

Brief Report

Arachidonic Acid Production by *Mortierella alpina* on Brewery Spent Malt Enriched with Vegetable Oils Using Solid-State Fermentation

Silvia Stredanská ^{1,*} , Janka Kubincová ² , Mária Kopuncová ², Eugen Kiss ¹, Stanislav Baxa ¹ 
and Miroslav Stredanský ³

¹ Division of Food Technologies and Innovation, Department of Food Science, Science, Research and Innovation Section, National Agricultural and Food Centre, Kostolná 5, 900 01 Modra, Slovakia; stanislav.baxa@nppc.sk (S.B.)

² Division of Food Chemistry and Analysis, Department of Food Science, Science, Research and Innovation Section, National Agricultural and Food Centre, Priemyselná 4, 821 08 Bratislava, Slovakia; janka.kubincova@nppc.sk (J.K.); maria.kopuncova@nppc.sk (M.K.)

³ Biorealis, s.r.o., Komenského 13, 900 01 Modra, Slovakia; miroslav.stredansky@biorealis.sk

* Correspondence: silvia.stredanska@nppc.sk

Abstract

Arachidonic acid (AA) is a high-value long-chain polyunsaturated fatty acid commonly produced by submerged fermentation of oleaginous fungi. Solid-state fermentation (SSF) using low-cost agro-industrial by-products represents a promising alternative approach for sustainable microbial lipid production. In this study, spent malt, a by-product of the brewing industry, was evaluated as a solid substrate for AA production by *Mortierella alpina* under SSF conditions. Four *M. alpina* strains were screened for growth, lipid accumulation, and AA production, and *M. alpina* 959 was selected as the most promising strain. The effects of nitrogen supplementation and oil incorporation on biomass formation, lipid accumulation, and AA productivity were subsequently investigated. Sunflower oil incorporation into spent malt-based substrates improved AA productivity under the most favorable conditions, resulting in an AA proportion of 38.6% of total fatty acids and 102.5 mg AA g^{−1} of final dry mass of the SSF system (FDW-SC). Comparison of selected vegetable oils further demonstrated that the lipid source affected AA production, while having only a limited effect on fungal growth and total lipid accumulation. Overall, this study demonstrates the feasibility of spent malt-based SSF for AA production by *M. alpina* and highlights the valorization of brewery spent malt as a low-cost agro-industrial by-product for the production of value-added microbial lipids.

Keywords: arachidonic acid; *Mortierella alpina*; solid-state fermentation; spent malt; agro-industrial by-products; microbial lipid; single cell oil



Academic Editor: Massimiliano Fabbricino

Received: 13 July 2026

Revised: 7 August 2026

Accepted: 20 August 2026

Published: 21 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Polyunsaturated fatty acids (PUFA) have attracted considerable attention due to their nutritional and physiological importance. Among them, arachidonic acid (AA; 20:4 n−6) is a valuable long-chain PUFA with essential roles in human development and as a precursor of bioactive lipid mediators [1–3]. Its importance in infant growth and neural development has led to its routine incorporation into infant formula, making AA a commercially important product of microbial fermentation [4].

Historically, AA was obtained from animal-derived sources, but the limited AA proportion of those materials restricted their suitability for industrial production. Consequently, microbial production has become an established approach for AA manufacture [5]. Currently, commercial AA is produced mainly by submerged fermentation of the oleaginous filamentous fungus *Mortierella alpina* cultivated on glucose-based media, followed by extraction of the intracellular oil from the harvested biomass [1,6]. However, the high cost of cultivation media remains a major limitation, highlighting the need for inexpensive substrates and alternative cultivation strategies. Compared with submerged fermentation, solid-state fermentation (SSF) offers advantages such as lower energy demand and reduced wastewater generation [7].

In SSF, microorganisms grow on moist solid materials containing little or no free water, conditions that resemble the natural ecological niche of filamentous fungi and may promote enhanced metabolite production [8]. This cultivation strategy also enables the utilization of inexpensive agricultural residues and food-processing by-products, contributing to more sustainable bioprocesses.

Building on our previous study demonstrating AA production from cereal substrates under SSF conditions [9], several agro-industrial residues and nutrient-enriched solid matrices have been explored as substrates for microbial lipid production under SSF conditions, including materials supporting AA and other PUFA biosynthesis [10–22]. Recent studies have further investigated alternative SSF approaches for PUFA production by *M. alpina* [8,23]. Beyond substrate selection, vegetable oil supplementation has been shown to influence fatty acid (FA) profiles in *M. alpina*, affecting the production of valuable PUFA such as dihomogamma-linolenic acid and eicosapentaenoic acid [24,25]. These studies highlight the importance of both substrate selection and lipid availability in shaping PUFA production under SSF conditions.

One of the major limitations of SSF is the compaction of moist substrates, which can reduce bed porosity, restrict oxygen transfer, and negatively affect fungal growth and product formation. This challenge can be addressed by using suitable solid matrices that improve substrate structure and support fungal development. Spent malt, one of the major by-products of the brewing industry, represents a promising low-cost fermentation matrix due to their structural properties and nutritional composition, supporting their use in SSF processes [16,18,20,21,26]. Moreover, their nutritional composition and ability to incorporate added nutrients and lipid sources provide opportunities for modulating microbial lipid production during SSF.

In the present study, spent malt was evaluated as a solid matrix for AA production by *M. alpina* under SSF conditions. The effects of nutritional conditions and oil-enriched substrate formulations on fungal growth, lipid accumulation, and AA productivity were investigated to identify factors influencing AA production within the developed SSF system.

Although previous studies have demonstrated the potential of agro-industrial residues as substrates for AA production by *M. alpina* under SSF conditions, most approaches have focused on substrate selection, nutrient optimization, or process parameters. The present study introduces an oil-enriched SSF strategy using spent malt as a brewery by-product and evaluates the potential of external lipid sources to enhance AA-enriched lipid production. The incorporation of vegetable oils was investigated as a strategy to provide additional lipid precursors and improve the efficiency of AA production by combining substrate valorization with targeted lipid supplementation.

2. Materials and Methods

2.1. Fungal Strains

Four *Mortierella alpina* strains (MSCL 1254, MSCL 1271, MSCL 959, and CCF 2861) were used in this study. The strain CCF 2861 was obtained from the Culture Collection of Fungi, Charles University (CCF; Prague, Czech Republic), whereas strains MSCL 1254, MSCL 1271, and MSCL 959 were obtained from the Microbial Strain Collection of Latvia, University of Latvia (MSCL; Riga, Latvia).

2.2. Culture Storage and Pre-Cultivation

All *M. alpina* strains were maintained on malt extract agar (MEA) at 4 °C and pre-cultivated on MEA plates at 25 °C until active growth was achieved.

2.3. Inoculum Preparation

Due to variable sporulation capacity among strains, inocula were prepared as mycelial suspensions from actively growing agar cultures under identical conditions.

2.4. General Solid-State Fermentation (SSF) Conditions

The spent malt used as the solid substrate was obtained from a local brewery. The material had a particle size of approximately 1–5 mm, was dried at 60 °C until constant weight, and stored in sealed containers under dry conditions until use. The prepared substrate had a dry matter content of approximately 97%. Prior to fermentation, the lipid content of the spent malt substrate was determined and represented approximately 5% of the dry substrate mass.

For SSF cultivation, 4 g of spent malt substrate was placed into 100 mL Erlenmeyer flasks, supplemented with 10 mL of nutrient solution, and enriched with oils according to the experimental design. Oil supplementation levels expressed as g flask^{−1} corresponded to fixed supplementation ratios relative to the initial dry substrate mass (S_0), as all SSF experiments used the same substrate loading. The prepared SSF systems were sterilized by autoclaving at 121 °C for 20 min prior to inoculation. The initial moisture content of the prepared SSF systems before inoculation was approximately 60–70%, depending on the nutrient solution composition and oil supplementation. Each flask was inoculated with 1 mL of mycelial suspension. SSF was performed under static aerobic conditions at 25 °C for 14 days.

2.5. Experimental Design

The experimental design included strain screening and evaluation of nitrogen conditions and oil-enriched substrates. A complete nutrient formulation was used for strain screening, whereas individual nutrient components were modified in subsequent experiments as described below.

2.5.1. Screening of Strains

The strains were cultivated under the general SSF conditions described above, using two different oil-enriched substrate formulations. Medium 1 contained 1 g of olive pomace oil and 1 g of broccoli oil, whereas Medium 2 contained 2 g of olive pomace oil. Olive pomace oil was selected as an oil source due to its origin as a by-product of the olive oil industry and its reported use as an alternative lipid source in microbial lipid production. The complete nutrient solution contained (g L^{−1}): glucose 5.0, yeast extract 10.0, peptone 10.0, MgSO₄·7H₂O 2.0, and K₂HPO₄ 2.0. The reported concentrations represent the final concentrations in the nutrient solution added at 10 mL per flask.

2.5.2. Evaluation of Organic Nitrogen Supplementation and Cultivation Conditions

Based on the results of the strain screening, *M. alpina* MSCL 959 was selected for subsequent experiments. The influence of different organic nitrogen sources on biomass, lipid accumulation, and FA composition was evaluated under the general SSF conditions described above. Yeast autolysate, a by-product derived from brewery yeast processing, was included among the evaluated organic nitrogen sources and was supplied as a suspension containing 108 g L⁻¹ dry matter. The basal nutrient solution described in Section 2.5.1 was used, and the experimental design is summarized in Tables 1 and 2.

Table 1. Composition of organic nitrogen sources in the nutrient solution (g L⁻¹).

Nitrogen Sources	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6	Medium 7	Medium 8
Yeast extract	10	10	20	20	10	-	-	-
Peptone	10	10	20	20	-	10	-	-
L-lysine	-	-	-	-	8	-	-	-
Yeast autolysate	-	-	-	-	-	12.5	30.0	50.0

Table 2. Experimental design matrix showing olive pomace oil incorporation levels medium (g flask⁻¹).

	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6	Medium 7	Medium 8
Olive pomace oil	2	3	2	3	2	2	2	3

2.5.3. Sunflower Oil-Enriched Substrate Formulation

The effect of sunflower oil incorporation (1.0–3.5 g flask⁻¹) into the SSF substrate on biomass, lipid accumulation, and AA production was evaluated using the basal nutrient solution described in Section 2.5.1 (Medium 1 in Table 1) under SSF conditions. This condition was selected as a reference formulation to evaluate the specific effect of sunflower oil supplementation while maintaining a constant nutrient background. All other cultivation parameters (substrate, temperature, and incubation time) were kept constant as described in Section 2.4.

2.5.4. Oil-Enriched Substrates with Different Vegetable Oils

The effect of different vegetable oils (sesame, flaxseed, and broccoli oil) on biomass formation, lipid accumulation and AA production was evaluated under SSF conditions. Each oil-enriched substrate contained 2.5 g of oil per flask. Due to the high erucic acid content of broccoli oil, it was applied as a 1:1 (*w/w*) mixture with sunflower oil, corresponding to a total oil amount of 2.5 g per flask prior to application in the cultivation experiments. All other cultivation parameters were kept constant as described in Section 2.5.3. Previously obtained data for sunflower oil under identical cultivation conditions were included as reference treatments.

2.6. Final Dry Mass Determination of the SSF System (FDW-SC)

At the end of cultivation, the entire content of the Erlenmeyer flask (substrate with fungal biomass) was dried at 70 °C for 13 h and subsequently weighed. The obtained value was expressed as the final dry mass of the SSF system (FDW-SC, g), including both the

residual substrate and fungal biomass. S_0 denotes the initial dry mass of the solid substrate loaded into each flask prior to inoculation (g).

2.7. Lipid Extraction and Determination of Lipid Content

Total lipids were extracted from FDW-SC using the Folch method [27]. Lipid content was determined from the entire SSF system, with uninoculated substrate controls used to estimate background lipid contribution from the substrate. Lipids were expressed relative to both FDW-SC and S_0 , representing lipid content of the fermented solid system and substrate-based lipid productivity, respectively.

2.8. Fatty Acid Methyl Ester (FAME) and GC-FID Analysis

FA composition was determined as fatty acid methyl esters (FAMES). Total lipids extracted by the Folch method [27] were transmethylated according to Ichihara and Fukubayashi [28] using methanolic HCl. FAMES were analyzed by GC-FID using a Shimadzu GC-2010 Plus system (Shimadzu, Kyoto, Japan) equipped with an AOC-20i autosampler and a DB-23 capillary column (60 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness; Agilent Technologies, Santa Clara, CA, USA). The injector and detector temperatures were set at 250 °C. Samples (1 μ L) were injected in split mode (100:1). The oven temperature program was as follows: initial temperature 130 °C (1 min), increased at 15 °C min⁻¹ to 170 °C, then at 2 °C min⁻¹ to 235 °C (7.5 min); total run time was 43.2 min. Helium was used as the carrier gas. FA were identified by comparison with the Supelco 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO, USA) and expressed as percentages of total fatty acids (TFA). For each cultivation condition, lipid extracts from three biological replicates were pooled prior to FAME preparation and GC-FID analysis. Consequently, FA composition was determined as a single representative value for each cultivation condition and was not subjected to statistical analysis. AA production parameters were expressed relative to both the final dry mass of the SSF system (FDW-SC) and the initial dry substrate mass (S_0). Calculations were based on the assumption that total fatty acids represented 75% of total lipid mass, which falls within the range commonly reported for oleaginous fungi (70–90%).

2.9. Data Processing and Statistical Analysis

All cultivations were performed in three biological replicates, and results are presented as mean $\pm$ standard deviation (SD). Basic data processing and graphical representation were performed using Microsoft Excel (Microsoft Office, Redmond, WA, USA). Statistical significance among cultivation conditions was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's HSD test ($p < 0.05$).

3. Results and Discussion

3.1. Strain Screening Under SSF Conditions

The initial screening evaluated four *Mortierella alpina* strains for fungal growth, lipid accumulation, and AA production under SSF conditions. One of the screening regimes included broccoli oil as an alternative lipid source to evaluate whether the incorporation of an oil source with a different FA composition could influence AA production among the tested strains. All tested strains showed comparable FDW-SC, ranging from 5.12 to 5.46 g flask⁻¹ (Table 3).

Lipid accumulation and AA production differed among the tested strains. *M. alpina* MSCL 959 showed the highest lipid accumulation (1.68–1.72 g flask⁻¹; 30.89–31.69% FDW-SC) and the highest observed AA proportion, reaching 38.0–40.1% TFA (Table 3). This strain also exhibited the highest observed values of AA production relative to FDW-SC (90.3–92.7 mg AA g⁻¹ FDW-SC) and substrate-based AA yield relative to the initial sub-

strate mass (78.0–80.5 mg AA g⁻¹ S₀) (Figure S1). Because FDW-SC represents the entire fermented solid system, including residual spent malt and fungal biomass, this parameter should be interpreted as AA production per unit of fermented solid material rather than as a direct biomass-specific productivity measure.

Table 3. Screening of *Mortierella alpina* strains for fungal growth, lipid accumulation, and AA production under SSF conditions.

STRAINS	Medium	FDW-SC (g flask ⁻¹)	Lipid Accumulation (g flask ⁻¹)	Lipid Content (% FDW-SC)	AA (% TFA)	AA/FDW-SC (mg AA g ⁻¹ FDW-SC)	AA/S ₀ (mg AA g ⁻¹ S ₀)
<i>M. alpina</i> MSCL 1254	Medium 1	5.24 ± 0.14	1.56 ± 0.02	29.71 ± 1.22	28.7	63.9	53.3
	Medium 2	5.29 ± 0.14	1.50 ± 0.04	28.35 ± 1.41	30.2	64.2	53.9
<i>M. alpina</i> MSCL 1271	Medium 1	5.26 ± 0.04	1.41 ± 0.03	26.72 ± 0.47	28.7	57.5	48.1
	Medium 2	5.30 ± 0.11	1.45 ± 0.01	27.37 ± 0.58	33.0	67.6	57.0
<i>M. alpina</i> MSCL 959	Medium 1	5.43 ± 0.07	1.72 ± 0.02	31.69 ± 0.17	38.0	90.3	78.0
	Medium 2	5.46 ± 0.12	1.68 ± 0.01	30.89 ± 0.90	40.1	92.7	80.5
<i>M. alpina</i> CCF 2861	Medium 1	5.12 ± 0.08	1.49 ± 0.03	29.19 ± 0.62	26.1	57.1	46.5
	Medium 2	5.38 ± 0.06	1.61 ± 0.08	29.95 ± 1.80	35.5	79.8	68.3

Medium 1: Olive pomace oil (1 g) + broccoli oil (1 g) per flask. Medium 2: Olive pomace oil (2 g) per flask. Values for FDW-SC, lipid accumulation, and lipid content are presented as mean ± standard deviation (SD) of independent experiments (*n* = 3). Fatty acid composition was determined from pooled lipid extracts of three biological replicates and is presented as a single representative value for each cultivation condition. FDW-SC represents the final dry mass of the solid-state fermentation system, including residual spent malt substrate and fungal biomass. S₀ denotes the initial dry substrate mass prior to cultivation. AA represents arachidonic acid proportion expressed as a percentage of total fatty acids (% TFA). Lipid content was calculated relative to FDW-SC.

The observed strain-dependent variation in AA production highlights the importance of strain selection as a first step in developing SSF-based lipid production processes. Similar strain-dependent differences in AA production and accumulation have been reported previously for *M. alpina*, indicating that strain selection is an essential step for optimizing fungal lipid production [9,13]. Although all tested *M. alpina* strains were able to grow under the applied SSF conditions, their different capacities for lipid accumulation and AA production indicate distinct metabolic potential among strains. The combined evaluation of AA proportion and productivity-related parameters identified *M. alpina* MSCL 959 as the most promising strain for further optimization of the oil-enriched SSF system.

3.2. Effect of Organic Nitrogen Supplementation Conditions on AA Production

Different nitrogen supplementation regimes combined with varying olive pomace oil levels affected fungal growth and lipid accumulation (Table 4). Media M2, M4, and M8 produced significantly higher FDW-SC and lipid accumulation than the remaining media according to Tukey's HSD test (Table S1). The highest lipid content was observed in M2 and M4, whereas M8 showed intermediate values. FDW-SC reached 6.21–6.40 g flask⁻¹ and lipid accumulation reached 2.31–2.46 g flask⁻¹.

The highest observed AA proportions were observed in M1 and M3 (40.7 and 41.6% TFA, respectively; Table 4). However, media promoting higher biomass and lipid formation showed comparable AA production relative to FDW-SC, ranging from 82.4 to 92.6 mg AA g⁻¹ (Figure S2). These results indicate that the tested combinations of nitrogen supplementation and oil incorporation mainly influenced fungal growth and lipid accumulation, while AA enrichment remained relatively stable under the evaluated conditions.

Table 4. Effect of different organic nitrogen sources on fungal growth, lipid accumulation, and arachidonic acid (AA) production by *Mortierella alpina* MSCL 959 under solid-state fermentation (SSF).

Medium No.	FDW-SC (g flask ⁻¹)	Lipid Accumulation (g flask ⁻¹)	Lipid Content (% FDW-SC)	AA (% TFA)	AA/FDW-SC (mg AA g ⁻¹ FDW-SC)	AA/So (mg AA g ⁻¹ So)
M1	5.31 ± 0.20	1.56 ± 0.05	29.42 ± 0.37	40.7	89.8	75.9
M2	6.25 ± 0.28	2.46 ± 0.09	39.39 ± 0.73	27.9	82.4	70.7
M3	5.29 ± 0.25	1.53 ± 0.12	28.95 ± 3.60	41.6	90.5	73.7
M4	6.21 ± 0.20	2.33 ± 0.13	37.55 ± 1.92	32.8	92.6	76.7
M5	5.18 ± 0.22	1.51 ± 0.09	29.21 ± 2.00	36.9	80.8	66.8
M6	5.17 ± 0.20	1.57 ± 0.03	30.28 ± 1.10	36.1	82.0	67.1
M7	5.30 ± 0.13	1.65 ± 0.03	31.20 ± 1.21	33.3	77.9	64.6
M8	6.40 ± 0.22	2.31 ± 0.21	36.10 ± 2.01	28.5	77.2	65.1

Values for FDW-SC, lipid accumulation, and lipid content are presented as mean ± SD ($n = 3$). FA composition and AA-derived parameters are reported as representative values for each cultivation condition. FDW-SC represents the final dry mass of the solid-state fermentation system, including residual spent malt substrate and fungal biomass. S_0 denotes the initial dry substrate mass prior to cultivation. The composition of the cultivation media is described in Tables 1 and 2.

Yeast autolysate, a by-product derived from brewery yeast processing, showed comparable performance to conventional organic nitrogen sources such as yeast extract and peptone, supporting its potential as an alternative nitrogen source for SSF-based AA production. Similar effects of medium composition optimization on AA production by *M. alpina* under SSF conditions have been reported previously, where cultivation parameters and nutrient composition significantly influenced ARA yield [10].

3.3. Effect of Sunflower Oil-Enriched Substrate Formulation on AA Production

Incorporation of sunflower oil into the SSF substrate significantly increased fungal growth, lipid accumulation, and lipid content (Table 5). FDW-SC increased progressively from 4.35 to 6.67 g flask⁻¹, while lipid accumulation increased from 0.81 to 2.88 g flask⁻¹ and lipid content from 18.55 to 43.14% FDW-SC. Tukey's HSD test confirmed a gradual increase in these parameters, with the highest values obtained at 3.5 g sunflower oil per flask (Table S2).

In contrast, AA proportion did not follow the same trend. The highest value (43.0% TFA) was observed at 1.5 g sunflower oil, after which it gradually decreased to 23.1% TFA at the highest incorporation level (Table 5).

AA production relative to FDW-SC and substrate-based AA yield showed their highest observed values at 2.5 g sunflower oil (102.5 mg AA g⁻¹ FDW-SC and 87.1 mg AA g⁻¹ S_0 , respectively) before declining at higher incorporation levels (Figure S3). Overall, incorporation of 2.5 g sunflower oil per flask provided the most favorable balance between fungal growth, lipid accumulation, and AA productivity.

Previous SSF studies have shown that lipid supplementation can influence AA production by *M. alpina*. Rayaroth et al. [12] reported an AA yield of 41.29 mg g⁻¹ dry substrate after optimization of a soybean meal-based SSF system supplemented with flaxseed oil. Similarly, Slaný et al. [14] obtained a maximum AA yield of 32.1 mg g⁻¹ fermented bio-product using *M. alpina* CCF 2861 cultivated on cereal-based substrates enriched with an animal fat by-product. Compared with these previous SSF studies, the cultivation strategy applied in the present work resulted in higher AA productivity, highlighting the potential of spent malt-based oil-enriched substrates for AA production. These findings indicate that external lipid sources can modify PUFA production under SSF conditions, but their effects depend on the lipid source and its interaction with the cultivation system.

Table 5. Effect of sunflower oil incorporation (g flask^{-1}) on biomass formation, lipid accumulation, and AA production in *Mortierella alpina* MSCL 959 cultivated under solid-state fermentation.

Medium No.	Sunflower Oil Supplementation (g flask^{-1})	FDW-SC (g flask^{-1})	Lipid Accumulation (g flask^{-1})	Lipid Content (% FDW-SC)	AA (% TFA)	AA/FDW-SC (mg AA g^{-1} FDW-SC)	AA/So (mg AA g^{-1} So)
M1	1.0	4.35 ± 0.18	0.81 ± 0.02	18.55 ± 0.65	29.9	41.6	34.2
M2	1.5	4.80 ± 0.28	0.93 ± 0.05	19.25 ± 0.68	43.0	63.5	52.7
M3	2.0	5.40 ± 0.16	1.59 ± 0.03	28.78 ± 0.99	41.0	88.6	74.5
M4	2.5	5.77 ± 0.19	2.04 ± 0.19	35.41 ± 1.25	38.6	102.5	87.1
M5	3.0	6.09 ± 0.27	2.41 ± 0.11	39.55 ± 0.90	31.2	92.6	77.3
M6	3.5	6.67 ± 0.17	2.88 ± 0.15	43.14 ± 0.95	23.1	74.7	64.0

FDW-SC represents the final dry mass of the solid-state fermentation system, including residual spent malt substrate and fungal biomass.

The lower AA proportion observed at higher sunflower oil incorporation levels may result from an increased contribution of the added oil to the total lipid pool, reducing the relative AA proportion despite increased lipid accumulation. This dilution effect suggests that, although higher oil availability promoted overall lipid accumulation, it did not proportionally increase fungal AA enrichment. In addition, external lipid sources may influence lipid metabolism and fatty acid distribution by affecting the availability and incorporation of exogenous fatty acids into fungal lipids. Previous studies have shown that supplementation with different lipid sources can modify fatty acid profiles in *M. alpina* depending on the oil composition and cultivation conditions [24,25].

3.4. Effect of Vegetable Oil-Enriched Substrate Formulation on AA Production

Different vegetable oils were compared at a constant incorporation level of 2.5 g flask^{-1} to evaluate the influence of lipid source composition on AA production under SSF conditions (Table 6). All tested oils showed comparable effects on fungal growth and lipid accumulation, with FDW-SC values ranging from 5.69 to $5.82 \text{ g flask}^{-1}$ and lipid content from 34.14 to 36.51% FDW-SC.

In contrast, AA-related parameters differed among oil supplements. Sunflower oil resulted in the highest observed AA proportion (38.6% TFA), AA production relative to FDW-SC ($102.5 \text{ mg AA g}^{-1}$ FDW-SC), and substrate-based AA yield ($87.1 \text{ mg AA g}^{-1} \text{ So}$), whereas the other tested oils showed lower AA productivity. These results indicate that the performance of the oil-enriched SSF substrate depends not only on the amount of incorporated oil but also on the FA composition of the oil source. Similar effects of exogenous oil supplementation on FA profiles in *M. alpina* have been reported, demonstrating that different vegetable oils can influence PUFA composition [24,25]. Overall, the results suggest that the FA profile of the incorporated oil source may influence AA production in *M. alpina* under SSF conditions. The tested oils differed in their major fatty acid composition, with sunflower and sesame oils containing predominantly oleic acid ($18:1 \text{ n-9}$) and linoleic acid ($18:2 \text{ n-6}$), whereas flaxseed oil was characterized by a higher proportion of α -linolenic acid ($18:3 \text{ n-3}$). These differences may affect fatty acid availability and lipid metabolism in *M. alpina*, contributing to the observed variation in AA production.

Previous studies have demonstrated the feasibility of AA production by *Mortierella* spp. under SSF conditions using various agricultural and agro-industrial substrates.

Early work by Stredanská et al. [9] demonstrated AA production by *M. alpina* on solid substrates, while subsequent studies investigated solid-state column reactors [7], spent mushroom substrate [11], date waste [15], oil cakes [10], soybean meal-based substrates [12], animal fat by-products [14], and other alternative solid-state systems for PUFA production by *M. alpina* [23]. The present results further demonstrate that spent malt represents a suitable brewery by-product for SSF-based AA production by *M. alpina*.

Table 6. Influence of vegetable oil type on fungal growth, lipid accumulation, and fatty acid-related parameters of *Mortierella alpina* MSCL 959 under SSF conditions.

Medium No.	Oil Incorporation (2.5 g flask ⁻¹)	FDW-SC (g flask ⁻¹)	Lipid Accumulation (g flask ⁻¹)	Lipid Content (% FDW-SC)	AA (% TFA)	AA/FDW-SC (mg AA g ⁻¹ FDW-SC)	AA/So (mg AA g ⁻¹ So)
M1	sunflower oil (1.25 g) + broccoli oil (1.25 g)	5.71 ± 0.17	2.08 ± 0.16	36.51 ± 1.94	17.2	47.1	39.6
M2	sesame oil	5.82 ± 0.18	1.99 ± 0.22	34.14 ± 2.73	15.8	40.5	34.7
M3	flaxseed oil	5.69 ± 0.23	2.06 ± 0.16	36.14 ± 2.21	20.7	56.1	47.0
M4–Stage 3 reference	sunflower oil	5.77 ± 0.19	2.04 ± 0.19	35.41 ± 1.25	38.6	102.5	87.1

Data for sunflower oil incorporation were obtained under the optimized condition in Stage 3 and included as a reference condition. FDW-SC represents the final dry mass of the solid-state fermentation system, including residual spent malt substrate and fungal biomass.

4. Conclusions

Among the tested strains, *Mortierella alpina* MSCL 959 was identified as the most suitable strain for AA production under spent malt-based SSF. Nitrogen nutrition and oil incorporation significantly affected fungal growth and lipid accumulation and influenced the observed AA productivity parameters. The FA composition of the incorporated oil further influenced AA production without markedly affecting fungal growth or total lipid accumulation. Overall, these findings demonstrate that brewery spent malt can serve not only as a low-cost SSF substrate but also as an effective platform for producing AA-rich microbial lipids, supporting the sustainable valorization of brewing by-products. The flexibility of FA profiles observed under SSF conditions further suggests that this cultivation system may also be suitable for producing other valuable PUFA.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation12080394/s1>, Figure S1. FDW-SC-based AA production and substrate-based AA yield of *Mortierella alpina* strains (MSCL 1254, MSCL 1271, MSCL 959, and CCF 2861) cultivated under solid-state fermentation (SSF) with two oil incorporation regimes: olive pomace oil (2 g per flask) and olive pomace oil + broccoli oil (1 g + 1 g per flask). (A) AA production relative to final dry mass of the SSF system (mg AA g⁻¹ FDW-SC). (B) Substrate-based AA yield (mg AA g⁻¹ S₀). Figure S2. FDW-SC-based AA production (A) and substrate-based AA yield (B) of *Mortierella alpina* MSCL 959 cultivated under solid-state fermentation (SSF) with different organic nitrogen sources. AA production was expressed relative to the final dry mass of the SSF system (mg AA g⁻¹ FDW-SC) and relative to the initial substrate mass (mg AA g⁻¹ S₀). Figure S3. FDW-SC-based AA production (A) and substrate-based AA yield (B) of *Mortierella alpina* MSCL 959 cultivated under solid-state fermentation (SSF) with different sunflower oil incorporation levels. (A) AA production relative to final dry mass of the SSF system (mg AA g⁻¹ FDW-SC) and (B) substrate-based AA yield (mg AA g⁻¹ S₀). Table S1. Tukey's HSD grouping of cultivation parameters under different nitrogen supplementation conditions in *Mortierella alpina* MSCL 959 cultivated under solid-state fermentation (SSF) conditions. Table S2. Tukey's HSD grouping of cultivation parameters under different sunflower oil incorporation levels in *Mortierella alpina* MSCL 959 cultivated under solid-state fermentation (SSF) conditions.

Author Contributions: Conceptualization, S.S.; methodology, E.K., J.K., M.S. and M.K.; project administration, S.B.; resources, S.B.; validation, E.K., J.K., M.S. and M.K.; formal analysis, E.K., J.K., M.S. and M.K.; investigation, S.S.; writing—original draft preparation, S.S.; writing—review and editing, S.S.; All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union NextGenerationEU through the Recovery and Resilience Plan of the Slovak Republic, project No. 09I03-03-V04-00548. The APC was funded by project No. 09I03-03-V04-00548.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting the findings of this study are available within the article and its Supplementary Materials. Further inquiries can be addressed to the corresponding author.

Acknowledgments: During the preparation of this manuscript, the authors used the free version of ChatGPT (OpenAI)-5.6 Luna, OpenAI (provided by OpenAI, San Francisco, California, U.S.; available at <https://openai.com/blog/chatgpt>) (accessed on 10 July 2026) to partially aid with language editing, text refinement, and improvement of scientific writing. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflicts of Interest: Author Miroslav Stredanský was employed by Biorealis, s.r.o. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

AA	arachidonic acid
FA	fatty acids
FAME	fatty acid methyl esters
FDW-SC	final dry mass of the solid-state fermentation system
PUFA	polyunsaturated fatty acids
SD	standard deviation
S _O	initial dry substrate mass
SSF	solid-state fermentation
TFA	total fatty acids

References

1. Mamani, L.C.D.; Magalhães, A.I., Jr.; Ruan, Z.; de Carvalho, J.C.; Soccol, C.R. Industrial production, patent landscape, and market trends of arachidonic acid-rich oil of *Mortierella alpina*. *Biotechnol. Res. Innov.* **2019**, *3*, 103–119. [\[CrossRef\]](#)
2. Tallima, H.; El Ridi, R. Arachidonic acid: Physiological roles and potential health benefits—A review. *J. Adv. Res.* **2018**, *11*, 33–41. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Hanna, V.S.; Hafez, E.A.A. Synopsis of arachidonic acid metabolism: A review. *J. Adv. Res.* **2018**, *11*, 23–32. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Zhang, H.; Cui, Q.; Song, X. Research advances on arachidonic acid production by fermentation and genetic modification of *Mortierella alpina*. *World J. Microbiol. Biotechnol.* **2021**, *37*, 4. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Ratledge, C. Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie* **2004**, *86*, 807–815. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Ren, Z.; Armenta, R.E.; Brooks, M.S.-L. Arachidonic acid production by *Mortierella alpina* MA2-2: Optimization of combined nitrogen sources in the culture medium using mixture design. *Bioresour. Technol. Rep.* **2025**, *29*, 102049. [\[CrossRef\]](#)
7. Jang, H.-D.; Yang, S.-S. Polyunsaturated fatty acids production with a solid-state column reactor. *Bioresour. Technol.* **2008**, *99*, 6181–6189. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Liu, T.; Li, P.; Ou, Z.; Feng, Y.; Wang, B.; Yu, T.; Zhu, Y.; Yu, L. Insights into the special physiology of *Mortierella alpina* cultured by agar supported solid state fermentation in enhancing arachidonic acid enriched lipid production. *Sci. Rep.* **2025**, *15*, 15967. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Stredanská, S.; Slugeň, D.; Stredanský, M.; Grego, J. Arachidonic acid production by *Mortierella alpina* grown on solid substrates. *World J. Microbiol. Biotechnol.* **1993**, *9*, 511–513. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Ghobadi, Z.; Hamidi-Esfahani, Z.; Azizi, M.H. Statistical optimization of arachidonic acid synthesis by *Mortierella alpina* CBS 754.68 in a solid-state fermenter. *Food Sci. Nutr.* **2022**, *10*, 436–444. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Antimanon, S.; Chamkhuy, W.; Sutthiwattanakul, S.; Laoteng, K. Efficient production of arachidonic acid of *Mortierella* Sp. by solid-state fermentation using a combinatorial medium with spent mushroom substrate. *Chem. Pap.* **2018**, *72*, 2899–2908. [\[CrossRef\]](#)

12. Rayaroth, A.; Tomar, R.S.; Mishra, R.K. One step selection strategy for optimization of media to enhance arachidonic acid production under solid state fermentation. *LWT* **2021**, *152*, 112366. [\[CrossRef\]](#)
13. Stressler, T.; Eisele, T.; Rost, J.; Haunschild, E.-M.; Kuhn, A.; Fischer, L. Production of polyunsaturated fatty acids by *Mortierella alpina* using submerge and solid state fermentation. *Chem. Ing. Tech.* **2013**, *85*, 318–322. [\[CrossRef\]](#)
14. Slaný, O.; Klempová, T.; Shapaval, V.; Zimmermann, B.; Kohler, A.; Čertík, M. Biotransformation of animal fat-by products into ARA-enriched fermented bioproducts by solid-state fermentation of *Mortierella alpina*. *J. Fungi* **2020**, *6*, 236. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Asadi, S.Z.; Khosravi-Darani, K.; Nikoopour, H.; Bakhoda, H. Production of arachidonic acid and eicosapentaenoic acid by *Mortierella alpina* CBS 528.72 on date waste. *Food Technol. Biotechnol.* **2018**, *56*, 197–207. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Stredansky, M.; Conti, E.; Salaris, A. Production of polyunsaturated fatty acids by *Pythium ultimum* in solid-state cultivation. *Enzym. Microb. Technol.* **2000**, *26*, 304–307. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Emelyanova, E.V. γ -linolenic acid production by *Cunninghamella japonica* in solid state fermentation. *Process Biochem.* **1996**, *31*, 431–434. [\[CrossRef\]](#)
18. Conti, E.; Stredansky, M.; Stredanska, S.; Zanetti, F. gamma-Linolenic acid production by solid-state fermentation of Mucorales strains on cereals. *Bioresour. Technol.* **2001**, *76*, 283–286. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Qiao, W.; Tao, J.; Luo, Y.; Tang, T.; Miao, J.; Yang, Q. Microbial oil production from solid-state fermentation by a newly isolated oleaginous fungus, *Mucor circinelloides* Q531 from mulberry branches. *R. Soc. Open Sci.* **2018**, *5*, 180551. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Stredansky, M.; Conti, E.; Stredanska, S.; Zanetti, F. γ -Linolenic acid production with *Thamnidium elegans* by solid-state fermentation on apple pomace. *Bioresour. Technol.* **2000**, *73*, 41–45. [\[CrossRef\]](#)
21. Jangbua, P.; Laoteng, K.; Kitsubun, P.; Nopharatana, M.; Tongta, A. Gamma-linolenic acid production of *Mucor rouxii* by solid-state fermentation using agricultural by-products. *Lett. Appl. Microbiol.* **2009**, *49*, 91–97. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Slaný, O.; Klempová, T.; Marcinčák, S.; Čertík, M. Production of high-value bioproducts enriched with γ -linolenic acid and β -carotene by filamentous fungi *Umbelopsis isabellina* using solid-state fermentations. *Ann. Microbiol.* **2020**, *70*, 5. [\[CrossRef\]](#)
23. Ferreira, M.; Fernandes, H.; Peres, H.; Oliva-Teles, A.; Belo, I.; Salgado, J.M. Polyunsaturated fatty acids production by solid-state fermentation on polyurethane foam by *Mortierella alpina*. *Biotechnol. Prog.* **2021**, *37*, e3113. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Shimizu, S.; Akimoto, K.; Kawashima, H.; Shinmen, Y.; Yamada, H. Production of dihomogamma-linolenic acid by *Mortierella alpina* 1S-4. *J. Am. Oil Chem. Soc.* **1989**, *66*, 237–241. [\[CrossRef\]](#)
25. Shimizu, S.; Kawashima, H.; Akimoto, K.; Shinmen, Y.; Yamada, H. Conversion of linseed oil to an eicosapentaenoic acid-containing oil by *Mortierella alpina* 1S-4 at low temperature. *Appl. Microbiol. Biotechnol.* **1989**, *32*, 1–4. [\[CrossRef\]](#)
26. Stredansky, M.; Conti, E.; Navarini, L.; Bertocchi, C. Production of bacterial exopolysaccharides by solid substrate fermentation. *Process Biochem.* **1999**, *34*, 11–16. [\[CrossRef\]](#)
27. Folch, J.; Lees, M.; Sloane Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [\[CrossRef\]](#)
28. Ichihara, K.; Fukubayashi, Y. Preparation of fatty acid methyl esters for gas-liquid chromatography. *J. Lipid Res.* **2010**, *51*, 635–640. [\[CrossRef\]](#) [\[PubMed\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.