Bioresource Technology Reports 30 (2025) 102119

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

Tailoring the fatty acid profile of microbial triglycerides in *Ustilago maydis* by adapting the cultivation conditions

Paul Richter [a,b], Marie Maßjosthusmann [a], Thomas Seidel [a], Leonard Walter [a], Katharina Miebach [a,b], Marcel Mann [a,b], Jochen Büchs [a,b], Kerstin Schipper [c,b], Michael Feldbrügge [c,b], Janis Goeke [d], Dominik Marcel Wieland [d], Heiko Hayen [d], Jørgen Barsett Magnus [a,b,*]

[a] *Aachener Verfahrenstechnik – Chair of Biochemical Engineering, RWTH Aachen University, 52074 Aachen, Germany*
[b] *Bioeconomy Science Center (BioSC), 52425 Jülich, Germany*
[c] *Institute of Microbiology, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany*
[d] *Institute of Inorganic and Analytical Chemistry, University of Münster, 48149 Münster, Germany*

ARTICLE INFO

Keywords:
Microbial triglycerides
Fatty acid composition
Tailoring
pH
Oxygen availability
Ustilago maydis

ABSTRACT

Plant oils are found in a wide range of consumer products. However, concerns have been raised regarding the environmental impact of their production. This study explores a sustainable alternative by investigating how cultivation parameters influence the fatty acid composition of microbial triglycerides in *Ustilago maydis*. Batch cultivations were performed with varying carbon sources, oxygen availability, pH, organic acid addition, and nutrient limitations to assess their impact. Different carbon sources had a modest influence, while nutrient limitations significantly altered the fatty acid composition. Lower oxygen supplies reduced unsaturation and increased chain length. Moreover, a neutral pH favored longer chains, while a basic pH increased unsaturation. The addition of citric acid significantly boosted palmitic acid content by 36 %. These findings demonstrate the versatility of microbial triglyceride production and provide insights into tailoring fatty acid profiles for specific applications, to provide a well-suited sustainable alternative to common plant oils.

1. Background

Vegetable oils are utilized in a multitude of consumer products, ranging from food items to cosmetics and cleaning products (Gunstone, 2011). However, their widespread use has become a topic of concern due to the environmental impact associated with their production. The expansion of plantations for oil crops like palm and soy often results in deforestation, particularly in rainforests, which contributes to habitat destruction and the loss of biodiversity (Carlson and Garrett, 2018). Such deforestation is not only detrimental to the environment but also to indigenous species and communities. Furthermore, the extensive transportation distances contribute to an elevated carbon footprint, giving rise to additional environmental concerns (Carlson and Garrett, 2018; Uusitalo et al., 2014).

Despite the diverse sources of vegetable oils, including palm, coconut, soybean, and sunflower, they all share a similar chemical structure. At their core, vegetable oils consist of triglycerides, which consist of glycerol and three fatty acids. The specific composition of fatty acids varies among different oils, influencing their physical properties and applications. However, this fundamental structure remains consistent across all plant oils, highlighting their interchangeable nature in various industrial applications (Gunstone, 2011; Krist, 2013).

Microbial oils are emerging as a highly promising alternative to traditional plant-based oils, offering significant advantages in terms of sustainability (Patel et al., 2020). In addition to requiring significantly less land and water, microbial oil production can be strategically localized, as fermentation facilities can be constructed directly at sites where triglycerides are needed. This has the dual benefit of reducing transportation costs and environmental impacts. Furthermore, microbial oils possess the potential for customizable fatty acid compositions, rendering them suitable for a multitude of applications (Athenaki et al., 2018; Ratledge, 2004). These include the imitation of prevalent oils such as palm and soybean, as well as the substitution of costly plant oils, including olive and coconut oil, in high-end products.

* Corresponding author at: Aachener Verfahrenstechnik – Chair of Biochemical Engineering, RWTH Aachen University, 52074 Aachen, Germany.
E-mail address: Jorgen.magnus@avt.rwth-aachen.de (J.B. Magnus).

https://doi.org/10.1016/j.biteb.2025.102119
Received 11 February 2025; Received in revised form 8 April 2025; Accepted 11 April 2025
Available online 20 April 2025

The most commonly utilized microorganisms for microbial oil production include *Mortierella isabellina*, *Yarrowia lipolytica*, and *Rhodotorula toruloides* (Athenaki et al., 2018; Patel et al., 2020), but numerous other oil-producing microorganisms have been identified (Athenaki et al., 2018). Modifications to their fatty acid profiles are frequently achieved through genetic modifications (Beopoulos et al., 2009; Howard G. Damude et al., 2009). However, some studies indicate that the fatty acid composition of the microbial oil produced in *Y. lipolytica* can be adjusted not only by genetic engineering but also by altering cultivation conditions (Ratledge, 2004).

A newly discovered oleaginous organism is *Ustilago maydis*, which has demonstrated particular potential for triglyceride production, as comparable oil yields to those of established *Y. lipolytica* strains have been observed (Aguilar et al., 2017; Richter et al., 2024). The objective of this study is to investigate whether altering the cultivation conditions can be used to tailor the fatty acid profile of the triglyceride produced by *U. maydis*. By examining how these parameters influence the fatty acid composition, this study contributes to targeted industrial microbial oil production. This approach offers two significant advantages. First, microbial oils can be tailored to mimic different plant oils or to generate novel triglyceride mixtures. Second, this can be achieved with remarkable speed simply by adjusting cultivation parameters, providing a more flexible alternative to genetically engineering one strain to achieve one specific fatty acid profile. This adaptability opens new possibilities for optimizing microbial triglyceride production for various applications. The ability to fine-tune triglyceride compositions to meet specific needs could provide a sustainable alternative to plant-based oils while reducing environmental impact. Further advances in cultivation strategies and metabolic engineering could increase production efficiency and broaden the scope of industrial applications.

2. Material and methods

2.1. Microorganism

The organism cultivated in the experiments was *U. maydis* MB215Δcyp1Δemt1, deposited at DSM17147 as MB215cyp1emt1 (Hewald et al., 2005) and obtained from Philipps University of Marburg, Marburg, Germany. The strain *U. maydis* MB215Δcyp1Δemt1 was genetically modified to eliminate both the synthesis of ustilagic acid and mannosylerythritol lipids by deletion of the genes *cyp1* and *emt1* encoding the enzymes for central catalytic steps in the two glycolipid biosynthesis pathways. These genetic modifications have minimized the formation of byproducts and resulted in a substantial intracellular accumulation of triglycerides. Its yeast-like growth characteristics enable straightforward cultivation, and lipid biosynthesis can be induced via the limitation of nitrogen, phosphorus, or sulfur availability. The organism was stored in a 9 $g\bullet L^{-1}$ sodium chloride solution with a glycerol concentration of 200 $g\bullet L^{-1}$ at −80 °C.

2.2. Media composition

A modified version of the Verduyn mineral medium (Verduyn et al., 1992) was used for all cultivations, if not stated otherwise, with the following composition: 100 $g\bullet L^{-1}$ glucose, 1.6 $g\bullet L^{-1}$ $(NH_4)_2SO_4$, 0.5 $g\bullet L^{-1}$ KH_2PO_4, 0.2 $g\bullet L^{-1}$ $MgSO_4 \bullet 7H_2O$, 0.01 $g\bullet L^{-1}$ $FeCl_3 \bullet 6H_2O$ and 1 $mL\bullet L^{-1}$ trace element solution, which contained: 15 $g\bullet L^{-1}$ EDTA, 3 $g\bullet L^{-1}$ $FeSO_4 \cdot 7H_2O$, 0.84 $g\bullet L^{-1}$ $MnCl_2 \cdot 2H_2O$, 4.5 $g\bullet L^{-1}$ $ZnSO_4 \cdot 7H_2O$, 0.3 $g\bullet L^{-1}$ $CuSO_4 \cdot 5H_2O$, 0.3 $g\bullet L^{-1}$ $CoCl_2 \cdot 6H_2O$, 0.4 $g\bullet L^{-1}$ $Na_2MoO_4 \cdot 2H_2O$, 4.5 $g\bullet L^{-1}$ $CaCl_2 \cdot 2H_2O$, 1 $g\bullet L^{-1}$ H_3BO_3 and 0.1 $g\bullet L^{-1}$ KI. For the experiments with carbon sources other than glucose, glucose was substituted with 100 $g\bullet L^{-1}$ glucose equivalents of sucrose, fructose, xylose, arabinose, and galactose, normalized with the molar amount of carbon atoms. Nitrogen limitation was employed as a trigger for oil production in all cultivations except for the ones specified below. In the phosphorus-limited cultivation, the concentration of the following media components in the Verduyn medium was adjusted: 2.5 $g\bullet L^{-1}$ $(NH_4)_2SO_4$, 0.13 $g\bullet L^{-1}$ KH_2PO_4, and additionally 0.3 $g\bullet L^{-1}$ K_2SO_4 were added. In the sulfur-limited cultivation, the concentration of the following media components in the Verduyn medium was adjusted: 0 $g\bullet L^{-1}$ $(NH_4)_2SO_4$, 0.04 $g\bullet L^{-1}$ $MgSO_4$, and additionally 2 $g\bullet L^{-1}$ NH_4Cl. Due to strong acidification of the culture while producing triglycerides, the medium in the cultivations with shake flasks was buffered with 0.4 M 2-(N-morpholino)-ethane sulfonic acid (MES) buffer, which was adjusted with 5 M NaOH to a starting pH value of 6.5. In the experiments with other pH values the MES buffer was replaced. For the cultivations at pH 7 and 8 0.4 M 3-(N-morpholino)propanesulfonic acid (MOPS) buffer and at pH 9 and 10 0.2 M carbonate buffer was used. For cultivation at pH 5.5, a stirred tank bioreactor was controlled to the desired pH with 5 M NaOH. All media components were sterilized by filtration with a 0.2 µm cut-off filter and supplemented directly before cultivation. A modified media composition was utilized for the precultures, with the carbon source concentration adjusted to 20 $g\bullet L^{-1}$ glucose and the $(NH_4)_2SO_4$ concentration adjusted to 5 $g\bullet L^{-1}$.

2.3. Cultivation conditions

The cultivations in shake flask scale were performed in an in-house built Respiratory Activity Monitoring System (RAMOS). Detailed information about the setup and technology can be found in (Anderlei et al., 2004; Anderlei and Büchs, 2001). A commercial version of the RAMOS device is available from HiTec Zang GmbH (Herzogenrath, Germany) or Adolf Kühner AG (Birsfelden, Switzerland). The cultivation process was performed in 250 mL RAMOS shake flasks, which were filled with 20 mL of media and inoculated from a preculture with an initial optical density at 600 nm of 0.1. Prior to inoculation, the preculture was washed with 9 $g\bullet L^{-1}$ sodium chloride solution, to prevent the transfer of media components into the main cultivation. The cultivation was performed in a temperature-controlled shaker (Kuhner Shaker GmbH, Herzogenrath, Germany) at 30 °C. To ensure an adequate oxygen supply, the shake flasks were shaken at 350 rpm, with a shaking diameter of 50 mm. The precultures were subjected to the same cultivation conditions, with the exception of the inoculation method. The inoculation was performed using an initial optical density of 0.1, derived from a cryo-culture.

The cultivations in benchtop fermenter scale were performed in a 2 L New Brunswick BioFlo®/CelliGen® benchtop stirred tank bioreactor (Eppendorf SE, Hamburg, Germany). The temperature was maintained at 30 °C, and dissolved oxygen tension (DOT) was monitored with a VisiFerm™ DO 225 pO_2 sensor (Hamilton Bonaduz AG, Bonaduz, Switzerland). Oxygen and carbon dioxide concentrations, necessary for calculating the oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR), and respiratory quotient (RQ), were determined using a Rosemount X-Stream NGA 2000 exhaust gas analyzer (Emerson Electric Co., St. Louis, USA). The bioreactor was equipped with one six-bladed Rushton turbine, 51 mm in diameter, along with four baffles. The pH was continuously monitored using an EasyFerm Plus K8 200 pH sensor (Hamilton Bonaduz AG, Bonaduz, Switzerland). Cultivation and oil production were carried out in batch mode, analogous to the shake flask experiment, with the same adapted, but unbuffered Verduyn mineral medium.

2.4. Parameter investigation by decoupling growth from triglyceride production

To isolate the effect of varying cultivation conditions on the organisms' growth, different strategies were used, depending on the modified cultivation parameter. An easy-to-implement strategy was employed to evaluate the influence of oxygen availability and organic acid supplementation. For both tested parameters, cultures were grown under standard conditions during the exponential growth phase of the culture. After depletion of the nitrogen source, the corresponding parameter was modified so that it coincided with the entire triglyceride production

phase. In accordance, acetic acid and citric acid were supplemented in a concentration of 10 and 38.4 g•L^{-1} (0.2 M) respectively, following the onset of nitrogen limitation.

To adjust the oxygen availability for evaluation, the wells of a microtiter plate were filled with varying volumes of culture broth. The maximum oxygen transfer capacity (OTR_{max}) is dependent on the filling volume of the microtiter plate well, allowing for the establishment of different OTR_{max} levels through the variation of the filling volumes. This dependency was described by Lattermann in 2014 in the following Equation (Lattermann et al., 2014):

$$OTR_{max} = 2.5 \bullet 10^{-7} \bullet Peri^{6} \bullet n^{2.37} \bullet V_L^{-0.64} + 3.3 \bullet 10^{-4} \quad (1)$$

Eq. (1) specifies the maximum oxygen transfer capacity in 48-well microtiter plates at in-phase operating conditions. It quantifies the dependency of the OTR_{max} and the perimeter of the wells (*Peri*), the shaking frequency (n), and the filling volume of the well V_L. Using this equation, the shaking frequency in the exponential growth phase was selected at 1000 rpm to ensure that the OTR_{max} of the system with the highest filling volume (2 mL) could enable unlimited growth until nitrogen depletion. Once the nitrogen source was depleted, the shaking frequency was decreased to 525 rpm, allowing for the establishment of distinct OTR_{max} levels in the cultivations, according to the varied filling volumes. This procedure enabled the testing of different oxygen availabilities within a single experiment.

A similar approach was employed to investigate the impact of pH and low osmolality on triglyceride formation. However, given the limitations of pH control at the flask scale, which necessitates the use of a buffer, the method for separating the growth and product formation phases was adapted to accommodate these constraints. Consequently, a resting cell assay (RCA) (Klein, 1955) was conducted for the triglyceride production phase during the pH value experiments in the shake flask. For this purpose, the cultivations were conducted under identical conditions (pH 6.5), until the nitrogen source was depleted. The cultures were then harvested, washed, and transferred to a medium lacking a nitrogen source, which had an appropriately adjusted pH value with a buffer, and cultivated further. In the absence of a nitrogen source, triglyceride production was initiated immediately after the transfer of the cells at the corresponding pH value of the used buffer. To validate the tested pH values (pH 6.5, 7, 8, 9, and 10) in the shake flask, two pH values were tested in a bioreactor (pH 5.5 and 6.5). As the bioreactor allows for more flexible pH control, the experiments at pH 6.5 allowed an evaluation of the RCA in the shake flask. Accordingly, the exponential growth phase was conducted at pH 6.5, and following the depletion of nitrogen, the pH set point of the bioreactor's pH control was adjusted to the respective pH values (pH 5.5 and 6.5). The cultivations were monitored using the RAMOS device and the off-gas analysis of the bioreactor.

2.5. Offline analysis

The quantification of the carbon sources was carried out via high-performance liquid chromatography (HPLC). HPLC measurements were performed in the Prominence HPLC system (Shimadzu, Duisburg, Germany). The HPLC system was equipped with the following columns and detectors: a pre-column (40 × 8 mm) containing organic acid resin, purchased from CS-Chromatography Service (Langerwehe, Germany), and a column (250 × 8 mm) also containing organic acid resin from CS-Chromatography Services (Langerwehe, Germany). A refractive index detector model RID-20 A, manufactured by Shimadzu (Duisburg, Germany), was also utilized. The mobile phase consisted of 5 mM H_2SO_4. The flow rate was regulated to 0.8 mL•min^{-1} at 35 °C, and an injection volume of 20 µL was used. For the purpose of HPLC sample preparation, the cultivation broth was first centrifuged at 14,000 rpm for 10 min and then filtered through a 0.2 µm cut-off filter.

Dry cell weight (DCW) was measured by transferring 1 mL of fermentation broth into a pre-weighed reaction tube, centrifuging for 10 min at 14,000 rpm to separate the biomass, decanting the supernatant, and drying the pellet at 80 °C. The tube was then cooled in a desiccator and re-weighed, with the weight difference representing the DCW.

For the quantification of triglycerides, 2.4 mL of the culture broth was sonicated with the Fisherbrand™ Model 120 Sonic Dismembrator equipped with a 1/8″ Microtip (Fisher Scientific, Schwerte, Germany) to disrupt the cells. Subsequently, 2 mL of this crude cell extract was utilized in an extraction process according to a modified protocol of Matyash et al. (2008), which involved the use of 5 mL of methyl-tert-butyl ether (MTBE) and 1.5 mL of methanol (Matyash et al., 2008). Following phase separation by centrifugation (10 min at 14,000 rpm), the solvent phase was collected in dried, weighed glass vials and evaporated at room temperature. After the evaporation process, the glass vials were re-weighed.

2.6. Determination of fatty acid distribution by GC-FID

The following solvents and standards were used for the determination of fatty acid distribution by GC-FID: LC-grade MTBE and FAME37 standard were purchased from Merck KGaA (Darmstadt, Germany). The derivatization agent trimethylsulfonium hydroxide (TMSH) (0.2 mol•L^{-1} in methanol) was obtained from Macherey-Nagel (Düren, Germany). Heptadecanoic acid was purchased from Biozol (Eching, Germany).

For sample preparation, the dried samples were weighed and subsequently dissolved in MTBE to achieve a concentration of 33.3 mg•mL^{-1}. From these solutions, 30 µL was diluted with 60 µL of MTBE. An internal standard was prepared by adding 10 µL of a 1 mg•mL^{-1} heptadecanoic acid solution. The transesterification process was initiated by the addition of a 100 µL TMSH solution, resulting in the formation of fatty acid methyl esters (FAME). This methodology was employed for all the results presented in this study and the solutions were contiguously used for GC analysis. The samples were analyzed using a GC-2010 gas chromatograph system equipped with an AOC-20i autosampler (both Shimadzu Corp, Kyoto, Japan). The separation of FAMEs was conducted on a polar FAMEWAX column (Restek GmbH, Pennsylvania, USA) with crossbond polyethylene glycol as stationary phase (30 m, 0.25 mm, 0.25 µm). The GC-flow was operated at a constant linear velocity of 45.0 cm·s^{-1} with hydrogen 5.0 as carrier gas (Westfalengas, Münster, Germany). Samples (1 µL) were injected in split-mode (split ratio 1:20) at a temperature of 250 °C. A linear gradient was employed for the column oven: commencing at 100 °C increasing by 4 °C•min^{-1} to 240 °C which was maintained for 5 min. Before each measurement, an equilibration time of 1 min was applied. Chromatograms were obtained by flame ionization detection (FID) with a detector temperature of 250 °C and a sampling rate of 100 ms. Data acquisition was performed using LabSolutions version 5.110 (Shimadzu Corp, Kyoto, Japan). For further data processing and interpretation Microsoft Office LTSC Professional Plus 2021 was used (Microsoft Corp, Redmond, USA). To compare retention times and thereby determine the fatty acid compositions of the samples, a FAME37 standard was measured before and after each sample batch. The standard was therefore diluted 1:10 with MTBE and used for measurements.

For every analyzed chain length, the response factor of the FID detector was calculated. The response of FID detection for fatty acids is dependent on their respective chain length and degree of unsaturation. In this study, we present the distribution of 36 fatty acid species, which necessitates the consideration of a variable detector response. Thus, the response factors for all 36 species were determined utilizing the FAME37 standard. Therefore, the non-diluted FAME37 standard was measured 12 times with a split-ratio of 1:50. Peaks were integrated, resulting areas averaged and referred to the area of stearic acid (C18:0) within the FAME37 standard. The response factor was calculated according to Eq. (2):

$$R_f = \frac{A \cdot W_{c18:0}}{A_{C18:0} \cdot W} \quad (2)$$

With R_f = Response factor, A = Peak area [pA · s], W = Particular FAME weight % in FAME37 standard.

3. Results and discussion

The cultivations described in this publication were carried out identically to the reference process, except for the adjusted parameters. Then the cultivation with these adjusted parameters is compared to the reference process. An outline of the reference process can be found in the subsequent section.

3.1. Influence of the cultivation time on the fatty acid composition

The data of the reference process, measured both online and offline, is illustrated in Fig. 1. This reference data serves as the foundation for comparing the tested parameters of this study. Furthermore, the influence of cultivation time on the fatty acid composition was investigated using this reference process. For this purpose, samples were analyzed for their fatty acid composition at 24-h intervals. This temporal aspect could play a significant role in the variation of fatty acid composition.

The metabolic behavior of the organism during the experiment can be followed via its respiratory activity. This is demonstrated in Fig. 1A in the form of the oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR), and respiration quotient (RQ). In the first phase of the process, *U. maydis* grows exponentially until the secondary substrate nitrogen is depleted at 20 h (indicated by the vertical dashed line). The depletion of nutrients in this instance has been shown to be a necessary condition for the accumulation of triglycerides, as the reduced growth leads to the conversion of excess carbon sources into triglycerides for energy storage. As a result, after 20 h, the growth ceases. This is followed by a short transition phase (Klement et al., 2012), which is visible in the OTR as a plateau formation, and the start of triglyceride production. During the triglyceride production, which lasts approximately 130 h, the activity of the culture exhibits a gradual decline, visible in the OTR (indicated by the second vertical dashed line). The triglyceride concentration (Fig. 1B) initially rises steeply at the beginning of the production phase and then approaches a maximum value determined by the biomass concentration until the end. The RQ is above 1 for the whole production phase indicating the production of a reduced product, in this case, triglycerides. Upon the exhaustion of the carbon source, the activity and, consequently, the OTR drops slightly, marking the onset of a metabolic turnover, as evidenced by the RQ falling under 1. At this time point, the

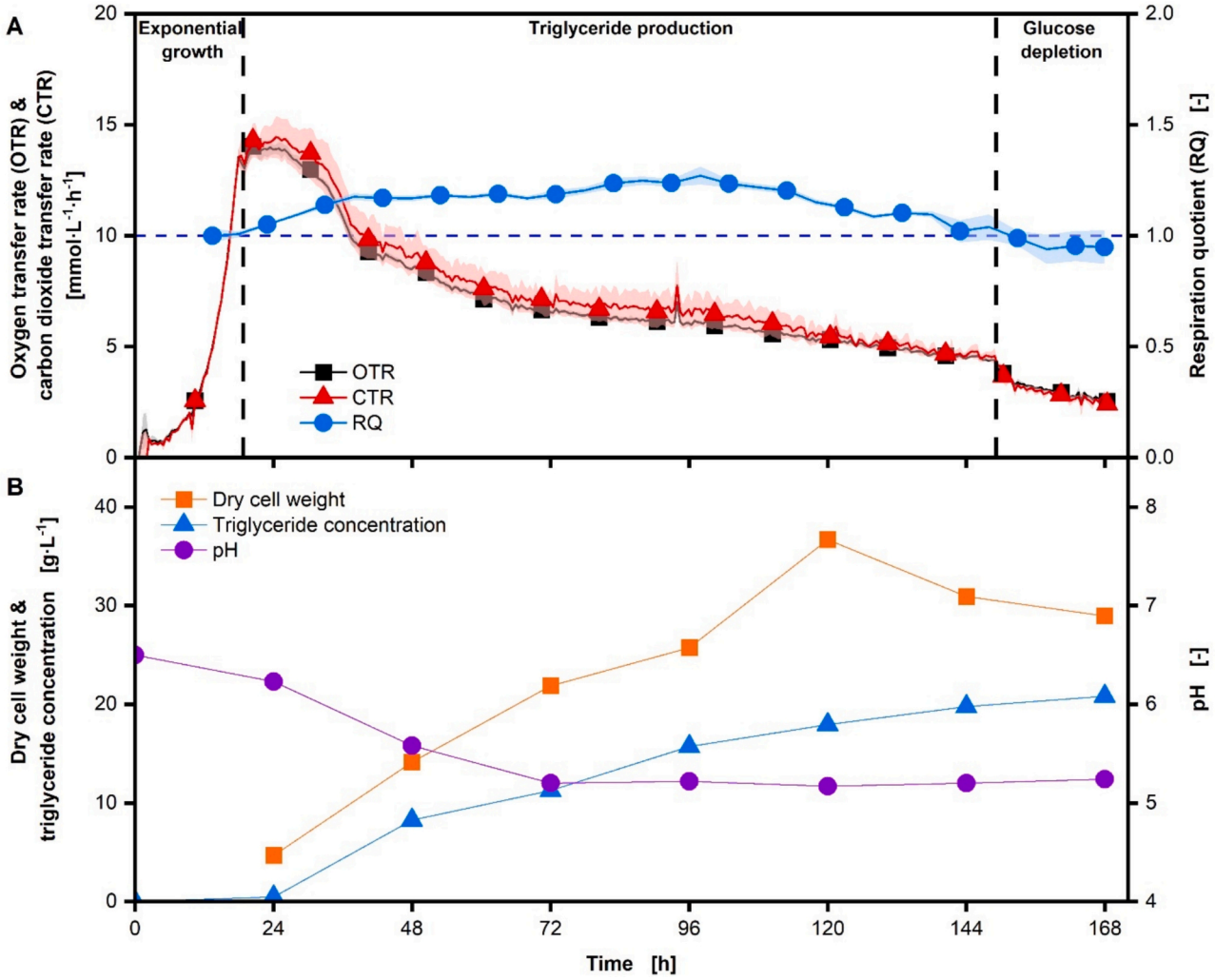

Fig. 1. Cultivation data of the triglyceride production of *U. maydis* MB215Δcyp1Δemt1 with a sampling interval of 24 h. The respiratory activity of the cultivation, in the form of OTR, CTR, and RQ is presented in panel A. Cultivations were conducted in duplicate, with the average values represented as a line and the min/max values illustrated as error shadows. For clarity, every fifth measuring point is indicated by a symbol. The phases of the cultivation are indicated via vertical dashed lines. In panel B offline analytics are presented. Cultivation conditions: 250 mL shake flasks; V_L = 20 mL; n = 350 rpm; d_0 = 50 mm; modified Verduyn medium; nitrogen-limited; $C_{carbon\ source}$ = 100 g•L^{-1} glucose; pH = 6.5.

cultivation process should be stopped to achieve the highest triglyceride concentration.

The offline analysis of the samples taken is depicted in Fig. 1B. To monitor the biomass concentration DCW was measured. In this data set one problem of oil producing organisms becomes clear. Biomass measurements like DCW are influenced by intracellular triglyceride accumulation. Without sufficient nitrogen supply, the growth of *U. maydis* stops after about 20 h of cultivation time, indicated for example by the abrupt plateau formation in the OTR (Fig. 1A). The observable increase in DCW after the nitrogen depletion results from the intracellular accumulation of triglycerides, resulting in higher DCWs. The pH value decreased to a value of 5.2 after 72 h and remained constant throughout the remaining cultivation time. This is mainly caused by the production of organic acids like malic acid or itaconic acid (data not shown).

The resulting triglyceride production in this reference cultivation follows a saturation curve and achieves a concentration of approximately 20 g•L^{-1}. This final concentration is also comparable with already published values of 18–20 g•L^{-1} with *U. maydis* (Richter et al., 2024). The productivity of the culture is highest after nitrogen limitation at 20 h and then slowly decreases. The extracted triglyceride samples were analyzed by GC-FID to determine their fatty acid composition. The proportions of the different fatty acids in the produced triglyceride mixture are shown in Fig. 2.

Fig. 2 shows the different sample times and the respective fatty acid composition as percentages of the produced triglycerides. A clear trend can be recognized by comparing the results at the different sampling times, especially in the main fatty acids palmitic acid (C16:0, red), oleic acid (C18:1, blue with vertical lines), and linoleic acid (C18:2, blue with hashed marks). The resulting fatty acid compositions demonstrate a notable decrease in short-chain C16:0 and an accompanying increase in longer-chained fatty acids, namely C18:1 and C18:2. This phenomenon also results in an increase in the proportion of unsaturated fatty acids over time. Another effect worth mentioning is the unchanged composition regarding myristic acid (C14:0) and the longer chained fatty acids (C > 20).

For easier understanding, the fatty acid compositions of every sample were compared to the sample taken after 48 h. The percentage change in relation to the fatty acid chain length and the degree of unsaturation was calculated and is shown in Fig. 3.

When evaluating the change in fatty acid saturation and overall chain length in the triglyceride mixture, the influence of the cultivation time on the fatty acid composition becomes evident. In Fig. 3, with increasing cultivation time a higher degree of unsaturation and a slightly increased chain length can be observed. Although the cultivation time of triglycerides seems to be a decisive factor, it has only rarely been investigated in connection with the fatty acid composition of microbial triglycerides. At present, the knowledge of the influence of cultivation time on fatty acid composition is limited. In 2020 Utami et al. were able to show that the cultivation time influences the fatty acid composition of the triglycerides formed in the filamentous fungus *Aspergillus oryzae* (Utami et al., 2020). Contrary to our data, a slight increase in palmitic acid and a constant proportion of stearic, oleic, and linolenic acid over the cultivation period was observed. However, a direct comparison of these results is difficult due to the different organisms and significantly different cultivation conditions, for example, a much shorter observed time window. What can be compared well with the literature is the high proportion of C18:1 in the triglyceride produced with the reference conditions. This is something that is often found in the literature regarding microbial triglycerides from many different organisms, like *Y. lipolytica* or *R. toruloides* (Athenaki et al., 2018; Papanikolaou and

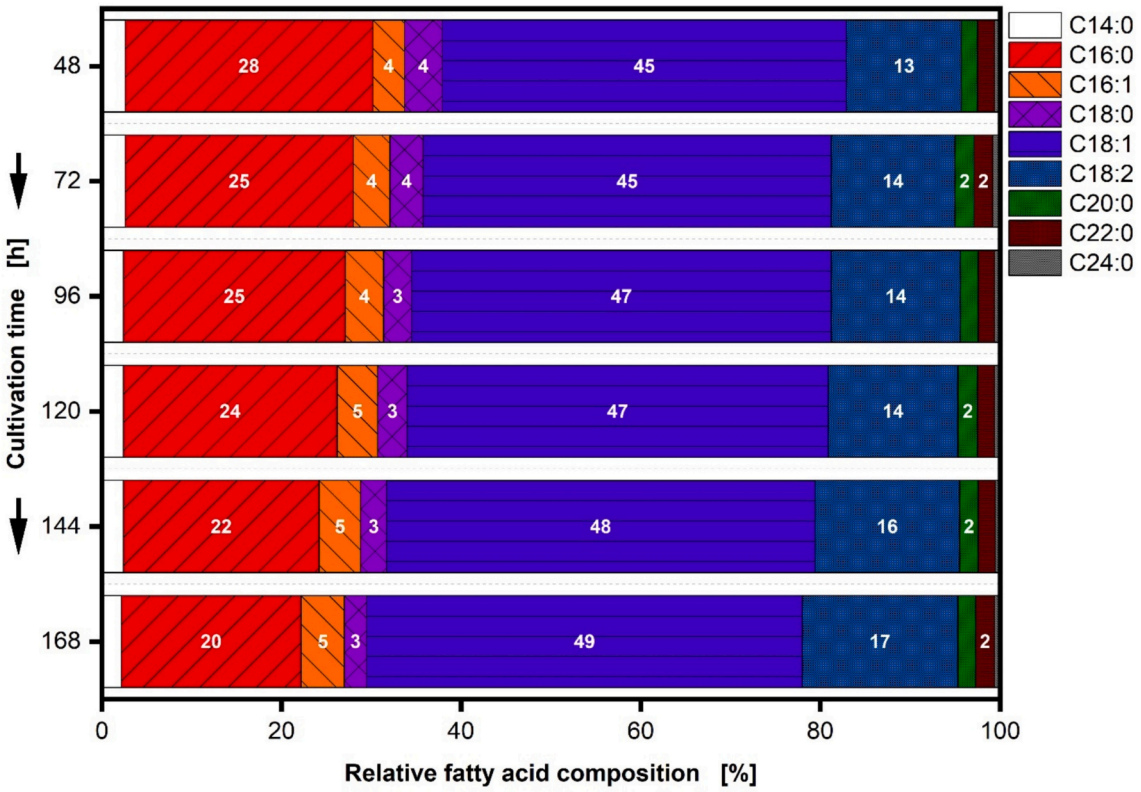

Fig. 2. Influence of the cultivation time on the fatty acid profile in the triglyceride mixture produced by *U. maydis* MB215Δcyp1Δemt1 under standard cultivation conditions. The data presented originates from the cultivation shown in Fig. 1. The x-axis represents the different fatty acids attached to the glycerol backbone in weight percent. The different fatty acid species are presented by the color code on the right with the first number representing the total length of the carbon chain and the second number for the number of double bonds in the fatty acid chain. Values are only depicted if their value exceeds 2 *w*/w %. Cultivation conditions: 250 mL shake flasks; V_L = 20 mL; *n* = 350 rpm; d_0 = 50 mm; modified Verduyn medium; nitrogen-limited; $C_{carbon\ source}$ = 100 g•L^{-1} glucose; pH = 6.5.

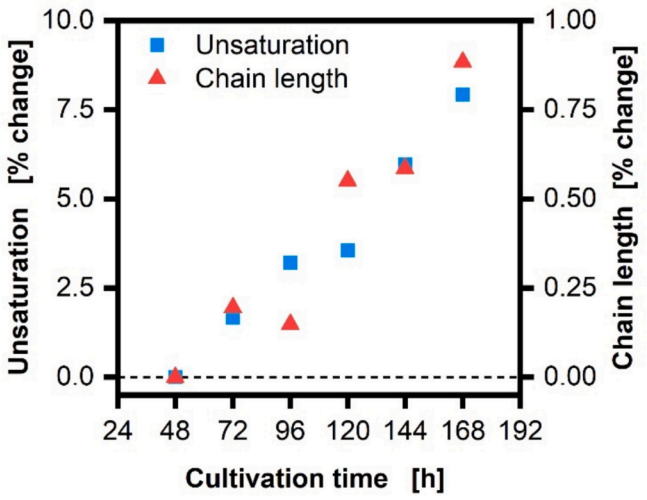

Fig. 3. Influence of the cultivation time on the fatty acid profile in the triglyceride mixture produced by *U. maydis* MB215Δcyp1Δemt1 under standard cultivation conditions. Change in fatty acid profile is illustrated regarding the change of the degree of unsaturation and the overall chain length, compared to the sampling point at 48 h. The degree of unsaturation represents the proportion of all unsaturated fatty acids in the total triglyceride mixture and was calculated using the proportion of each fatty acid and their degree of unsaturation, whereas the chain length refers to the total length of the fatty acids present. The data presented originates from the cultivation shown in Fig. 1.

Aggelis, 2011).

The experiment, shown in Figs. 1–3, demonstrates that for a valid comparison between different cultivation conditions, the cultivation time must be kept consistent. Any variation will affect the comparison and potentially lead to less accurate results.

3.2. *Influence of the carbon source on the fatty acid composition*

The carbon source represents a fundamental component of a bioprocess, which typically remains unaltered and is not subject to modification during an ongoing fermentation. To assess the impact of varying carbon sources on the fatty acid profile a comparison to a reference cultivation with the same cultivation parameters as in Fig. 1 was used. Glucose was replaced by sucrose, fructose, xylose, arabinose, and galactose. Each carbon source was normalized to the molar amount of carbon atoms in the reference cultivation and then added at the corresponding concentration. Given that the cultivation time affects the fatty acid composition, the cultivations were terminated after a production phase of 120 h (equals 144 h cultivation time). The resulting online monitoring data from these cultivations are presented in the supplementary information in Fig. S 1. The results of the fatty acid composition analysis are depicted in Fig. 4.

The organism displays the capacity to grow and produce triglycerides on a diverse range of carbon sources, including hexose sugars such as glucose, fructose, sucrose, and galactose, as well as pentose sugars, such as xylose and arabinose (Helm et al., 2023; Müller et al., 2018; Richter et al., 2024). The fungal growth patterns, which were recorded by measuring the respiratory activity (Fig. S 1), remain largely consistent across these different carbon sources, with the only observable variation being the length of the lag phase. Following nitrogen limitation, no significant differences in respiration activity were observed, regardless of the carbon source, as can be recognized in Fig. S 1. The fatty acid composition of the triglycerides produced from glucose, fructose, and sucrose is nearly identical. This is likely due to the fact that

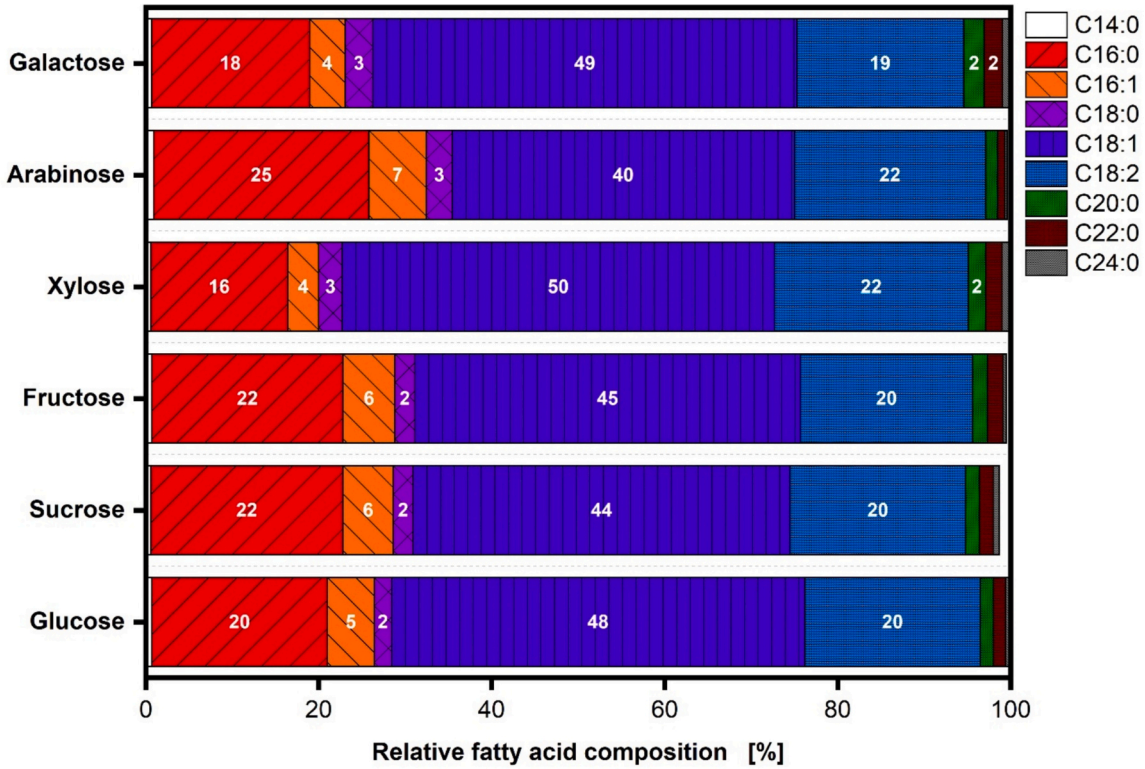

Fig. 4. Fatty acid composition of the analyzed triglyceride samples derived from cultivations on different carbon sources depicted in Fig. S1. The x-axis represents the different fatty acids attached to the glycerol backbone in weight percent. The different fatty acid species are presented by the color code on the right with the first number representing the total length of the carbon chain and the second number for the number of double bonds in the fatty acid chain. Values are only depicted if their value exceeds 2 *w*/w %. Cultivation conditions: 250 mL shake flasks; $V_L = 20$ mL; $n = 350$ rpm; $d_0 = 50$ mm; modified Verduyn medium; nitrogen-limited; $C_{carbon\ source} = 100$ g•L^{-1} glucose equivalents; pH = 6.5.

all of them are metabolized via glycolysis. Similarly, galactose, another hexose, also yields a very similar fatty acid profile. The utilization of pentoses, specifically xylose and arabinose, results in slight variations in the fatty acid composition, particularly in the proportions of palmitic acid (C16:0) and oleic acid (C18:1). The content of palmitic acid varied by ±5 %, while that of oleic acid exhibited fluctuations of up to ±10 % in comparison to the reference cultivation on glucose. These changes, although significant, remain relatively minor in comparison to other biological parameters tested in this study. It is noteworthy that palmitic acid levels decline when xylose is utilized yet increase when arabinose is used. This suggests the existence of a subtle distinction in the metabolic processes of *U. maydis* when dealing with these two C5 sugars. This hypothesis can be supported by the two partly divided metabolic pathways used for arabinose and xylose degradation in yeast (Fonseca et al., 2007; Zha et al., 2021). Nevertheless, the precise mechanism underlying this variation remains unclear. It seems probable that *U. maydis* follows a broad, conserved biosynthetic pathway for triglyceride production across different carbon sources. The synthesis pathway appears to be robust for hexoses, yielding highly consistent triglyceride profiles, while the slight changes observed using pentose sugars reflect a mild metabolic adaptation to these less common carbon sources. These conserved fatty acid synthesis pathways have also been confirmed in the literature for other oleaginous microorganisms. It has been shown that the carbon source has an influence on the fatty acid composition, but this influence is relatively small depending on the carbon source used (Papanikolaou and Aggelis, 2011; Sajbidor et al., 1988; Stutzenberger and Jenkins, 1995).

3.3. *Influence of the nutrient limitation triggering the triglyceride production*

Similar to the carbon source used, the limiting nutrient that triggers triglyceride synthesis is often predetermined during the development of the fermentation process. To examine how different limiting nutrients affect the fatty acid composition of the produced triglyceride, nitrogen-limited cultivation was performed in comparison to phosphorus-limited and sulfur-limited cultivation. Due to necessary adjustments in nutrient and carbon source concentrations, the cultivation times in this experiment are shorter than in the already presented reference cultivation (Fig. 1). A comparison of the fatty acid compositions of this experiment is, therefore, only possible within this experiment. The online-monitored cultivation data can be found in the supplementary information in Fig. S 2. The variation of the limiting nutrient results in changes in the OTR courses (Grebe et al., 2024), which differs from the nitrogen-limited course of the reference cultivation. The resulting fatty acid compositions are depicted in Fig. 5.

The analysis of the fatty acid composition presented in Fig. 5 reveals distinct variations based on the type of nutrient limitation applied to the cultivation. Here, phosphorus-limited and sulfur-limited cultivations are compared to nitrogen-limited reference cultivation. Notably, the influence on palmitic acid (C16:0) levels remains relatively low, with only a 5 % increase observed under sulfur-limited conditions. In contrast, significant reductions in oleic acid (C18:1) are evident when either sulfur or phosphorus limitations are present. Interestingly, an increase in linoleic acid (C18:2) is observed, particularly under phosphorus-limiting conditions. This leads to linoleic acid becoming the predominant fatty acid under phosphorus-limited conditions. It can be deduced that different limiting nutrients result in the production of diverse triglyceride profiles. For other organisms like oleaginous algae, this nutrient-dependent fatty acid synthesis was already demonstrated for the nutrients nitrogen and phosphorus (Gong et al., 2013). However, as the actual focus of Gong et al., 2013 was on the production of eicosapentaenoic acid, it showed that phosphorus limitation leads to higher palmitic and oleic acid concentrations than nitrogen limitation but did not quantify these results. Su et al. (2016) described in their paper that phosphate limitation in the oil-producing algae *Porphyridium purpureum* leads to increased formation of unsaturated fatty acids, which is in good agreement with our results (Su et al., 2016). In 1993, Granger et al.

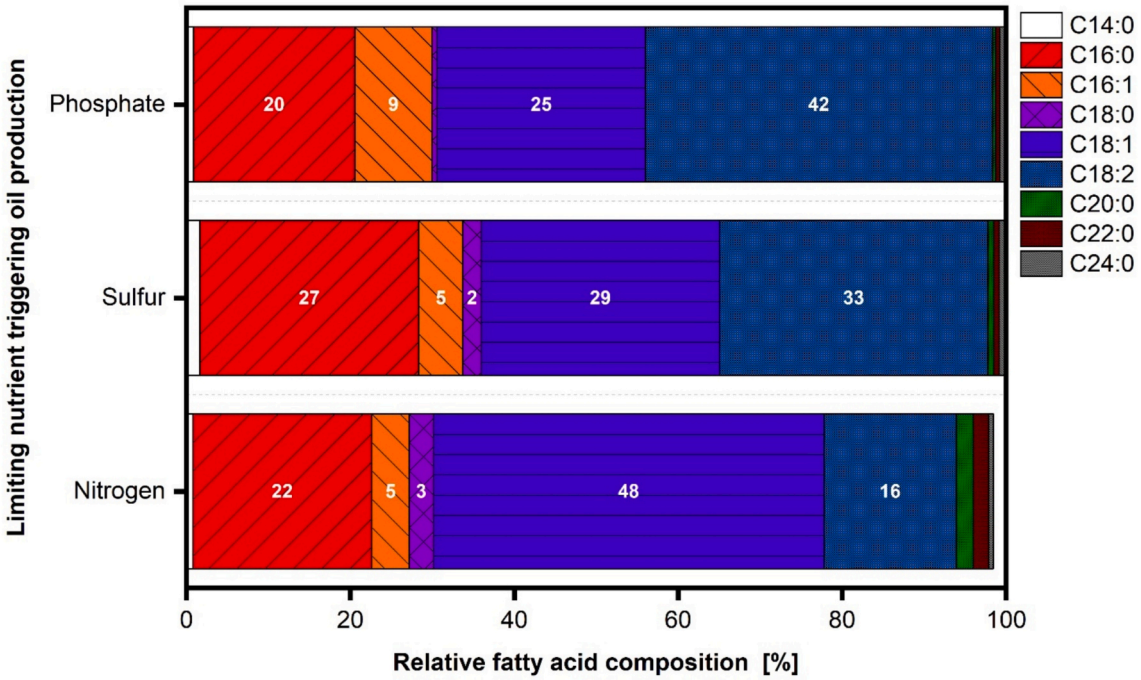

Fig. 5. Fatty acid composition of the analyzed triglyceride samples derived from cultivations with different nutrient limitations depicted in Fig. S 2. The x-axis represents the different fatty acids attached to the glycerol backbone in weight percent. The different fatty acid species are presented by the color code on the right with the first number representing the total length and the second number for the number of double bonds in the fatty acid chain. Values are only depicted if their value exceeds 2 *w*/w %. %. Cultivation conditions: 250 mL shake flasks; V_L = 20 mL; n = 350 rpm; d_0 = 50 mm; modified Verduyn medium with varied nutrient limitations; $C_{carbon\ source}$ = 100 g•L^{-1} glucose equivalents; pH = 6.5.

described that different nutrient limitations interact with the fatty acid composition in *Rhodotorula glutinis*, resulting in higher α-linolenic acid (C18:3) concentrations when comparing a nitrogen-limited to a phosphorus-limited cultivation. Unfortunately, the study did not provide a comprehensive description of total fatty acid compositions but only examined the influence of the limitations on the formation of α-linolenic acid. (Granger et al., 1993). In addition, Zhuang et al., 2022 were able to show the influence of nutrient limitations on both the desaturase and elongase for the production of bioactive lipids (Zhuang et al., 2022). Based on the presented results and the assumptions made in the literature, it can be assumed that certain enzymes, for example, elongases and desaturases, that are essential for achieving higher levels of unsaturated fatty acids may not be synthesized as effectively under nitrogen limitation in *U. maydis*.

3.4. Influence of the oxygen availability on the fatty acid composition

The first parameter investigated in this section is oxygen availability. As described in the Material and Methods section, to ensure better comparability, sufficient oxygen levels were maintained during the growth phase of the organisms. To study the impact of oxygen supply during triglyceride production, oxygen availability was intentionally reduced to varying limiting levels.

The resulting OTR courses from different cultivations are presented in the supplementary information (Fig. S3). These courses clearly distinguish between the two phases, the growth phase and the production phase, as OTR reflects the oxygen availability within the system. Additionally, the analyzed fatty acid profiles corresponding to these conditions are also included in the supplementary information (Fig. S4). From this figure, several overarching trends can be derived, which are illustrated in Fig. 6 as changes in fatty acid saturation and chain length. The two trends are primarily caused by a decrease in the proportion of palmitic acid (C16:0) and oleic acid (C18:1), as well as an increase in the proportion of stearic acid (C18:0) and linoleic acid (C18:2). Additionally, an increase of over 100 % in the longer-chain fatty acids (>C20) was observed, with lower oxygen supplies. The nonlinear trend of the unsaturation degree is primarily caused by the C18:0 content, as it increases substantially with decreasing oxygen availability, but decreases again at maximal oxygen transfer capacity lower than 5 mmol•L^{-1}•h^{-1}.

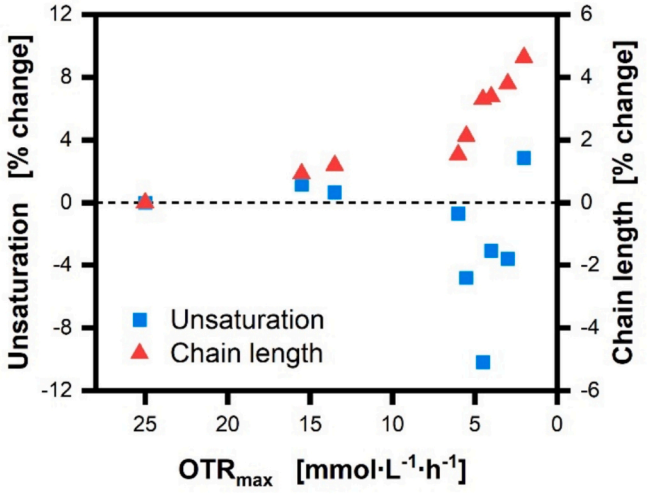

Fig. 6. Influence of the maximal oxygen transfer capacity on the fatty acid profile in triglyceride mixture produced by *U. maydis* MB215Δcyp1Δemt1. Change in the fatty acid profile is illustrated regarding the degree of unsaturation and the overall chain length, compared to the standard cultivation procedure. All cultivations were stopped at the same time point, to prevent the already shown temporal influence on the fatty acid composition. The degree of unsaturation represents the proportion of all unsaturated fatty acids in the total triglyceride mixture and was calculated using the proportion of each fatty acid and their degree of unsaturation, whereas the chain length refers to the total length of the fatty acids present. The data presented originates from the cultivation shown in Fig. S 3 and the resulting fatty acid composition shown in Fig. S 4. Cultivation conditions: 48 well microtiter plate; V_L = 0.5–2 mL; n_1 = 1000 rpm; n_2 = 525 rpm d_0 = 3 mm; modified Verduyn medium; nitrogen limited; $C_{carbon\ source}$ = 100 g•L^{-1} glucose; pH = 6.5; OTR_{max} = varied by adjusting the filling volume (calculated using Eq. (1)).

To calculate the unsaturation and chain length change, the established reference cultivation conditions (see Figs. 1–3), which provided a maximal oxygen transfer capacity of 25 mmol•L^{-1}•h^{-1}, were used. From Fig. 6 two distinct trends of oxygen availability on the fatty acid composition emerge. As oxygen availability declines, the length of the produced fatty acids increases. A contrasting trend is observed in the unsaturation degree of the fatty acids. As oxygen availability decreases, the unsaturation also decreases. However, after reaching a minimum saturation level, at a maximum oxygen transfer capacity of 5 mmol•L^{-1}•h^{-1}, the degree of saturation increases once more.

Some of the observed phenomena have been demonstrated in other studies of oleaginous organisms. In agreement with our results, Davies et al. (1990) demonstrated that a reduction in oxygen availability in the oleaginous yeast *Apiotrichum curvatum* resulted in an increased formation of stearic acid (C18:0) and a reduced formation of oleic acid (C18:1). However, in the same experiments an increase in C16:0 and a decrease in C18:2 content has been observed resulting from a reduction in oxygen availability (Davies et al., 1990). Another study compares anaerobic with aerobic fatty acid production in the fungus *Mucor rouxii* and links the different fatty acid profiles to the oxygen dependence of various desaturases involved in fatty acid synthesis (Ruenwai et al., 2010). Accordingly, it can be assumed that the influence of oxygen availability on the fatty acid composition depends at least partly on the used organism and its enzyme apparatus. The organism dependence has already been shown by comparing different organisms with different oxygen availabilities (Jirout, 2015). Because oxygen availability interacts in so many ways with the metabolic balance of aerobic organisms, it is difficult to conclude universal trends for all organisms. Nevertheless, the results presented here establish a consistent trend indicating how the fatty acid profile is dependent on oxygen availability for *U. maydis*.

There are also minor restrictions on the use of oxygen availability for tailoring the fatty acid composition. The method can only be used effectively within a certain range of maximum oxygen transfer capacities, as limiting oxygen supply in general slows down the metabolism. As a result, insufficient oxygen availability results in a process that is not economically viable.

3.5. Influence of the pH in the triglyceride production phase on the fatty acid composition

One of the most readily modifiable parameters during production processes in a bioreactor is the pH value. As outlined in the Materials and Methods section, to isolate the impact of pH on fatty acid composition, the growth phase was maintained at a pH of 6.5. The triglyceride production phase was then conducted at various pH levels, ranging from 5.5 to 10, to test the influence of pH on triglyceride production. In Fig. S5, for the growth phase, a mean course for all cultivations is depicted, whereas for the production phase, individual OTR courses are displayed. The fatty acid compositions of the different pH values are shown in the supplementary information (Fig. S6) and the derived trends of the pH value are depicted in Fig. 7.

The fatty acid composition, specifically chain length and degree of unsaturation, was normalized to the reference process, where a pH of 6.5 was maintained throughout both, the growth and production phase. The data presented in Fig. 7 reveal a positive correlation between pH and the degree of unsaturation of the fatty acids produced. As the pH increased, so did the unsaturation level. Even at the higher pH values of 9 and 10, the culture continued to produce triglycerides although with decreased

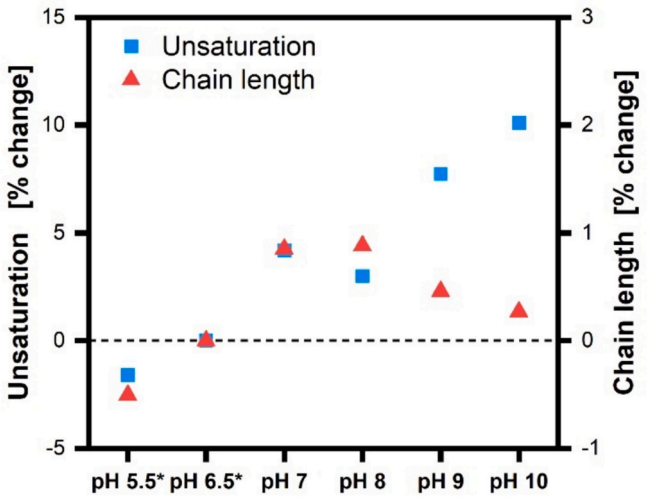

Fig. 7. Influence of the pH value on the fatty acid profile in triglyceride mixtures produced by *U. maydis* MB215Δcyp1Δemt1. Change in the fatty acid profile is illustrated regarding the degree of saturation and the overall chain length, compared to the standard cultivation procedure at pH 6.5. The degree of unsaturation represents the proportion of all unsaturated fatty acids in the total triglyceride mixture and was calculated using the proportion of each fatty acid and their degree of unsaturation, whereas the chain length refers to the total length of the fatty acids present. All cultivations were stopped at the same time point, to eliminate the already shown temporal influence on the fatty acid composition. The data presented originates from the cultivation shown in Fig. S5 and the resulting fatty acid composition shown in Fig. S6. Cultivation conditions: 250 mL shake flasks; V_L = 20 mL; n = 350 rpm; d_0 = 50 mm; modified Verduyn medium; nitrogen limited; $C_{carbon\ source}$ = 100 g•L^{-1} glucose; pH = varied.

productivity. This suggests that pH manipulation is a viable tool to manipulate fatty acid saturation during fermentation. Regarding chain length, the longest fatty acid chains were observed at a neutral pH value of 7. Deviations from neutrality, both towards acidic and basic conditions, led to the production of shorter fatty acid chains. This trend indicates that extreme pH values, either too acidic or too basic, might disrupt the enzymatic processes involved in elongation, resulting in shorter fatty acids. The two identifiable trends are primarily attributable to a shift from C18:1 to C18:2, as illustrated in the supplementary information (Fig. S6). The proportion of C16:0 remains constant at 20–22 % over the majority of pH values, but rises to 27 % at pH 5.5, suggesting that there may be even more significant deviations at even more acidic pH values. Very similar trends were reported in the literature for the organism *R. glutinis*. A comparatively constant proportion of C16:0 and an increasing overall degree of unsaturation with increasing pH value were observed (Johnson et al., 1992). The influence of pH on fatty acid composition, in general, has been investigated for certain fatty acids and different organisms, but it appears that the effects and deducible trends are quite organism-specific (Ochoa-Alfaro et al., 2019; Sakarika and Kornaros, 2016).

The results show that pH adjustment during the production phase has a significant impact on both the saturation and chain length of the produced triglycerides. Higher pH levels promote unsaturation, while neutral pH favors the formation of longer chains. Given the ease of pH control during the production process, these findings highlight the potential of using pH manipulation to tailor the fatty acid profile to specific industrial or consumer needs.

3.6. Supplementation of organic acids and their influence on the triglyceride production and fatty acid composition

Another parameter that could be readily adjusted to modify the fatty acid profile is the introduction of organic acids to the fermentation process. The acids were only incorporated after nitrogen limitation, i.e., at the onset of triglyceride production, and could then serve as an additional carbon source for triglyceride production. The fatty acid compositions of the produced triglycerides are depicted in Fig. 8.

Fig. 8 compares the fatty acid composition of triglycerides produced in the presence of different organic acids, normalized to the reference process where no additives were used (pH 6.5, stopped after 144 h). When acetic acid was added, the fatty acid composition remained nearly identical to that of the reference process. There were no significant changes in the proportions of C16:0, C18:1, or C18:2, suggesting that acetic acid does not strongly influence the enzymatic pathways involved in fatty acid synthesis under the tested conditions. Literature suggests that acetic acid should indeed influence the fatty acid composition of produced triglycerides (Huang et al., 2018) but is also dependent on the applied concentration. It is therefore plausible that the used acetic acid concentration of 10 g•L^{-1} was insufficient to induce significant alterations in the fatty acid profile. In contrast to acetic acid supplementation, the addition of citric acid increased the proportion of C16:0 by 36 % compared to the reference process, significantly altering the triglyceride profile. This increase in C16:0 was accompanied by a marked reduction in both C18:1 and C18:2 proportions. Additionally, the C16:1 content showed a slight increase. These changes indicate that citric acid strongly influences the fatty acid biosynthetic pathways, favoring the production of saturated, shorter-chained fatty acids at the expense of longer-chain unsaturated fatty acids. The relationship between the use of citric acid as a carbon source and fatty acid synthesis has been described at the metabolic level for bacteria (Kelly and Hughes, 2001), as well as in general for oleaginous organisms (Ratledge, 2004; Ratledge and Wynn, 2002). The latter study underscores the significant impact of citric acid, as ATP citrate lyase and the associated acetyl-CoA, which is essential for fatty acid synthesis, are directly related to the presence of citric acid. Therefore, it is reasonable to conclude that the introduction of supplementary citric acid will have a significant effect on fatty acid synthesis. Unfortunately, Ratledge, 2004 does not provide any data regarding the fatty acid composition of experiments conducted with the addition of citric acid. Consequently, it is impossible to determine the precise impact that the addition of citric acid should have on the fatty acid composition from this publication (Ratledge, 2004). Another study, which examined the fatty acid composition of neutral fats during parallel accumulation of citric acid in *Aspergillus niger*, did not observe any significant alteration in the fatty acid composition (Jernejc et al., 1989). However, it is questionable whether this process is at all comparable to the one presented here. In addition, as with the other parameters tested, there will also be a certain dependency on the respective organism.

The presented results demonstrate that different organic acids can lead to significant changes in the fatty acid composition of triglycerides produced by *U. maydis*. This highlights the importance of medium composition in controlling and potentially tailoring fatty acid profiles during triglyceride production.

4. Conclusion and outlook

This study highlights the impact of various cultivation parameters on the fatty acid profile of triglycerides produced by *U. maydis*, underscoring their potential for targeted applications in the production of plant oil substitutes.

The initial assessment reveals that cultivation time significantly influences fatty acid composition, with longer durations leading to more unsaturated and longer fatty acid chains. Consequently, it is essential to take cultivation time into account when comparing different production conditions. The impact of the carbon source is modest, but nutrient limitations (nitrogen, phosphorus, sulfur) significantly alter the fatty acid profiles, highlighting the importance of nutrient management. Oxygen availability is a critical factor in aerobic metabolism, wherein reduced oxygen levels can increase chain length but decrease unsaturation until a threshold is reached. However, low oxygen levels can

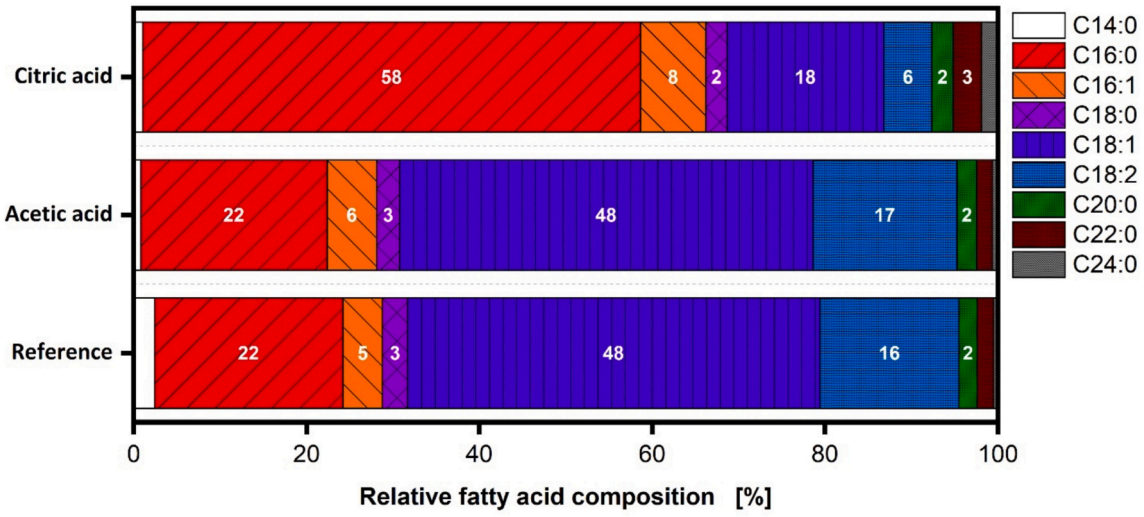

Fig. 8. Fatty acid composition of the analyzed triglyceride samples derived from cultivations with different supplemented organic acids in the triglyceride production phase. Acetic acid and citric acid were supplemented in a concentration of 10 and 38.4 g•L^{-1} (0.2 M) respectively, following the onset of nitrogen limitation. The x-axis represents the different fatty acids attached to the glycerol backbone in weight percent. The different fatty acid species are presented by the color code on the right with the first number standing for the total length and the second number for the number of double bonds in the fatty acid chain. Values are only depicted if their value exceeds 2 *w*/w %. Cultivation conditions: $C_{carbon\ source}$ = 100 g•L^{-1} glucose; nitrogen-limited; pH = 6.5.

impair metabolic rates, which may limit their suitability for industrial processes. Furthermore, pH adjustments have been observed to affect fatty acid profiles, with higher pH enhancing unsaturation and neutral pH achieving higher overall chain lengths. The effects of organic acid supplementation vary depending on the specific acid in question. The presence of acetic acid has no significant impact on the composition, while the addition of citric acid was observed to increase the palmitic acid content by 36 %. The potential for utilizing the diverse range of organic acids serves to further underscore the adaptability of microbial oil production processes.

In summary, the manipulation of cultivation parameters such as time, carbon source, nutrient limitation, oxygen levels, pH, and organic acid supplementation can effectively tailor microbial triglyceride composition. These modifications hold potential for replicating diverse plant oils like palm or avocado oil through tailored microbial processes. Despite necessary economic considerations at extreme parameter adjustments, *U. maydis*-derived triglycerides offer consistent and customizable alternatives to conventional plant oils.

CRediT authorship contribution statement

Paul Richter: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Marie Maßjosthusmann:** Investigation. **Thomas Seidel:** Investigation. **Leonard Walter:** Investigation. **Katharina Miebach:** Writing – review & editing, Methodology, Conceptualization. **Marcel Mann:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Jochen Büchs:** Writing – review & editing, Supervision, Funding acquisition. **Kerstin Schipper:** Writing – review & editing. **Michael Feldbrügge:** Writing – review & editing, Funding acquisition. **Janis Goeke:** Validation, Methodology, Investigation. **Dominik Marcel Wieland:** Writing – review & editing, Investigation. **Heiko Hayen:** Writing – review & editing, Conceptualization. **Jørgen Barsett Magnus:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Maja Sophie Kreuznacht for performing GC-FID measurements and processing the raw data, as well as Prof. Dr. Michael Bölker and Dr. Björn Sandrock (Faculty of Biology, Philipps University of Marburg, Marburg, Germany) for providing the strain of *U. maydis* used in this work.

Funding

The scientific activities of the Bioeconomy Science Center were financially supported by the Ministry of Culture and Science within the framework of the NRW Strategieprojekt BioSC (No. 005-2012-0107). Open Access funding enabled and organized by Projekt DEAL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2025.102119.

Data availability

Data will be made available on request.

References

Aguilar, L.R., Pardo, J.P., Lomelí, M.M., Bocardo, O.I.L., Juárez Oropeza, M.A., Guerra Sánchez, G., 2017. Lipid droplets accumulation and other biochemical changes induced in the fungal pathogen *Ustilago maydis* under nitrogen-starvation. Arch. Microbiol. 199, 1195–1209. https://doi.org/10.1007/s00203-017-1388-8.

Anderlei, T., Büchs, J., 2001. Device for sterile online measurement of the oxygen transfer rate in shaking flasks. Biochem. Eng. J. 157–162. https://doi.org/10.1016/s1369-703x(00)00116-9.

Anderlei, T., Zang, W., Papaspyrou, M., Büchs, J., 2004. Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. Biochem. Eng. J. 187–194. https://doi.org/10.1016/S1369-703X(03)00181-5.

Athenaki, M., Gardeli, C., Diamantopoulou, P., Tchakouteu, S.S., Sarris, D., Philippoussis, A., Papanikolaou, S., 2018. Lipids from yeasts and fungi: physiology, production and analytical considerations. J. Appl. Microbiol. 124, 336–367. https://doi.org/10.1111/jam.13633.

Beopoulos, A., Cescut, J., Haddouche, R., Uribelarrea, J.-L., Molina-Jouve, C., Nicaud, J.-M., 2009. *Yarrowia lipolytica* as a model for bio-oil production. Prog. Lipid Res. 48, 375–387. https://doi.org/10.1016/j.plipres.2009.08.005.

Carlson, K.M., Garrett, R.D., 2018. Environmental Impacts of Tropical soybean and Palm Oil Crops. Oxford research encyclopedia of environmental science. https://doi.org/10.1093/acrefore/9780199389414.013.234.

Damude, Howard G., Gillies, Peter John, Macool, Daniel Joseph, Picataggio, Stephen K., Walters, Dana M., Pollak, James John, Ragghianti, Zhixiong Xue, Yadav, Narendra S., Zhang, Hongxiang, Zhu, Quinn Qun, 2009. High arachidonic acid producing strains of *Yarrowia lipolytica*. Patent number: US7932077B2.

Davies, R.J., Holdsworth, J.E., Reader, S.L., 1990. The effect of low oxygen uptake rate on the fatty acid profile of the oleaginous yeast *Apiotrichum curvatum*. Appl. Microbiol. Biotechnol. 33, 569–573. https://doi.org/10.1007/BF00172553.

Fonseca, C., Romão, R., Rodrigues de Sousa, H., Hahn-Hägerdal, B., Spencer-Martins, I., 2007. L-Arabinose transport and catabolism in yeast. FEBS J. 274, 3589–3600. https://doi.org/10.1111/j.1742-4658.2007.05892.x.

Gong, Y., Guo, X., Wan, X., Liang, Z., Jiang, M., 2013. Triacylglycerol accumulation and change in fatty acid content of four marine oleaginous microalgae under nutrient limitation and at different culture ages. J. Basic Microbiol. 53, 29–36. https://doi.org/10.1002/jobm.201100487.

Granger, L.-M., Perlot, P., Goma, G., Pareilleux, A., 1993. Effect of various nutrient limitations on fatty acid production by *Rhodotorula glutinis*. Appl. Microbiol. Biotechnol. 38, 784–789. https://doi.org/10.1007/BF00167145.

Grebe, L.A., Lichtenberg, P.G., Hürter, K., Forsten, E., Miebach, K., Büchs, J., Magnus, J. B., 2024. Phosphate limitation enhances malic acid production on nitrogen-rich molasses with *Ustilago trichophora*. Biotechnol. Biofuels Bioprod. 17, 92. https://doi.org/10.1186/s13068-024-02543-z.

Gunstone, F., 2011. Vegetable Oils in Food Technology: Composition, Properties and Uses, 2nd ed. Wiley-Blackwell, Oxford. 353 pp. ISBN: 9781444339918.

Helm, T., Niehoff, P.-J., Gätgens, J., Stausberg, T., Pichler, B., Häßler, T., Wiechert, W., Büchs, J., Wierckx, N., Noack, S., 2023. Introducing molasses as an alternative feedstock into itaconate production using *Ustilago* sp. N. Biotechnol. 77, 30–39. https://doi.org/10.1016/j.nbt.2023.06.003.

Hewald, S., Josephs, K., Bölker, M., 2005. Genetic analysis of biosurfactant production in *Ustilago maydis*. Appl. Environ. Microbiol. 71, 3033–3040. https://doi.org/10.1128/AEM.71.6.3033-3040.2005.

Huang, X.-F., Shen, Y., Luo, H.-J., Liu, J.-N., Liu, J., 2018. Enhancement of extracellular lipid production by oleaginous yeast through preculture and sequencing batch culture strategy with acetic acid. Bioresour. Technol. 247, 395–401. https://doi.org/10.1016/j.biortech.2017.09.096.

Jernejc, K., Vendramin, M., Cimerman, A., 1989. Lipid composition of *Aspergillus Niger* in citric acid accumulating and nonaccumulating conditions. Enzyme Microb. Technol. 11, 452–456. https://doi.org/10.1016/0141-0229(89)90142-7.

Jirout, J., 2015. The effect of oxygen on fatty acid composition of soil micromycetes. Ecol. Indic. 53, 125–128. https://doi.org/10.1016/j.ecolind.2015.01.035.

Johnson, V., Singh, M., Saini, V.S., Sista, V.R., Yadav, N.K., 1992. Effect of pH on lipid accumulation by an oleaginous yeast: *Rhodotorula glutinis* IIP-30. World J. Microbiol. Biotechnol. 8, 382–384. https://doi.org/10.1007/BF01198749.

Kelly, D.J., Hughes, N.J., 2001. The citric acid cycle and fatty acid biosynthesis. In: Mobley, H.L.T., Mendz, G.L., Hazell, S.L. (Eds.), *Helicobacter pylori:* Physiology and Genetics. ASM Press, Washington, DC, pp. 135–146.

Klein, H.P., 1955. Synthesis of lipids in resting cells of Saccharomyces cerevisiae. J. Bacteriol. 69, 620–627. https://doi.org/10.1128/jb.69.6.620-627.1955.

Klement, T., Milker, S., Jäger, G., Grande, P.M., Domínguez de María, P., Büchs, J., 2012. Biomass pretreatment affects *Ustilago maydis* in producing itaconic acid. Microb. Cell Fact. 11, 43. https://doi.org/10.1186/1475-2859-11-43.

Krist, S., 2013. Lexikon der pflanzlichen Fette und Öle. Springer Vienna, Vienna, 843 pp. (ISBN: 978-3-7091-1004-1).

Lattermann, C., Funke, M., Hansen, S., Diederichs, S., Büchs, J., 2014. Cross-section perimeter is a suitable parameter to describe the effects of different baffle geometries in shaken microtiter plates. J. Biol. Eng. 8, 18. https://doi.org/10.1186/1754-1611-8-18.

Matyash, V., Liebisch, G., Kurzchalia, T.V., Shevchenko, A., Schwudke, D., 2008. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J. Lipid Res. 49, 1137–1146. https://doi.org/10.1194/jlr.D700041-JLR200.

Müller, M.J., Stachurski, S., Stoffels, P., Schipper, K., Feldbrügge, M., Büchs, J., 2018. Online evaluation of the metabolic activity of *Ustilago maydis* on (poly)galacturonic acid. Journal of biological engineering 12, 34. https://doi.org/10.1186/s13036-018-0128-1.

Ochoa-Alfaro, A.E., Gaytán-Luna, D.E., González-Ortega, O., Zavala-Arias, K.G., Paz-Maldonado, L.M.T., Rocha-Uribe, A., Soria-Guerra, R.E., 2019. pH effects on the lipid and fatty acids accumulation in *Chlamydomonas reinhardtii*. Biotechnol. Prog. 35, e2891. https://doi.org/10.1002/btpr.2891.

Papanikolaou, S., Aggelis, G., 2011. Lipids of oleaginous yeasts. Part I: Biochemistry of single cell oil production. Eur. J. Lipid Sci. Technol. 113, 1031–1051. https://doi.org/10.1002/ejlt.201100014.

Patel, A., Karageorgou, D., Rova, E., Katapodis, P., Rova, U., Christakopoulos, P., Matsakas, L., 2020. An overview of potential oleaginous microorganisms and their role in biodiesel and omega-3 fatty acid-based industries. Microorganisms 8. https://doi.org/10.3390/microorganisms8030434.

Ratledge, C., 2004. Fatty acid biosynthesis in microorganisms being used for Single Cell Oil production. Biochimie 86, 807–815. https://doi.org/10.1016/j.biochi.2004.09.017.

Ratledge, C., Wynn, J.P., 2002. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv. Appl. Microbiol. 51, 1–51. https://doi.org/10.1016/s0065-2164(02)51000-5.

Richter, P., Panchalingam, J., Miebach, K., Schipper, K., Feldbrügge, M., Mann, M., 2024. Studying microbial triglyceride production from corn Stover saccharides unveils insights into the galactose metabolism of *Ustilago maydis*. Microb. Cell Fact. 23, 204. https://doi.org/10.1186/s12934-024-02483-1.

Ruenwai, R., Cheevadhanarak, S., Rachdawong, S., Tanticharoen, M., Laoteng, K., 2010. Oxygen-induced expression of Delta(6)-, Delta(9)- and Delta(12)-desaturase genes modulates fatty acid composition in *Mucor rouxii*. Applied microbiology and biotechnology86, 327–334. https://doi.org/10.1007/s00253-009-2338-4.

Sajbidor, J., Certík, M., Dobroňová, S., 1988. Influence of different carbon sources on growth, lipid content and fatty acid composition in four strains belonging to *mucorales*. Biotechnol. Lett. 10, 347–350. https://doi.org/10.1007/BF01026163.

Sakarika, M., Kornaros, M., 2016. Effect of pH on growth and lipid accumulation kinetics of the microalga *Chlorella vulgaris* grown heterotrophically under sulfur limitation. Bioresour. Technol. 219, 694–701. https://doi.org/10.1016/j.biortech.2016.08.033.

Stutzenberger, F.J., Jenkins, T.C., 1995. Effect of carbon source on growth temperature and fatty-acid composition in *Thermomonospora curvata*. World journal of microbiology & biotechnology 11, 621–624. https://doi.org/10.1007/BF00361003.

Su, G., Jiao, K., Li, Z., Guo, X., Chang, J., Ndikubwimana, T., Sun, Y., Zeng, X., Lu, Y., Lin, L., 2016. Phosphate limitation promotes unsaturated fatty acids and arachidonic acid biosynthesis by microalgae *Porphyridium purpureum*. Bioprocess Biosyst. Eng. 39, 1129–1136. https://doi.org/10.1007/s00449-016-1589-6.

Utami, T.S., Arbianti, R., Nayanda, C., Nuraliyah, A., Wijanarko, A., 2020. The effect of time cultivation in production of AA and EPA from *Aspergillus oryzae* with three-stage fermentation strategy. AIP Conf. Proc. 2230, 20014. https://doi.org/10.1063/5.0002777.

Uusitalo, V., Väisänen, S., Havukainen, J., Havukainen, M., Soukka, R., Luoranen, M., 2014. Carbon footprint of renewable diesel from palm oil, jatropha oil and rapeseed oil. Renew. Energy 69, 103–113. https://doi.org/10.1016/j.renene.2014.03.020.

Verduyn, C., Postma, E., Scheffers, W.A., van Dijken, J.P., 1992. Effect of benzoic acid on metabolic fluxes in yeasts: a continuous-culture study on the regulation of respiration and alcoholic fermentation. Yeast (Chichester, England) 8, 501–517. https://doi.org/10.1002/yea.320080703.

Zha, J., Yuwen, M., Qian, W., Wu, X., 2021. Yeast-based biosynthesis of natural products from xylose. Front. Bioeng. Biotechnol. 9, 634919. https://doi.org/10.3389/fbioe.2021.634919.

Zhuang, X.-Y., Zhang, Y.-H., Xiao, A.-F., Zhang, A.-H., Fang, B.-S., 2022. Key Enzymes in Fatty Acid Synthesis Pathway for Bioactive Lipids Biosynthesis. Front. Nutr. 9, 851402. https://doi.org/10.3389/fnut.2022.851402.