SQW (62.41%), *L. squarrosulus* LSF (63.64%), which is almost comparable to the presently investigated mushroom. In comparison, Ravikrishnan et al. (2015), Manjunathan et al. (2017), Adeoye-Isijola et al. (2018) and Roy et al. (2020) reported much less amount of linoleic acid in *Lentinus polychrous* (25.30%), *L. tuber-regium* (7.44%), *L. squarrosulus* SQW (37.29%), *L. squarrosulus* LSF (24.21%), respectively. Linoleic acid is known to act as a precursor of alcohol in fungi (1-octen-3-ol) and is one of the principal aromatic compounds in most fungi contributing to mushroom flavour (Maga, 1981). It is also reported as a potential cytotoxic agent against HeLa cell possessing antibacterial activity as well (Lee et al., 2002; Mei et al., 2006).

Conclusion

The results revealed that the cultivated sporophores of *Lentinus sajor-caju* are quite rich in essential fatty acids and unsaturated fatty acids, with linoleic acid, oleic acid and palmitic acid being present in substantial amounts. It also possesses higher amounts of unsaturated fatty acids in comparison to saturated fatty acids, which is quite significant from a nutritional standpoint. Therefore, it can form an important constituent of the human diet.

Acknowledgement

The authors are grateful to the Head Department of Botany, Punjabi University, Patiala, Punjab, India for providing laboratory facilities.

The work was funded by the University Grants Commission with a grant under the DRS Phase – III (SAP) Program. The senior author is also indebted to the University Grants Commission for the award of fellowship under the UGC-BSR scheme.

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Recommended for publication by M.M. Sukhomlyn

Лата, Атрі Н.С. **Профіль жирних кислот автохтонного штаму *Lentinus sajor-caju* (*Basidiomycota*).** *Український ботанічний журнал*, 78(5): 327–334. https://doi.org/10.15407/ukrbotj78.05.327

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Реферат. Метою цього дослідження було встановити склад жирних кислот їстівного гриба *Lentinus sajor-caju*, який широко споживається у світі, на прикладі вирощеного в культурі автохтонного штаму природного походження. Нами встановлено наявність 26 жирних кислот, включаючи насичені жирні кислоти (SFA-27,69%), мононенасичені (MUFA-5,42%) і поліненасичені жирні кислоти (PUFA-65,06%) у різній кількості в межах від 0,01% до 60,62%. Виявлено, що серед усіх досліджених жирних кислот переважала лінолева кислота (60,62%), а пальмітинова (17,6%) та олеїнова (3,95%) кислоти були відповідно другою і третьою за їхнім вмістом у цього гриба.

Ключові слова. *Lentinus sajor-caju*, газова хроматографія, мононенасичені жирні кислоти, насичені жирні кислоти, плодові тіла, поліненасичені жирні кислоти, полум'яно-іонізаційний детектор