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Development and Production of High-Oleic Palm Oil Alternative by Fermentation of Microalgae

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Abstract: The development of high-oleic palm oil alternatives through microbial fermentation offers a sustainable solution to the environmental challenges associated with traditional palm oil cultivation. In this study, a *Prototheca moriformis* microalgae strain was optimized via classical strain improvement techniques to produce a high-oleic palm oil with fatty acid and triacylglycerol (TAG) profiles similar to those of conventional high-oleic palm oil. Iterative rounds of mutagenesis and screening enhanced the palmitic acid content from 28 to 30–32% and oleic acid from 60 to 55–57% of total fatty acids, with an oil yield of 136.5 g/L and an oil content of 69.45% of the dry cell weight. The scalability of this process was demonstrated across fermentation scales ranging from 1 L to 50 L. The TAG profile showed elevated unsaturated TAG species, meeting the quality and nutritional requirements of industrial applications. These findings highlight the potential of microbial systems to address the growing demand for high-value nutritional oils while alleviating the environmental and socio-economic impacts of tropical oil crop cultivation. The application of *P. moriformis* fermentation provides a transformative approach to advancing sustainability and resilience in global fat and oil production.

Keywords: algal oil; fermentation; high-oleic palm oil; microalgae

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1. Introduction

The demand for high-oleic acid oils is driven by their superior oxidative stability, making them ideal for food processing, cosmetics, and biodiesel applications while also offering health benefits such as improved cardiovascular health. However, major oils typically lack a high abundance of oleic acid and to meet market demands; significant research has focused on developing high-oleic soy, rapeseed, and camelina oils, primarily through genetic modifications and, more recently, gene editing [1]. In the context of oil palms, interspecific OxG hybrids were developed through conventional breeding to address bud rot disease. Some of these cultivars exhibit high-oleic genotypes, resulting in hybrids that produce oils with over 55% oleic acid content and 33% saturated fatty acid content [2,3]. These OxG hybrids are a product of crossing the American oil palm (*Elaeis oleifera*) with the African oil palm (*Elaeis guineensis*) and constitute 12% of the total oil palm plantation area in Colombia [4]. Notably, these hybrids are highly productive, with an average production of 38 metric tons per hectare per year of fresh fruit bunches (FFBs) in certain regions of Colombia, and some plantations obtain nearly 45 metric tons per hectare per year [5]. In addition to their high productivity and superior oil quality, OxG hybrids exhibit a slow growth rate. However, they require assisted pollination using pollen from *E. guineensis* to produce well-formed bunches with oil content comparable to *E. guineensis* cultivars [6]. This necessary assisted pollination is both expensive and labor-intensive, accounting for approximately 18% of the crop's total annual cost, which negatively impacts the crop's economic viability [7]. Tissue culture techniques, including large-scale micropropagation, have been explored to address these limitations, ensuring the efficient multiplication of high-quality planting materials [8]. Recent genomic and agronomic studies have further enhanced the understanding of OxG hybrid performance under diverse environmental conditions, solidifying its role as a sustainable crop. The oil derived from OxG hybrids has garnered attention due to its unique composition and broad industrial applicability. Its high oleic acid content, reduced saturated fat levels, and excellent oxidative stability make OxG oil particularly valuable for food processing, cosmetics, and biodiesel production [2,9,10]. In the premium market segment, the oil meets rising consumer demand for healthier edible oils, especially in health-conscious markets. Its oxidative stability extends the shelf life of processed foods and makes it suitable for high-temperature cooking, a critical feature for industrial and household applications [10]. Globally, OxG oil production is concentrated in Latin America, with Colombia, Brazil, and Ecuador leading the supply chain. These countries capitalize on the hybrid's resilience to local diseases and environmental challenges.

The rising demand for high-oleic palm oil has further highlighted the need for sustainable alternatives to tropical oil crops. Microbial fermentation, particularly utilizing microalgae such as *Prototheca moriformis*, offers a transformative solution. Our recent advancements [11,12] underscore the remarkable versatility of *P. moriformis* as a technology and production platform, capable of generating oils with diverse compositions tailored for specific industrial applications. By employing genetic engineering and/or classical strain optimization, *P. moriformis* has proven to be a scalable and efficient lipid production platform in both temperate and tropical regions alike such as Brazil, the United States, Canada, Japan, and Europe.

We have previously demonstrated [11] that *P. moriformis* can be genetically engineered to produce oils mimicking human milk fat, achieving the precise incorporation of palmitic acid into the *sn*-2 position of triacylglycerols (TAG) with high fidelity. The process consistently achieved oil titers exceeding 150 g/L of fermentation broth, highlighting its industrial scalability. Similarly, we have optimized *P. moriformis* for high-oleic acid production, enhancing oleic acid levels in excess of 85% of total fatty acids while maintaining high oil productivity up to 145 g/L [12]. These studies illustrate the flexibility of *P. moriformis* to produce oils with diverse functional properties, such as high-oleic oils and structured lipids, depending on the specific strategy employed. The scalability and robustness of *P. moriformis*, demonstrated through fermentation trials, further emphasize its suitability for industrial applications. Its ability to utilize various carbon sources and adapt to large-scale processes positions *P. moriformis* as a relevant technology in the sustainable production of high-value oils for applications in food, cosmetics, biofuels, and specialty chemicals.

This study aims to further leverage our *P. moriformis* production platform, applying classical strain optimization techniques, to develop a strain capable of producing an oil with a fatty acid and TAG composition comparable to high-oleic palm oil. The selection of *P. moriformis* for this study is supported by its superior performance in lipid productivity compared to other well-established oleaginous microorganisms, especially in terms of oil titer and scalability [12]. *P. moriformis* demonstrates exceptional lipid productivity, significantly higher than species such as the widely studied yeast *Yarrowia lipolytica* [12]. By tailoring the fatty acid profile and ensuring industrial scalability, this research seeks to reduce reliance on tropical oil crops, thereby mitigating environmental concerns such as deforestation and biodiversity loss. The resulting high-oleic algal oil holds the potential to meet the rigorous functional and nutritional requirements of diverse industries.

2. Materials and Methods

Palm oil samples: Crude high-oleic palm oil samples were obtained from Palmas de Tumaco (Bogotá, Colombia). Conventional red palm oil was obtained in a local market (San Francisco, CA, USA).

Strain development. The *P. moriformis* wild-type strain (UTEX 1533) was received from the University of Texas (Austin, TX, USA) and classical strain improvement initiated with a baseline of 28% and 60% palmitic and oleic acids, respectively (Table 1). The experimental process to develop the high-oleic palm oil alternative from the parental strain involved several optimization techniques targeting lipid productivity and fatty acid profiles. Initially, the parental strain was chemically mutagenized using 44 µM 4-nitroquinoline 1-oxide (4-NQO) at 32 °C for 30 min. Stop The mutagen was neutralized with sodium thiosulfate and removed by washing. Following recovery in limited sugar media, the cells were cultured in lipid production media at 38 °C for five days, a temperature higher than optimal to stress the cells and select mutants predisposed to higher palmitate production. Surviving cells were then subjected to a heat shock at 65 °C for 4 min, which eliminated more than 99% of the population, allowing the selection of mutants. The resulting subclones were screened successively for high glucose consumption within lipid production media using a hexose kinase-based microtiter plate assay (e.g., ThermoScientific#TR15421), followed by a high-throughput FAME assay to screen for fatty acid profiles among the more limited selection of high-glucose-consuming subclones. These clones underwent additional rounds of serial passaging and phenotypic stability assessment to refine the desired traits. After five rounds, a stable strain, designated as the high-oleic palm oil alternative strain, was identified. This strain demonstrated improved lipid production metrics, including enhanced oil content, dry cell weight, and lipid titer, alongside a significant increase in palmitate levels compared to the parental strain (see Table 1).

Table 1. Key performance indicators (KPIs) for the high-oleic palm oil alternative strain in 1, 20, and 50 L vessels. Runs were carried out using a final nitrogen concentration of 320 mM. Differences reflect the lower oxygen transfer rate (OTR) achievable in the larger vessel, necessitating a process that would put fewer cells in place to stay within the capabilities of the fermenter. For each parameter (oleic and palmitic acids, dry cell weight, oil titer, and content), a one-sample *t*-test was used to determine whether or not the mean of the 6 fermentation runs with the high-oleic palm oil alternative strain was equal to parental strain value or not.

Fermentation Run	Oleic Acid (% of Total FA)	Palmitic Acid (% of Total FA)	Dry Cell Weight (DCW, g/kg)	Oil Titer (g/L)	Oil Content (% of DCW)
Parental strain, 1 L	60.47	28.00	197.00	132.50	67.25
High-oleic palm oil alternative strain					
Run 1, 1 L	57.00	30.55	204.00	142.20	69.71
Run 2, 1 L	56.90	29.98	186.30	131.00	70.32
Run 3, 1 L	57.35	29.67	200.30	139.90	69.85
Run 4, 1 L	55.29	31.34	201.70	142.50	70.65
Run 5, 20 L	55.70	31.10	192.70	129.30	67.10
Run 6, 50 L	56.14	31.60	194.10	134.10	69.09
Average	56.40	30.71	196.52	136.50	69.45
SD	0.81	0.77	6.66	5.79	1.27
Coeff. of var. (%)	1.44	2.51	3.39	4.25	1.83
% of differences vs. parental strain	−6.7%	+9.7%	−0.2%	+3.0%	+3.3%
p-value	<0.001	<0.001	0.846	0.152	0.008

Culture media composition. The culture conditions and media used were described in a previous paper [12]. Briefly, the vegetative growth medium comprised macronutrients including NaH2PO4, K2HPO4, citric acid monohydrate, magnesium sulfate heptahydrate, calcium chloride dihydrate, and dextrose at 1.64, 1.98, 1.05, 1.23, 0.02, and 40 g/L, respectively. Ammonium sulfate served as the sole nitrogen source at 1.0 g/L. Antifoam (Sigma 204, Sigma-Aldrich, St Louis, MO, USA) was added to a final concentration of 0.225 mL/L. Trace minerals were prepared as a 1000 X stock solution comprising boric acid, zinc sulfate heptahydrate, manganese sulfate monohydrate, sodium molybdate dihydrate, nickel nitrate heptahydrate, citric acid monohydrate, copper (II) sulfate pentahydrate, and iron (II) sulfate heptahydrate at 0.91, 1.76, 1.23, 0.05, 0.04, 20.49, 0.05, and 0.75 g/L, respectively. A 1000 X vitamin stock comprised thiamine HCl, D-pantothenic acid hemicalcium salt, biotin, cyanocobalamin, riboflavin, and pyridoxine HCl at 3.0, 0.16, 0.0048, 0.00034, 0.015, and 0.0078 g/L, respectively. The lipid production medium was identical to the vegetative growth medium except that ammonium sulfate was supplied at 0.2 g/L. All solutions were filter-sterilized prior to use.

Fermentation of a *P. moriformis* high oleic palm alternative strain. The fermentation process was scaled from 1 L to 20 L as described previously [12]. Typical fermentation results obtained at the 1 and 20 and 50 L scales are provided in Table 1. The fermentation conditions utilized included an inoculation volume of 0.25–0.3% of the fermenter volume and pH and dissolved oxygen (DO) setpoints of 5.5–6.0 and 30%, respectively. The operating temperature was 28 °C throughout, with aeration and agitation managed to maintain DO. The fermentation medium was fortified to support the higher cell density in reactors by increasing vitamin and trace metals solutions 10- and 15-fold, respectively. Macronutrients (sodium phosphate, potassium phosphate, citric acid monohydrate, magnesium sulfate heptahydrate, and calcium chloride dihydrate) were increased to 7.13, 9.25, 2.1, 17.33, and 0.8 g/L, respectively. The glucose feed was comprised of a 55% wt:wt sterile solution.

Production of algal oil. After fermentation [12], drying of algal fermentation broth was carried out using a double-drum dryer (Buflovak Model ADDD operating at 70 psig steam and 1200 rpm; Buflovak, Tonawanda, NY, USA) followed by mechanical/solvent extraction utilizing a 6:1 hexane-to-biomass ratio carried out for up to 6 h in an MSE Pro planetary ball mill (10 L capacity). Micellae resulting from solvent extraction were subsequently roto-vaped to remove hexane, followed by degumming using citric acid (0.2% wt:wt of a 50% solution) at 130 °C for 10 min with agitation. The removal of gums was carried out by centrifugation at 3000 rpm for 10 min, after which de-gummed oil was decanted for further processing. This was followed by bleaching under vacuum (685.8 mm Hg) at a temperature of 90–110 °C using 2% (wt:wt) bentonite bleaching earth. The bleaching clay and oil were separated by filtration under vacuum followed by steam deodorization under vacuum (<25 in Hg (or 635 mm Hg) at 200 °C for 90 min), resulting in refined, bleached, and deodorized (RBD) oil.

Fatty acid (FA) analysis. The fatty acid composition of the algal and high-oleic palm oil samples were measured as their fatty acid methyl esters (FAMEs) following direct transesterification with a sulfuric acid methanol solution [12]. The samples were injected into an Agilent 8890 gas chromatography (GC) system equipped with a split/splitless inlet and flame ionization detector (Agilent Technologies, Palo Alto, CA, USA). An Agilent DB-WAX column (30 m $\times$ 0.32 mm $\times$ 0.25 μm) was used for chromatographic separation of the FAME peaks. A FAME standard mixture purchased from Nu-Chek Prep (Nu-Chek Prep Inc., Elysian, MN, USA) was injected to establish retention times. Response factor corrections were determined using various standard mixtures from Nu-Chek Prep. Methyl nonadecanoate (19:0) was used as an internal standard for quantitation of individual FAMEs.

Triacylglycerol (TAG) and diacylglycerol (DAG) analysis. TAG profiles were determined as described previously using an Agilent 1290 Infinity II UHPLC system coupled to a 6470B triple quadrupole mass spectrometer and APCI ionization source according to the parameters described previously [11,13]. Diacylglycerol (DAG) ion ratios were used to assess the relative abundance of TAG regio-isomers, and quantification was performed using a calibration curve of pure standards.

Statistical analysis. The fermentation performance data (Table 1) of the high-oleic palm oil alternative strain were tested for normality using the Shapiro–Wilk test. One-sample *t*-tests were then used to determine whether or not the mean of the 6 fermentation runs with the high-oleic palm oil alternative strain was equal to the parental strain value, for each parameter (oleic and palmitic acids, dry cell weight, oil titer, and content). The fatty acid composition and triacylglycerol (TAG) profile are presented as percentages of total fatty acids. Saturated and unsaturated fatty acid proportions were compared using chi-square tests (high-oleic palm oil alternative vs. high-oleic palm oil vs. regular palm oil). Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC, USA). The statistical significance was set at 0.05.

3. Results

3.1. Development of High-Oleic Palm Oil Alternative Strain

To develop the high-oleic palm oil alternative strain from the parental strain, a classical strain improvement strategy was employed. The parental strain cells were chemically mutagenized, followed by neutralization and recovery in limited sugar media. The cells were cultured in lipid production media for five days to induce stress conditions and select for mutants with improved lipid production. A subsequent heat shock eliminated over 99% of the population, allowing for further selection of robust mutants. Surviving clones were subjected to high-throughput screening for glucose consumption and fatty acid profiles, with selected clones undergoing multiple rounds of passaging to ensure phenotypic stability. The optimized high-oleic palm oil alternative strain exhibited a significant increase in palmitic acid content at the *sn*-1(3) position of TAGs compared with the parental strain. Fermentation performance demonstrated enhanced oil content and improved lipid production metrics, including increased lipid titer and dry cell weight, establishing its superior capability for industrial-scale lipid synthesis.

3.2. Fermentation Trials

The high-oleic palm oil alternative strain, developed from *Prototheca moriformis* through classical strain optimization, exhibited robust fermentation performance at both 1 and 20 L scales (Table 1). The optimized strain consistently delivered elevated oil yields and surpassed the parental strain in oil titer and productivity, with an average palmitic acid content of 30.71%, oleic acid content of 56.40%, and oil content of 69.45% on a dry cell weight (DCW) basis. Fermentation runs showed dry cell weights ranging from 186.30 to 204.00 g/kg, an average oil titer of 136.50 g/L with low coefficient of variation (4.25%), confirming the reproducibility of the process. Compared to the parental strain, the improved strain exhibited a 6.7% decrease in oleic acid ($p < 0.001$) and a 9.7% increase in palmitic acid ($p < 0.001$), along with a 3.3% increase in oil content ($p = 0.008$). No significant difference was observed in the dry cell weight ($p = 0.846$) and oil titer ($p = 0.152$), indicating comparable biomass production. These findings confirm a successful metabolic shift in the improved strain, enhancing its suitability as a sustainable and efficient alternative for high-oleic palm oil production.

3.3. Fatty Acid (FA) and Triacylglycerol (TAG) Profile

High-oleic palm oil has a fatty acid profile enriched in oleic acid (18:1 n-9, 49.21%) compared to regular palm oil (39.07%), while containing less palmitic acid (16:0, 32.71% vs. 42.83%) (Table 2). This higher unsaturated-to-saturated fatty acid ratio makes it a healthier alternative [14]. The high-oleic palm oil alternative strain closely mirrored this composition, with oleic acid levels ranging from 55 to 57%, surpassing those of the high-oleic palm oil, while the palmitic acid remained comparable (30–32%) (Tables 1 and 2). The total unsaturated (63.57%) and saturated (36.21%) fatty acid levels also closely aligned with those of the high-oleic palm oil (62.96% and 36.86%, respectively), confirming the strain's ability to replicate its desired properties (no statistically significant difference between the two strains' composition ($p = 0.926$)). The total unsaturated (63.57%) and saturated (36.21%) fatty acid levels of the high-oleic palm oil alternative strain differed from those of the regular palm oil (49.68% and 50.07%, respectively). The differences in proportions showed a trend toward significance ($p = 0.054$), but did not meet the conventional cutoff of $p < 0.05$.

Table 2. The fatty acid (FA) composition of the algal high-oleic palm oil alternative, high-oleic palm oil, and conventional palm oil. Results are expressed as % of total fatty acids. Saturated and unsaturated fatty acid proportions were compared using chi-square tests (high-oleic palm oil alternative vs. high-oleic palm oil and vs. regular palm oil).

Fatty Acids	High-Oleic Palm Oil Alternative	High-Oleic Palm Oil	Regular Palm Oil
12:0	0.03	0.06	0.40
14:0	1.24	0.50	1.00
16:0	32.05	32.71	42.83
16:1 n-7	1.34	0.38	0.18
17:0	0.07	0.10	0.10
18:0	2.41	3.08	4.85
18:1 n-9	55.53	49.21	39.07
18:2 n-6	6.30	12.84	10.39
18:3 n-3	0.30	0.40	0.28
20:0	0.28	0.28	0.36
20:1	0.11	0.14	0.15
22:0	0.08	0.06	0.07
24:0	0.05	0.09	0.08
Other FA	0.22	0.18	0.25
Saturated FA	36.21	36.86	49.68
Unsaturated FA	63.57	62.96	50.07
Comparison of Unsaturated/Saturated Proportions (Chi-Square):			
High-Oleic Palm Oil Alternative vs. High-Oleic Palm Oil: $p = 0.926$			
High-Oleic Palm Oil Alternative vs. Regular Palm Oil: $p = 0.054$			
High-Oleic Palm Oil vs. Regular Palm Oil: $p = 0.066$			

In addition, the high-oleic palm oil alternative exhibited distinct compositional shifts, with higher oleic acid (+12.8%) and lower linoleic acid (−50.9%) relative to high oleic palm oil, favoring a more monounsaturated lipid profile. Additionally, myristic acid (14:0, +148.0%) and palmitoleic acid (16:1, +252.6%) were elevated. Despite these changes, the overall fatty acid distribution remained comparable, reinforcing the alternative strain's suitability as a high-oleic palm oil replacement with enhanced monounsaturated content. These results confirm that the high-oleic palm oil alternative closely mimicked the fatty acid distribution of the high-oleic palm oil, while exhibiting an enhanced monounsaturated fatty acid profile.

This close compositional alignment underscores the effectiveness of the algal strain as an alternative to high-oleic palm oil.

The TAG profile of the high-oleic palm oil significantly differed from that of the regular palm oil (p = 0.006), with a higher proportion of unsaturated–unsaturated–unsaturated (Unsat–Unsat–Unsat) TAG species (20.32% vs. 9.39%) and lower levels of saturated–saturated–saturated (Sat–Sat–Sat) TAG species (0.75% vs. 4.99%, see Table 3). This reflects the enhanced unsaturation characteristic of high-oleic palm oil, contributing to its improved functional and nutritional qualities [14].

Table 3. The triacylglycerol (TAG) composition of the high-oleic palm oil algal alternative, high-oleic palm oil, and conventional palm oil. Results are expressed as % of total TAG. Saturated and unsaturated fatty acid proportions were compared using chi-square tests (high-oleic palm oil alternative vs. high-oleic palm oil and vs. regular palm oil).

TAG Species	High-Oleic Palm Oil Alternative	High-Oleic Palm Oil	Regular Palm Oil
LLL	0.00	0.19	0.19
OLnL	0.00	0.15	0.00
PLnL	0.00	0.18	0.00
LOL	0.68	2.04	0.92
OOLn	0.21	0.00	0.00
LLP	0.77	3.38	2.92
MOL (+POLn)	0.58	0.00	0.00
POLn	0.38	0.45	0.00
MLP	0.00	0.18	0.24
OOL	4.26	6.21	2.58
POPo + PoLS	1.17	0.00	0.00
POL	7.41	14.16	11.12
OOM	3.03	0.00	0.00
PLP	2.30	5.81	7.43
MOP	1.48	0.00	0.89
OOO	15.17	11.73	5.70
SOL	0.00	0.00	1.32
sn-OOP	35.62	30.63	21.76
PLS	0.17	0.61	1.41
sn-POP	17.79	13.91	23.29
sn-PPO	0.56	1.83	2.41
PPP	0.28	0.66	4.08
OOS	2.81	2.75	3.66
POS	2.58	2.71	5.59
PPS	0.00	0.09	0.91
OOA	0.30	0.18	0.24
SOS + POA	0.30	0.29	0.60
Other TAG	2.15	1.86	2.74
Sat–Sat–Sat	0.28	0.75	4.99
Sat–Sat–Unsat	25.18	25.34	41.86
Sat–Unsat–Unsat	50.90	51.73	41.02
Unsat–Unsat–Unsat	20.32	20.32	9.39
Comparison of Unsaturated/Saturated Proportions (Chi-Square):			
High-Oleic Palm Oil Alternative vs. High-Oleic Palm Oil: p = 0.976			
High-Oleic Palm Oil Alternative vs. Regular Palm Oil: p = 0.004			
High-Oleic Palm Oil vs. Regular Palm Oil: p = 0.006			

Abbreviations used: M, myristic (14:0) acid; P, palmitic (16:0) acid; Po, palmitoleic (16:1 n-7) acid; S, stearic (18:0) acid; O, oleic (18:1 n-9) acid; L, linoleic (18:2 n-6) acid; Ln, alpha-linolenic (18:3 n-3) acid; A, arachidic (20:0) acid; Sat, saturated fatty acid; and Unsat, unsaturated fatty acid.

The high-oleic palm oil alternative strain exhibited a TAG composition closely matching that of the high-oleic palm oil ($p = 0.976$) and significantly different from that of the regular palm oil strain ($p = 0.004$). The primary TAG species in the high-oleic palm oil alternative and high-oleic palm oil remained OOP, POP, OOO, and OOL in descending abundance (Table 3, Figure 1). The alternative strain achieved a similar proportion of unsaturated–unsaturated–unsaturated (Unsat–Unsat–Unsat) TAG species (20.32%), exceeding that of the regular palm oil. Its saturated–saturated–saturated (Sat–Sat–Sat) TAG content (0.28%) was notably lower than that of the high-oleic palm oil (0.75%) and regular palm oil (4.99%). Additionally, the saturated–unsaturated–unsaturated (Sat–Unsat–Unsat) TAG species (50.90%) closely aligned with those of the high-oleic palm oil (51.73%) and remained higher than those of the regular palm oil (41.02%). These findings confirm the alternative strain's ability to replicate the desirable TAG distribution of high-oleic palm oil, reinforcing its potential as a sustainable alternative. Despite this overall similarity, the alternative strain exhibited notable TAG profile differences. Triolein (OOO, +29.3%), OOP (+16.3%), and POP (+27.9%) were higher, favoring a monounsaturated TAG-rich composition. Conversely, POL (−47.7%) and PLP (−60.4%) were lower, indicating a shift away from polyunsaturated TAG species. Several polyunsaturated TAG species (LLL, OLnL, PLnL, MLP) were entirely absent in the alternative strain. Meanwhile, OOLn (+100%), MOL (+100%), OOM (+100%), and POPo (+100%) increased, reflecting a redistribution of TAG species. Despite these variations, total unsaturated TAG content remained unchanged (20.32%), confirming the algae high-oleic palm oil alternative strain's ability to replicate the functional characteristics of high-oleic palm oil.

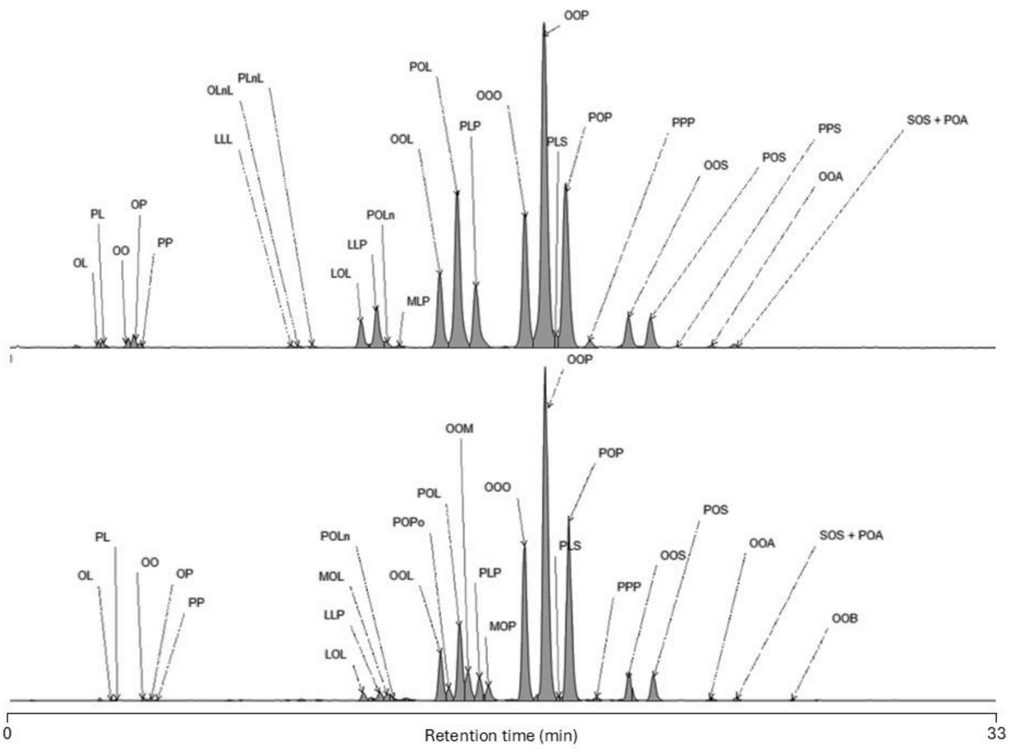

Figure 1. LC-MS chromatograms of triacylglycerol (TAG) and diacylglycerol (DAG) profiles of high-oleic palm oil (upper panel) and algal high-oleic palm oil alternative (lower panel). Abbreviations used: M, myristic (14:0) acid; P, palmitic (16:0) acid; Po, palmitoleic (16:1 n-7) acid; S, stearic (18:0) acid; O, oleic (18:1 n-9) acid; L, linoleic (18:2 n-6) acid; Ln, alpha-linolenic (18:3 n-3) acid; and A, arachidic (20:0) acids.

Crude high-oleic palm oil contained 2% diacylglycerols (DAGs, Figure 1), identified as dipalmitin (PP), diolein (OO), oleoyl–palmitoyl–glycerol (OP), oleoyl–linoleoyl–glycerol

(OL), and palmitoyl–linoleoyl–glycerol (PL). The occurrence of partial glycerides such as DAGs in high-oleic palm oil and its fractions arises from the characteristics of the raw materials and is a result of the oil's extraction from ripening fruits. In algal oil, the same DAG species were identified and the total amount was estimated to be 0.5% of the oil.

In summary, the high-oleic palm oil alternative algal strain demonstrated fatty acid and triacylglycerol profiles that were remarkably similar to those of the high-oleic palm oil, with nearly identical levels of unsaturated fatty acids and comparable TAG species distributions and a lower level of DAGs. These results highlight the strain's capacity to effectively replicate the overall composition of high-oleic palm oil, reinforcing its potential as a functional alternative to sources historically associated with deforestation, megafauna habitat loss, loss from disease/pests, and a lack of supply chain traceability and transparency in Southeast Asia (e.g., Indonesia, Malaysia, Thailand) and Colombia, reinforcing its potential as a sustainable and functional alternative.

4. Discussion

This study successfully demonstrated the development of a high-oleic palm oil alternative using the microalga *P. moriformis*. Through iterative strain optimization and the scaling of fermentation processes, the resulting algal oil closely matched the fatty acid and TAG profiles of the high-oleic palm oil. Key findings included an oleic acid content ranging from 55 to 57% of total fatty acids, with an oil yield of 136.5 g/L and an oil content of 69.45% of the dry cell weight (DCW). The TAG profile, characterized by high proportions of unsaturated TAG species, further reinforced the oil's functionality and nutritional value. These results highlight the potential of microbial fermentation to meet the growing demand for sustainable, scalable, and high-quality oil alternatives.

Our results align with previous studies that emphasize the utility of microbial fermentation for producing high-value lipids. Zhou and co-workers [11] demonstrated the scalability of *P. moriformis* to produce human milk fat analogs, achieving oil yields exceeding 150 g/L with customized fatty acid compositions. Similarly, studies by Silva and co-workers [15] and Ghazani and Marangoni [16] underlined the role of oleaginous microorganisms in generating high-oleic lipids with competitive industrial applications. The oleic acid content reported in this study (56.4%) is consistent with values achieved with other microbial species, such as *Yarrowia lipolytica* [17] and *Trichosporon capitatum* [18], which also exhibited fatty acid profiles tailored for nutritional and industrial purposes. Additionally, the TAG composition of the algal oil, with elevated unsaturated TAG species and reduced saturated TAG species, is comparable to findings from metabolic engineering studies on *Mortierella alpina* [19].

Earlier studies achieved oleic acid levels exceeding 86% of total fatty acids with an oil yield of 145 g/L at the industrial scale [12], while this work focused on replicating high-oleic palm oil's unique fatty acid and TAG profiles. Both studies underscore the robustness and flexibility of *P. moriformis* for sustainable oil production, with this study further emphasizing its capability to produce oils tailored for specific applications. This progress highlights the flexibility of classical strain improvement and fermentation scaling as tools to address evolving industry needs.

Microbial fermentation presents significant environmental benefits by reducing reliance on tropical oil crops such as palm oil. Utilizing oleaginous microorganisms like *Cutane-otrichosporon oleaginosus* (aka *Trichosporon oleaginosus*), researchers have developed sustainable oil production methods that offer a viable alternative to plant-derived oils [20]. The economic feasibility of microbial oil production might be further enhanced by utilizing low-cost substrates like glycerol [20]. In this context, microbial fermentation contributes to waste reduction and resource utilization by converting agricultural waste, such as cassava, potato, and yam peels, into enriched animal feed, thereby reducing environmental pollution [21]. Industrial by-products can also serve as fermentation substrates, promoting waste management and sustainability [22]. Despite these advantages, continued research and technological advancements are necessary to optimize fermentation processes, enhance yield efficiency, and develop cost-effective large-scale production systems to fully realize the potential of microbial solutions.

Furthermore, the environmental benefits of microbial fermentation were corroborated by Meijaard and co-workers [23], who emphasized the urgent need to reduce reliance on traditional tropical oil crops to mitigate deforestation and biodiversity loss. The scalability and adaptability demonstrated in this study's fermentation trials further supports the viability of microbial oil platforms as a sustainable alternative. The cultivation of traditional palm oil in Latin America has faced significant challenges, including deforestation, habitat destruction, and water scarcity [23]. Although advancements such as OxG hybrids [9] have improved disease resistance and oil quality, the environmental footprint of traditional cultivation remains a pressing issue. By contrast, the use of *P. moriformis* to produce high-oleic oil offers a transformative solution. First, the fermentation process eliminates the need for arable land in tropical regions, directly addressing the drivers of deforestation and habitat loss. Second, the process requires significantly less water compared to traditional palm oil cultivation, as demonstrated in recent studies [24,25]. Third, the sustainability of the process is influenced by the origin of the sugar feedstock, with sourcing from sustainably managed agricultural systems further enhancing its environmental benefits. Finally, the adaptability of microbial systems to non-tropical regions, as highlighted by Patel and co-workers [26], enables decentralized production, reducing transportation-related carbon emissions and de-risking supply chains. From a socio-economic perspective, the fermentation of *P. moriformis* could provide a sustainable livelihood for industries in non-tropical regions, while alleviating pressure on palm oil-producing nations. By leveraging microbial fermentation, stakeholders can align their operations with global sustainability goals, including the reduction in greenhouse gas emissions [25] and the preservation of biodiversity.

5. Conclusions

In conclusion, this study highlights the potential of *P. moriformis* as a sustainable and scalable species for producing high-oleic palm oil alternatives. By aligning closely with the fatty acid and TAG profiles of conventional high-oleic palm oil, this microbial approach offers a viable solution to mitigate the environmental challenges associated with traditional palm oil cultivation. The findings underscore the transformative potential of microbial fermentation in building sustainable and resilient supply chains for the global oil and fat industry.

6. Patents

The data presented in this paper are described in the WO2023212726A2 patent entitled "Regiospecific incorporation of fatty acids in triglyceride oil".

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