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Valorisation of Kopyor Coconut Waste for Fatty Acid Production by *Yarrowia lipolytica*

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ABSTRACT

Yarrowia lipolytica yeast is a versatile platform that can be used to produce high-value fatty acids from various feedstocks, including the underutilised kopyor coconut. This study aims to explore the differences in valuable lipid-based metabolites from different types of kopyor coconut through fermentation by *Y. lipolytica*. The experiment in this study used three levels of coconut oil concentration (2.5%, 5%, and 7.5%) derived from kopyor coconut waste (var. Genjah and Dalam), which was further processed to obtain oil, and variations in fermentation time (0, 24, 48, 72, and 96 h). The concentration of kopyor coconut oil added to the fermentation medium influenced the growth of *Y. lipolytica*; higher concentrations led to greater cell biomass. After 96 hours of incubation, fatty acids are detected in the cell extract. Substrate fermentation influences the fatty acid profile of *Y. lipolytica*. Yeast Nitrogen Base (YNB) media without coconut oil addition produced alkanes as the majority compound. Meanwhile, YNB with coconut oil addition from var. Genjah produced the usual various fatty acid profiles. The addition of coconut oil from var. Dalam to *Y. lipolytica* pellets resulted in the production of unusual fatty acids with complex derivation. These various free fatty acids hold promise for important applications in biodiesel, nutraceuticals, and food flavouring agents.

Keywords: Fatty acid, kopyor coconut, value-added compounds, *Yarrowia lipolytica*

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INTRODUCTION

Kopyor coconut is a rare and highly prized variety of coconut, characterised by its soft, jelly-like endosperm, which sets it apart from the more commonly consumed mature coconut. Kopyor coconut is a natural genetic mutation of the coconut plant (*Cocos nucifera* L.), which has soft-textured fruit flesh that does not stick to the shell. According to BPS data (2022), the area of kopyor plantations in Central Java has continued to increase from 2016 to 2021, reaching 477.46 hectares with a total production of 1.120.357 tons. Furthermore, the Oil Palm Research Centre developed kopyor coconut seedlings with tissue culture technology that is capable of producing 10-15 thousand seedlings per year.

Kopyor coconut has a unique nutritional profile compared to regular coconuts. While the exact values can vary based on factors like maturity and growing conditions, kopyor coconut generally contains 90% moisture content and a substantial amount of lipids, primarily in the form of medium-chain triglycerides, which are readily digested and absorbed, offering potential health benefits (Dunn et al., 2023). Kopyor coconut contains 30.71% fat, 4.93% protein, and 62.30% carbohydrates (Santoso et al., 1996). Although coconut kopyor is valued for its unique culinary properties, its production generates large amounts of waste, such as oil-rich coconut waste. The unique composition of kopyor coconut, particularly its moisture content and unique lipid profile, makes it a highly sought-after delicacy. However, it also poses challenges in processing and storage due to its perishable nature (Maskromo & Sudarsono, 2013). Kopyor coconut waste can be utilised to create products with high economic value. One approach is through microbial fermentation. This fermentation process can produce compounds with high added value and is environmentally friendly.

According to the Indonesian Oil Palm Research Institute (IOPRI), approximately 20% of kopyor coconuts are produced as waste during processing, demonstrating the potential of this resource for bioconversion (IOPRI internal report, 2020). Research on the utilisation of kopyor coconut waste holds great promise for maximising the utilisation of this valuable resource. Utilising kopyor coconut waste offers the opportunity to convert this underutilised resource into valuable products, such as fatty acids, using microorganisms such as *Y. lipolytica* (Wang et al., 2022). *Y. lipolytica* has been extensively studied as an oil-producing yeast and has been identified as safe (GRASS). This yeast appears to be a host for the production of many valuable compounds (Cao et al., 2022). *Y. lipolytica* can efficiently utilize a variety of carbon sources, including various oils and fatty substrates, to produce lipids and other valuable metabolites (Friedlander et al., 2016).

Many studies have shown the potential of *Y. lipolytica* to produce a wide range of fatty acids from various raw materials as a method for creating new and renewable energy sources (Liu et al., 2021; Lu et al., 2021; Pereira et al., 2022). Therefore, in this study, we aimed to investigate the potential of *Y. lipolytica* for the valorisation of kopyor coconut waste through the production of high-value fatty acids.

MATERIALS AND METHODS

Materials

The research uses *Y. lipolytica* JCM 2320, Yeast Extract Peptone Dextrose (Himedia), Yeast Nitrogen Base (Himedia), aluminium foil, aquadest, KH_2PO_4 (Merck), kopyor coconut waste from the Indonesian Oil Palm Research Institute. The kopyor coconut waste was further processed to extract kopyor coconut oil using the traditional wet method, followed by a filtration step to remove residual solids (Hutauruk, 2017).

Extraction of Kopyor Coconut Oil

The kopyor coconut oil used in this study was obtained through a traditional extraction method described by Simuang et al. (2004). The initial step is to produce the size of the coconut flesh by grating it. The grated coconut flesh is mixed with water to produce coconut milk. The coconut milk is heated at 70-90 °C to reduce the water content, leaving a thick pulp. The resulting oil is separated from the pulp by filtration. To optimise the filtration process, the pulp must be pressed to extract the oil. This process produces the oil used in the experiment.

Substrate Characterisation

Analysis of the physicochemical properties of kopyor coconut oil includes density measurement using an Anton Paar DMA 4500M densitometer in accordance with ASTM D4052, viscosity measurement using a Brookfield DV-III ultra-viscometer in accordance with ASTM D7042, carbon content analysis using spectrophotometry (Rayment & Higginson, 1992), and nitrogen content analysis using the Kjeldahl method (ACIAR, 1990).

Preparation for *Yarrowia lipolytica*

The growth medium for *Y. lipolytica* was YPD (Yeast Extract Peptone Dextrose) agar, which was prepared with 20 g L^{-1} yeast extract, 10 g L^{-1} peptone, 20 g L^{-1} glucose, and 15 g L^{-1} agar (Kim et al., 2013). The growth medium for *Y. lipolytica* was YPD (Yeast Extract Peptone Dextrose) agar, prepared with 20 g L^{-1} yeast extract, 10 g L^{-1} peptone, 20 g L^{-1} glucose, and 15 g L^{-1} agar (Kim et al., 2013). Ingredients were dissolved in distilled water, sterilised at 121 °C and 1 atm for 15 minutes, and poured into sterile petri dishes (15 mL each) to solidify. Liquid YPD medium was prepared with the same composition, omitting agar, and distributed into 100 mL Erlenmeyer flasks (10 mL each) post-sterilisation.

Y. lipolytica was pre-cultured by inoculating a yeast loop in 10 mL YPD in a 100 mL flask, incubating at 30 °C and 250 rpm for 96 hours. The culture was streaked on YPD agar, incubated at 30 °C for 24 hours, and used as stock (Marella et al., 2019).

Preparation of Fermentation Media

The fermentation media for *Y. lipolytica* were prepared following the method of Marella et al., (2019), with modifications for kopyor coconut oil. Kopyor coconut oil at concentrations of 2.5%, 5%, and 7.5% was mixed into the media, and the pH was adjusted to 5.5-6 (Yu et al., 2014). The media composition included 6.7 g L^{-1} YNB (Yeast Nitrogen Base without Amino Acids), 12 g L^{-1} KH_2PO_4, and 10 g L^{-1} glucose. Media preparation was conducted for two kopyor coconut oil varieties, *Dalam* and *Genjah*. Each fermentation medium was prepared at a volume of 150 mL.

Inoculation and Fermentation in Treatment Media

Y. lipolytica was inoculated into a 100 mL Erlenmeyer flask containing 10 mL YPD medium and incubated at 30 °C, 250 rpm for 24 hours in a shaker incubator (Yu et al., 2014). For fermentation, 10 mL of the inoculated YPD medium was added to the prepared fermentation media. The flask was sealed and incubated at 30 °C, 250 rpm for 120 hours (Marella et al., 2019).

Sample Preparation for Analysis

Samples of 5 mL were collected from each fermentation media treatment. For cell density analysis, the samples were directly placed in centrifuge tubes. For wet cell weight, microscopic, and flavour compound analysis, 5 mL samples were centrifuged at 10.000 rpm at 4 °C for 10 minutes to isolate the pellet. The pellet and supernatant were separated using a micropipette, and the pellet was stored at -20 °C for further analysis (Marella et al., 2019).

Cell Density Determination

Cell density was determined by diluting 5 mL of the sample 30 times, transferring the diluted sample into a cuvette, and measuring the absorbance at 600 nm using a UV-Vis spectrophotometer (Celinska, 2013). OD_{600} values were subsequently converted into biomass concentrations (g L^{-1}) using a published conversion factor for *Yarrowia lipolytica* CBS7504 (1 OD_{600} = 0.34 g L^{-1}) (Walker et al., 2021).

Wet Cell Weight Measurement

The wet cell weight was measured by weighing an empty centrifuge tube and then weighing the tube containing the cell pellet using an analytical balance. Before weighing, the pellets were washed with deionised water to remove residual medium components. Afterwards, the pellets were centrifuged again to obtain clean pellets. The difference in weight was recorded as the wet cell weight (Puspitasari & Habibah, 2021).

Microscopic Analysis

Microscopic analysis was conducted using pellets from the fermentation samples, which were washed twice with 2 mL of 10 mM KH_2PO_4 solution. The samples were then centrifuged at 10,000 rpm, 4 °C, for 10 minutes. A final wash with 3 mL distilled water was followed by another centrifugation. The pellet was smeared onto a glass slide, mixed with distilled water, and covered with a cover glass. The morphology of *Y. lipolytica* was observed under a microscope at 100× magnification (Marella et al., 2019).

Fatty Acid Compound Analysis

The fermented pellets were first lysed with 1 mL of distilled water using a sonicator at a speed of 30 for 5 minutes. Then, 3 mL of n-Hexane solvent was added, and the sample was lysed again under the same conditions. Following this, the sample was centrifuged at 10.000 rpm, 4 °C, for 10 minutes. The top layer of the centrifugation was collected (1 mL), filtered using a 0.45 µm syringe filter, and placed in a vial for gas chromatography-mass spectrometry (GC-MS) analysis.

Free fatty acid (FFA) compound analysis was conducted using gas chromatography-mass spectrometry (GC-MS). A 1 µL sample was injected into the GC injector, set to an initial temperature of 250 °C and operated in splitless mode for 2 minutes, then switched to split mode with a 50:1 ratio. Helium gas was used as carrier gas, with a constant flow rate of 1 mL/min. The gas chromatography column temperature was set at 50 °C for 1 minute, then increased at a rate of 10 °C per minute until it reached 210 °C, where it was maintained for 10 minutes. The temperature was then increased to 230 °C at 10 °C per minute and maintained for 5 minutes (Marella et al., 2019).

The compound yield was estimated by multiplying the relative area percentage of each compound by the total extract mass obtained from each sample, followed by normalisation to the corresponding wet cell weight (WCW) (Alseekh et al. 2021). The results were expressed as compound yield (g compound g^{-1} wet cell weight), as shown as in Equation 1.

$$Y_i = \frac{A_i}{100 \times m_{\mathrm{WCW}}} \qquad [1]$$

where:

- Y_i = yield of compound *i* (g compound g^{-1} wet cell weight)
- A_i = relative GC-MS peak area of compound *i* (%)
- m_{WCW} = wet cell weight (g)

Statistical Analysis

This study examined three factors: kopyor coconut varieties, media substitution percentage, and fermentation time. This study used *Genjah* and *Dalam* kopyor coconut varieties as media types, with the addition of kopyor coconut oil at three levels: 2.5%, 5%, and 7.5% (v/v) of the total growth medium. Fermentation time was observed at 24, 48, 72, and 96 hours. Each combination of coconut varieties, media substitution percentage, and fermentation time was repeated three times. All data were analysed using one-way analysis of variance (ANOVA) with a 95% confidence level. If a significant effect was found (P value <0.05), Duncan's test was used to assess the significance of the difference between treatments. Statistical analysis was performed using the SPSS Statistics 26.

RESULTS AND DISCUSSION

Physicochemical Properties of Kopyor Coconut Oil

The physicochemical properties of kopyor coconut oil can be seen in Table 1, which include viscosity, density, carbon, and nitrogen. The potential of kopyor coconut oil, particularly as a substrate for *Y. lipolytica*, can be seen from the listed parameters. This is important to note because viscosity and density can affect the dispersion of substrate availability in aqueous media, while nitrogen and carbon are nutrient sources for cell growth and metabolic activity (Singh et al. 2017).

Table 1
Physicochemical properties of kopyor coconut oil

	Density (kg/m^3)	Viscosity (mm^2/s)	Carbon (%)	Nitrogen (%)	Lipid (%)
Kopyor Coconut Oil	906.90	29.06	232.02	0.10	99.91

Effect of Substrate Concentration and Fermentation Time on *Yarrowia lipolytica* Cell Density on Kopyor Coconut Oil Substrate (Var. Genjah and Dalam)

The growth of *Y. lipolytica* on both kopyor coconut oil substrates (var. Genjah and var. Dalam) showed a comparable trend (Figure 2). The results from both substrates showed a decrease in cell density during the first 24 hours. This is closely related to the yeast adaptation phase to the media conditions. These results are in line with the Ginovart et al. (2011) study, which reported that yeast cells are metabolically active during the lag phase. The absorbance values at 48 hours from high to low concentrations in var. Genjah were respectively: 7.5% (0.095 ± 0.03), 5% (0.227 ± 0.07) and 2.5% (0.289 ± 0.012). Meanwhile, the absorbance values at 48 hours from high to low concentrations in var. Genjah at 7.5% concentration (0.095 ± 0.03), followed by 5% (0.227 ± 0.07) and 2.5% (0.289 ± 0.012). Similarly, for var. Dalam, the highest absorbance value was observed at 7.5%

(0.363 ± 0.01), with 5% (0.245 ± 0.006) and 2.5% (0.145 ± 0.014) following suit. Based on these results, it can be seen that substrate concentrations generally encourage more cell growth in the exponential phase. The increased cell density at high concentrations (7.5% and 5%) indicates that yeast is able to utilise fatty acids such as caprylic and myristic acids as carbon sources (Rigoun et al. 2017). These fatty acids have the advantage of being efficiently degraded, providing energy, and serving as building blocks for biomass production. The higher cell density than the control medium (YNB) also supports this conclusion.

At a concentration of 2.5%, cell density was lower than the control medium. For example, in var. Dalam, cell density peaked at 72 hours with an absorbance value of 0.145 ± 0.014, this indicates that the nutrients contained in the medium are starting to decrease and byproducts are also starting to form in large quantities. This occurred in both substrates with a significant decrease at low concentrations. Although the growth pattern was similar in both varieties, var. Dalam showed a higher cell density at all concentrations. The comparison of absorbance values in the two varieties with the highest concentration value was 0.363 ± 0.01 (var. Dalam) and 0.289 ± 0.012 (var. Genjah). This difference is due to the higher fatty acid content in var. Dalam. This is able to encourage better growth and biomass production (Soong et al., 2019). The following is a graph of *Y. lipolytica* cell density in fermentation media with kopyor coconut oil substrate (var. Genjah and Dalam).

The conclusion of this study is that *Y. lipolytica* can grow better on kopyor coconut oil substrate var. Dalam with high concentrations (7.5% and 5%).

To complement the growth curve data shown in Figure 1, the OD_{600} values obtained in this study were converted to estimated cell mass (g/L). The conversion was performed using a published factor, where 1 OD_{600} = 0.34 g/L for *Y. lipoytica* CBS7504 (Walker et al., 2021). Table 2 presents the obtained OD_{600} values along with the corresponding estimated cell mass at the observed peak growth for each substrate concentration. While this conversion provides only estimates, as actual values may vary depending on strain and culture conditions, it still provides a useful context for interpreting optical density-based growth patterns in relation to biomass calculation.

Table 2
OD_{600} values and corresponding estimated cell mass (g/L) of Yarrowia lipolytica *at peak growth under different substrate concentrations*

Yarrowia lipolytica	**OD_{600}**	**Cell Mass (g/L)**
Genjah and Dalam 0%	0.235±0.011	0.080±0.004
Genjah 2.5%	0.141±0.008	0.048±0.003
Genjah 5%	0.227±0.003	0.077±0.001
Genjah 7.5%	0.289±0.012	0.098±0.004
Dalam 2.5%	0.145±0.014	0.049±0.005
Dalam 5%	0.245±0.006	0.083±0.002
Dalam 7.5%	0.363±0.010	0.123±0.003

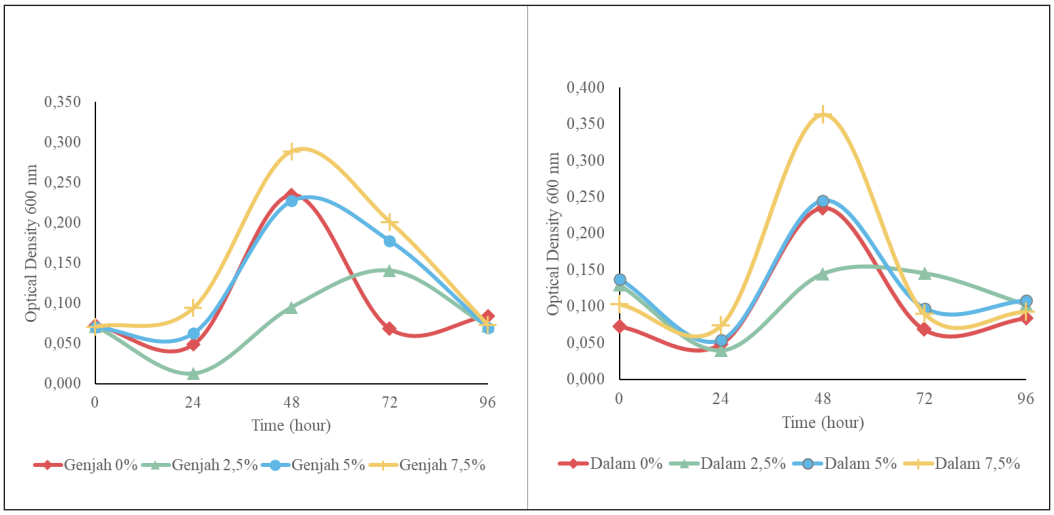

Figure 1. Cell density of *Yarrowia lipolytica* in fermentation medium + coconut oil kopyor var. Genjah and var. Dalam

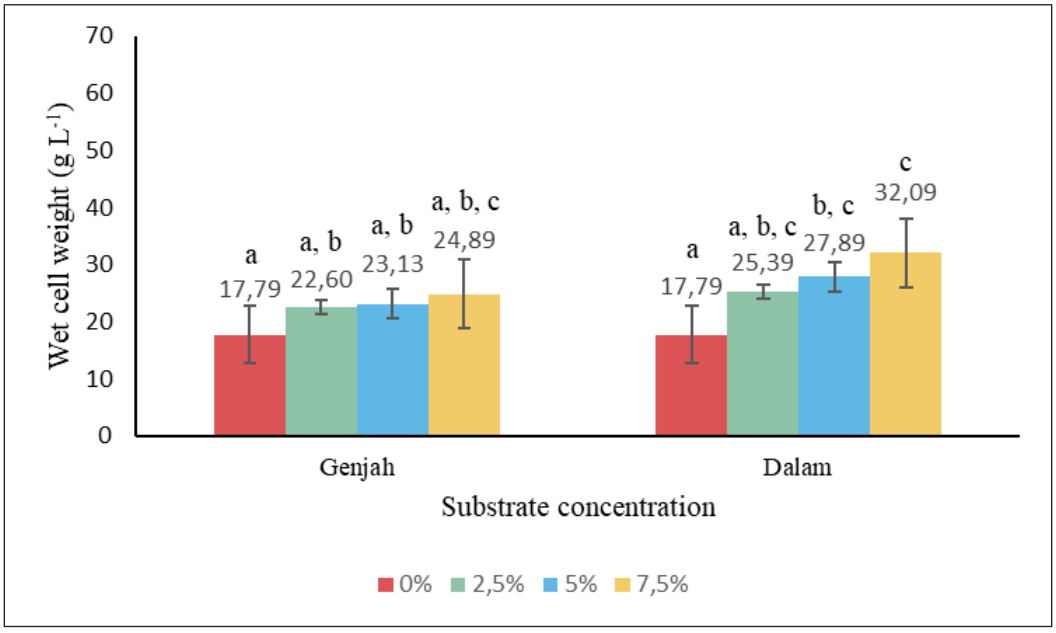

Figure 2. Wet weight curve of *Yarrowia lipolytica* cells on various media with a fermentation time of 72 hours

Note. Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at $\alpha = 0.05$

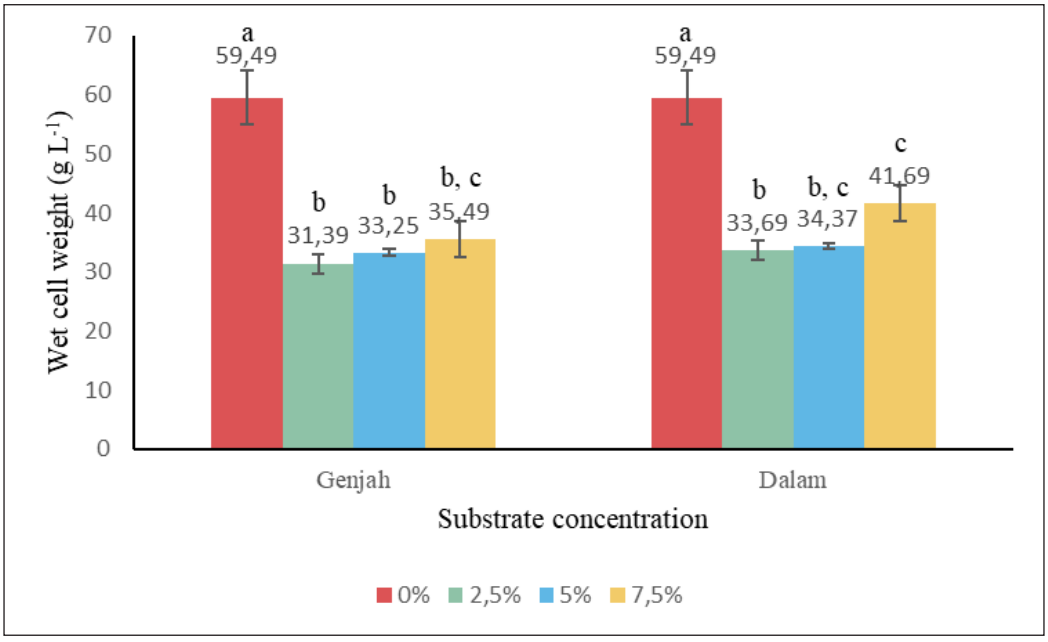

Figure 3. Wet weight curve of *Yarrowia lipolytica* cells on various media with a fermentation time of 96 hours

Note. Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at $\alpha = 0.05$

Wet Cell Weight of *Yarrowia lipolytica* Cells in Kopyor Coconut Oil Substrate

Wet cell weight provides a direct estimate of biomass concentration and can be used to obtain information about the growth of microorganisms such as *Y. lipolytica* (Choi et al., 2013). In this study, the wet weight of *Y. lipolytica* was measured using an analytical balance to observe biomass fluctuations during the fermentation process.

Fermentation of *Y. lipolytica* in kopyor coconut oil substitute medium showed an increase in wet weight from 72 to 96 hours (Figure 2 and Figure 3). This increase indicates cell growth and can be used as an important parameter to evaluate the performance of *Y. lipolytica* during fermentation.

However, wet cell weight does not always correlate directly with cell density. Wet weight is influenced by the rate of cell division (Bryan et al., 2009) as well as variations in the mass of individual cells. Variability in individual cell weight can lead to conditions where samples have high cell density but relatively low cell mass, or vice versa (Zhao et al., 2014).

ANOVA results indicated that fermentation on coconut oil substitution media produced significantly higher wet weight values compared to the control (YNB). Variations in oil type (var. Genjah and var. Dalam) and concentration significantly influenced wet weight at

72 hours ($p < 0.05$). Duncan's test revealed that var. Dalam at 5% and 7.5% concentrations yielded significantly higher wet weight increases.

At 96 hours, significant differences ($p<0.05$) were observed between treatments. Duncan's test identified var. Dalam at 7.5% concentration as the best treatment, with the highest wet weight among substituted media. However, compared to the control, this value remained lower. This indicates that var. Dalam media at 7.5% concentration has substantial potential, although the control remains more effective for wet weight production at this time point.

On average, the wet weight of *Y. lipolytica* cells increased by 31% from 72 to 96 hours in the substitution media. Across all concentrations, var. Dalam produced a higher wet cell weight compared to the var. Genjah. This difference is related to factors that influence cell division and growth, such as nutrient availability, temperature, pH, and fermentation medium composition (Talavera et al., 2024). However, an increase in wet weight does not always correlate with an increase in cell density. Physiological differences between cells can affect individual mass and cell density within the resulting biomass (Zhao et al., 2014). In many cases, higher wet weight reflects more intense metabolic activity, which may be related to increased production of fatty acid compounds (Braga et al., 2022).

Morphological Characteristics

Y. lipolytica is a dimorphic yeast that is able to grow in two morphological forms, namely yeast-like cells and short mycelium cells (Ruiz-Herrera & Sentandrey, 2002). In control medium (YNB), *Y. lipolytica* showed both yeast-like and short mycelial forms, with round to oval cells, some forming chains, and having a smooth surface. Morales-Vargas et al. (2012) stated that under natural conditions, *Y. lipolytica* generally grows as a mixture of yeast and short mycelial cells. However, under more controlled culture conditions, this microorganism can also grow as yeast-like cells or branching hyphae.

Microscopic observations of *Y. lipolytica* grown in kopyor coconut oil substitution media showed various morphological forms at different oil concentrations in Figure 4. At a 2.5% coconut oil substitution (var. Genjah), the cells displayed a mycelial form with pseudohyphae, forming elongated chains. This is consistent with Zinajarde et al. (2008), who observed that mycelial formation occurs when certain crude oils, such as coconut oil, are used as carbon sources. At a 5% coconut oil substitution, the cells adopted a yeast-like shape with round and uniform characteristics. At 7.5%, the cells transitioned back to mycelial form with pseudohyphae, forming chains, a finding also reported by Papanikolaou et al. (2007), who noted that the yeast-to-mycelium transition is common when fats containing lauric, myristic, palmitic, oleic, and stearic acids are used as carbon sources.

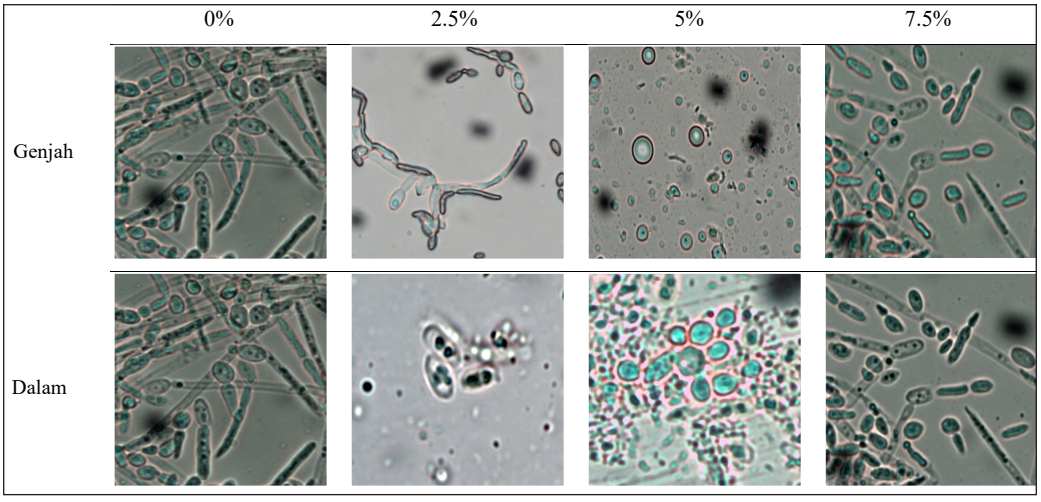

Figure 4. Microscopic observation of *Y. lipolytica* with a magnification of 100×

For the coconut oil, kopyor substitution with Var. Dalam, at 2.5%, the cells retained a yeast-like shape, with round to oval cells and uniform size. At 5%, the cells were yeast-like, with some cells attached in pairs. At 7.5%, the cells showed both yeast and mycelial forms, with pseudohyphae forming long chains. Similar to var. Genjah, these changes can be attributed to the types of carbon sources, such as glucose and fatty acids, and nitrogen sources like ammonium, as well as factors like low oxygen availability in the medium (Braga & Belo, 2022; Zinajarde et al., 2008).

In conclusion, *Y. lipolytica* can adapt its morphology in response to different fermentation conditions, particularly the composition of the media. However, as noted by Małajowicz and Kozłowska (2021), the shape of the yeast does not directly correlate with the production of bioflavour compounds, such as lactones, which are influenced by metabolic pathways, enzymatic activities, and environmental factors.

Profiling FFA Compound

In this study, *Y. lipolytica* was fermented using minimal media of YNB added with coconut oil Kopyor. Hydrophobic substrates like coconut oil can activate *Y. lipolytica* to produce extracellular lipase, and then the oil can be hydrolysed into fatty acids and glycerol. The incorporation of these fatty acids into the cytosol led to the activation of FAA1 (fatty acyl-CoA synthetase) in order to produce acyl-CoA (Rakicka et al., 2015). Acyl-CoA is a building block for fatty acyl-CoA to synthesise some compounds, such as essential fatty acids (EFA), alkanes, fatty alcohols, fatty acid ethyl esters (FAAE), and free fatty acids (FFA) (Liu et al., 2021). Meanwhile, hydrophilic substrates such as glucose and glycerol can be converted into fatty acids via the *de novo* pathway (Ledesma-Amaro & Nicaud, 2016).

To identify the fatty acid compounds produced during *Y. lipolytica* fermentation, we investigated the fatty acid profile from pellets using Gas chromatography-mass spectrometry (GC-MS) methods. The sample preparation focussed on the pellet fraction, as pellets are known to accumulate fatty acid compounds from kopyor fermentation. This is due to the production and accumulation of intracellular fatty acid compounds within yeast cells (Pensupa et al., 2023). Furthermore, Colacicco et al. (2022) reported that cell pellets retain cellular activity essential for fatty acid production, making cell biomass a rich source of these compounds. The use of pellets as samples can also improve the efficiency of fatty acid extraction. This is because supernatants typically contain various dissolved culture media components that do not always represent the total fatty acid production by the microbes (Naveira-Pazos et al. 2023).

The GC-MS chromatogram indicates the presence of various fatty acid compounds, with the identified compounds presented in Table 3. In the control medium, several dominant compounds detected included octadecane, hexadecane, and heneicosane. These hydrocarbons indicate active lipid biosynthesis by *Y. lipolytica*. Campos-Góngora et al. (2018) reported that saturated hydrocarbons can be formed as byproducts of the lipid degradation process. Furthermore, the presence of compounds such as octadecane and hexadecane may also indicate significant β-oxidation activity under the applied fermentation conditions (Vasiliadou et al., 2018).

In the fermentation medium substituted with kopyor coconut oil, various concentrations produced several dominant compounds such as lauric acid, stearic acid, palmitic acid, myristic acid, and oleic acid. These compounds are important components in lipid metabolism and highlight the potential of *Y. lipolytica* in producing valuable lipids for industrial applications. The presence of lauric, stearic, and oleic acids identified in this study is also aligned with the previous reports that certain fermentation conditions can enhance fatty acid production in *Y. lipolytica* (Wang et al., 2022). Palmitic and myristic acids, key products in lipid biosynthesis, underscore the influence of fermentation conditions on the metabolic pathways of *Y. lipolytica* (Soong et al., 2019).

Genjah 2.5% has the highest dodecanoic acid content among other fatty acids, where the lauric fraction has been reported to be used as a raw material source for renewable energy (Bukhari et al., 2022). The fatty acid content in *Y. lipolytica* can be utilised as an alternative energy source in certain modified engines. Liu et al., (2021)

Genjah 2.5% has the highest dodecanoic fatty acid content among the other fatty acids, where the laurate fraction is reported to be able to be used as a source of raw material for renewable energy (Bukhari et al., 2022). The fatty acid content within *Y. lipolytica* can be utilised as a source of alternative energy in certain modified engines. Liu et al. (2021) discussed the secretion of fatty acid esters by an engineered *Y. lipolytica* strain. This approach eliminates the need for transesterification, simplifying the process and potentially reducing costs.

Table 3
Compounds identified from the fermentation of Y. lipolytica *in various media substrates*

Sample	Retention Time (minutes)	Area (%)	Compound	Yield (g compound g^{-1} wet cell weight)
Control (YNB)	16.576	17.10	Octadecane ($C_{18}H_{38}$)	0.1710
	14.361	13.97	Hexadecane (C_6H_{34})	0.1397
	18.914	9.77	Heneicosane ($C_{21}H_{44}$)	0.0977
	32.022	3.86	Fumaric acid ($C_4H_4O_4$)	0.0386
	32.244	2.95	Glycerol tricaprylate ($C_{27}H_{50}O_6$)	0.0295
	16.348	0.78	Carbonic acid (H_2CO_3)	0.0078
Genjah 2.5%	14.188	41.55	Dodecanoic acid ($C_{12}H_{24}O_2$)	0.4155
	16.320	20.44	Octadecanoic acid ($C_{18}H_{36}O_2$)	0.2044
	18.534	12.48	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	0.1248
	21.527	10.23	Oleic acid ($C_{18}H_{34}O_2$)	0.1023
	25.774	0.05	Propanoic acid ($C_3H_6O_2$)	0.0005
	13.417	0.05	2,4-Di-tert-butylphenol ($C_{14}H_{22}O$)	0.0005
Genjah 5%	14.074	34.42	Dodecanoic acid ($C_{12}H_{24}O_2$)	0.3442
	16.259	19.92	Tetradecanoic acid ($C_{14}H_{28}O_2$)	0.1992
	18.486	14.64	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	0.1464
	21.475	12.55	Oleic acid ($C_{18}H_{34}O_2$)	0.1255
	13.413	0.29	2,4-Di-tert-butylphenol ($C_{14}H_{22}O$)	0.0029
Genjah 7.5%	14.077	38.62	Dodecanoic acid ($C_{12}H_{24}O_2$)	0.3862
	16.259	18.99	Tetradecanoic acid ($C_{14}H_{28}O_2$)	0.1899
	14.485	10.81	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	0.1081
Dalam 2.5%	14.023	32.14	Dodecanoic acid ($C_{12}H_{24}O_2$)	0.3214
	16.229	15.49	Tetradecanoic acid ($C_{14}H_{28}O_2$)	0.1549
	18.468	14.60	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	0.1460
	13.406	0.93	2,4-Di-tert-butylphenol ($C_{14}H_{22}O$)	0.0093
Dalam 5%	14.025	29.28	Dodecanoic acid ($C_{12}H_{24}O_2$)	0.2928
	16.278	17.65	i-Propyl 12-methyl-tridecanoate ($C_{17}H_{34}O_2$)	0.1765
	18.514	15.54	l-(+)-Ascorbic acid 2,6-dihexadecanoate ($C_{38}H_{68}O_8$)	0.1554
	13.420	0.09	2,4-Di-tert-butylphenol ($C_{14}H_{22}O$)	0.0009
Dalam 7.5%	14.225	21.27	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester ($C_{19}H_{36}O_5$)	0.2127
	21.561	12.59	6-Octadecenoic acid ($C_{18}H_{34}O_2$)	0.1259
	9.085	12.14	5-Butyl-1,3-oxathiolan-2-one	0.1214
	16.242	12.06	i-Propyl 12-methyl-tridecanoate ($C_{17}H_{34}O_2$)	0.1206

Table 4
Identified FFA compounds and their applications as fine chemicals in the food industry

Medium	Compounds	Applications
Control (YNB)	Octadecane Hexadecane Heneicosane	Production of lubricants, waxes and food packaging materials (Chayabutra & Ju, 2000)
	Fumaric acid	Corn and wheat tortilla flavour ingredients, acidity regulators, and preservatives (Ilica et al., 2018)
	Glycerol tricaprylate	Improved b-pinene flavour release characteristics and stabilisers (Koupantsis et al., 2016)
	Carbonic acid	Provides a carbonation effect on carbonated drinks, alcohol, and kombucha (Pelchat et al., 2014)
	n-Hexadecanoic acid	Affects texture and improves butter quality (Music et al., 2022).
	Oleic acid	Increases the release of aromatic aromas from the Maillard reaction (Benet et al., 2015)
Kopyor coconut oil	Propanoic acid	Gives a sweet taste and nutty aroma to cheese, as a preservative (Bücher et al., 2021)
	2,4-Di-tert-butylphenol	Natural antioxidant (Varsha et al., 2015)
	Tetradecanoic acid	It produces a waxy taste in the mouth that can be enjoyed when combined with other ingredients (Burdock and Carabin 2007)
	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Vitamin C has antioxidant and antimicrobial properties (Mumtaz et al. 2023)

In addition to producing fatty acid components that have the potential to be used as renewable energy feedstock, the utilisation of kopyor coconut waste through microbial fermentation also produces various value-added chemicals with potential for food applications (Table 4). *Y. lipolytica* can be a microbial cell factory for producing various fatty acid-based bioproducts, including nutraceuticals and biochemicals (Liu et al., 2021).

These findings indicate that the media composition and fermentation conditions are capable of directing the metabolism of *Y. lipolytica* toward the production of lipids and their derivatives. The presence of hydrocarbons such as octadecane and hexadecane reflects lipid degradation activity during the fermentation process. Meanwhile, the identification of fatty acids such as lauric, stearic, palmitic, myristic, and oleic acids demonstrates the yeast's ability to synthesise various lipid derivatives with commercial value.

Although *Y. lipolytica* produces relatively lower amounts of fatty acids compared to microalgae, this yeast still offers several advantages. One of the advantages is that the cultivation conditions are simpler. Microalgae require appropriate nutrient inputs, such as carbon, nitrogen, and light, to optimise lipid production (Mou et al., 2023; Udayan et al., 2023). In contrast, *Y. lipolytica* can utilise a broader range of substrates, including

organic waste, for lipid synthesis (Drzymała-Kapinos et al., 2022; Fabiszewska et al., 2021). Moreover, microalgae generally require more complex lipid extraction processes compared to *Y. lipolytica*, as they have thicker and more intricate cell walls. In contrast, *Y. lipolytica* biomass can directly produce lipids in a form that is more readily processable (Lee et al., 2021).

CONCLUSION

Based on the results and discussion, the level of coconut oil substitution in the fermentation media significantly affects the growth of *Y. lipolytica*. A substitution concentration of 7.5% results in a higher increase in *Y. lipolytica* growth compared to the control medium, while a 5% substitution resulted in relatively equivalent growth. Conversely, a 2.5% substitution leads to lower growth. Among the substrates tested, kopyor coconut oil from the Dalam variety consistently produced higher growth than the Genjah variety across all concentrations, as observed through cell density and wet cell weight parameters.

The wet weight of *Y. lipolytica* cells at 72 and 96 hours of fermentation was significantly influenced by the concentration and variation of the media. Substitution with 7.5% kopyor coconut oil (var. Dalam) resulted in the highest wet cell weight compared to the other treatment media, although it did not exceed the control media. Microscopic observations indicated that *Y. lipolytica* is able to adapt well to various fermentation conditions, as evidenced by diverse morphological forms. However, these morphological changes did not correlate with the production of fatty acid compounds.

Overall, utilising coconut waste through microbial fermentation using *Y. lipolytica* is a promising approach to transforming this underutilised resource. This process has the potential to produce high-value fatty acids that can be used as a renewable energy source and other high-value products for the nutraceutical and food industry.

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REFERENCES

Alseekh, S., Aharoni, A., Brotman, Y., Contrepois, K., D'Auria, J., Ewald, J., Ewald, J. C., Giavalisco, P., Haunsberger, L. N., Hu, M., James, S. I., Janocha, H., Jirasakulsirisin, M., Gaquerel, E., & Fernie, A. R. (2021). Mass spectrometry-based metabolomics: A guide for annotation, quantification and best reporting practices. *Nature Methods*, *18*(7), 747-756. https://doi.org/10.1038/s41592-021-01197-1

Braga, A., & Belo, I. (2022). Microbial production of flavours and fragrances by *Yarrowia lipolytica*. In *Springer eBooks* (pp. 1-28). Springer. https://doi.org/10.1007/978-3-030-81403-8_7-1

Bryan, A. K., Goranov, A., Amon, A., & Manalis, S. R. (2009). Measurement of mass, density, and volume during the cell cycle of yeast. *Proceedings of the National Academy of Sciences*, *107*(3), 999-1004. https://doi.org/10.1073/pnas.0901851107

Bücher, C., Burtscher, J., & Domig, K. J. (2021). Propionic acid bacteria in the food industry: An update on essential traits and detection methods. *Comprehensive Reviews in Food Science and Food Safety*, *20*(5), 4299-4323. https://doi.org/10.1111/1541-4337.12804

Bukhari, S. A. Z., Ullah, K., Ahmad, M., Zafar, M., Ullah, A., Sultana, S., Shah, S. N., & Ghoneim, S. S. M. (2022). Biodiesel from *Dodonaea* plant oil: Synthesis and characterisation—A promising nonedible oil source for bioenergy industry. *Frontiers in Bioengineering and Biotechnology*, *10*, Article 864415. https://doi.org/10.3389/fbioe.2022.864415

Burdock, G. A., & Carabin, I. G. (2007). Safety assessment of myristic acid as a food ingredient. *Food and Chemical Toxicology*, *45*(4), 517-529. https://doi.org/10.1016/j.fct.2006.10.009

Cao, L., Yin, M., Shi, T. Q., Lin, L., Ledesma-Amaro, R., & Ji, X. J. (2022). Engineering *Yarrowia lipolytica* to produce nutritional fatty acids: Current status and future perspectives. *Synthetic and Systems Biotechnology*, *7*(4), 1024-1033. https://doi.org/10.1016/j.synbio.2022.06.005

Chayabutra, C., & Ju, L. (2000). Degradation of n-hexadecane and its metabolites by *Pseudomonas aeruginosa* under microaerobic and anaerobic denitrifying conditions. *Applied and Environmental Microbiology*, *66*(2), 493-498. https://doi.org/10.1128/AEM.66.2.493-498.2000

Colacicco, M., Ciliberti, C., Agrimi, G., Biundo, A., & Pisano, I. (2022). Towards the physiological understanding of *Yarrowia lipolytica* growth and lipase production using waste cooking oils. *Energies*, *15*(14), Article 5217. https://doi.org/10.3390/en15145217

Drzymała-Kapinos, K., Mirończuk, A. M., & Dobrowolski, A. (2022). Lipid production from lignocellulosic biomass using an engineered *Yarrowia lipolytica* strain. *Microbial Cell Factories*, *21*(1), Article 226. https://doi.org/10.1186/s12934-022-01951-w

Dunn, E., Zhang, B., Sahota, V. K., & Augustin, H. (2023). Potential benefits of medium chain fatty acids in aging and neurodegenerative disease. *Frontiers in Aging Neuroscience*, *15*, Article 1230467. https://doi.org/10.3389/fnagi.2023.1230467

Fabiszewska, A. U., Zieniuk, B., Kozłowska, M., Mazurczak-Zieniuk, P. M., Wołoszynowska, M., Misiukiewicz-Stępień, P., & Nowak, D. (2021). Studies on upgradation of waste fish oil to lipid-rich yeast biomass in *Yarrowia lipolytica* batch cultures. *Foods*, *10*(2), Article 436. https://doi.org/10.3390/foods10020436

Friedlander, J., Tsakraklides, V., Kamineni, A., Greenhagen, E. H., Consiglio, A. L., MacEwen, K., Crabtree D. V., Afshar, J., Nugent, R.L., Hamilton, M.A., Joe Shaw, A., South, C.R., Stephanopoulos, G., & Brevnova, E. E. (2016). Engineering of a high lipid producing *Yarrowia lipolytica* strain. *Biotechnology for Biofuels*, *9*(1), 1-12. https://doi.org/10.1186/s13068-016-0492-3

Ilica, R., Kloetzer, L., Galaction, A., & Caşcaval, D. (2018). Fumaric acid: Production and separation. *Biotechnology Letters*, *41*(1), 47-57. https://doi.org/10.1007/s10529-018-2628-y

Kim, S., Park, D., Song, S. H., Wee, Y., & Jeong, G. (2013). Effect of fermentation inhibitors in the presence and absence of activated charcoal on the growth of *Saccharomyces cerevisiae*. *Bioprocess and Biosystems Engineering*, *36*(6), 659-666. https://doi.org/10.1007/s00449-013-0888-4

Koupantsis, T., Pavlidou, E., & Paraskevopoulou, A. (2016). Glycerol and tannic acid as applied in the preparation of milk proteins - CMC complex coacervates for flavour encapsulation. *Food Hydrocolloids*, *57*, 62-71. https://doi.org/10.1016/j.foodhyd.2016.01.007

Ledesma-Amaro, R., & Nicaud, J. M. (2016). *Yarrowia lipolytica* as a biotechnological chassis to produce usual and unusual fatty acids. *Progress in Lipid Research*, *61*, 40-50. https://doi.org/10.1016/j.plipres.2015.12.001

Lee, S. Y., Khoiroh, I., Vo, D. V. N., Senthil Kumar, P., & Show, P. L. (2021). Techniques of lipid extraction from microalgae for biofuel production: A review. *Environmental Chemistry Letters*, *19*(1), 231-251. https://doi.org/10.1007/s10311-020-01088-5

Liu, H., Song, Y., Fan, X., Wang, C., Lu, X., & Tian, Y. (2021). *Yarrowia lipolytica* as an oleaginous platform for the production of value-added fatty acid-based bioproducts. *Frontiers in Microbiology*, *11*, Article 608662. https://doi.org/10.3389/fmicb.2020.608662

Lu, R., Cao, L., Wang, K., Ledesma-Amaro, R., & Ji, X. J. (2021). Engineering *Yarrowia lipolytica* to produce advanced biofuels: Current status and perspectives. *Bioresource Technology*, *341*, Article 125877. https://doi.org/10.1016/j.biortech.2021.125877

Małajowicz, J., & Kozłowska, M. (2021). Factors affecting the yield in formation of fat-derived fragrance compounds by *Yarrowia lipolytica* yeast. *Applied Sciences*, *11*(21), Article 9843. https://doi.org/10.3390/app11219843

Marella, E. R., Dahlin, J., Dam, M. I., Ter Horst, J., Christensen, H. B., Sudarsan, S., Wang, G., Holkenbrink, C., & Borodina, I. (2019). A single-host fermentation process for the production of flavour lactones from non-hydroxylated fatty acids. *Metabolic Engineering*, *61*, 427-436. https://doi.org/10.1016/j.ymben.2019.08.009

Maskromo, I., & Sudarsono, S. (2013). Productivity of three dwarf kopyor coconut varieties from Pati, Central Java, Indonesia. *CORD*, *29*(2), 10-20. https://doi.org/10.37833/cord.v29i2.86

Morales-Vargas, A. T., Domínguez, A., & Ruiz-Herrera, J. (2012). Identification of dimorphism-involved genes of *Yarrowia lipolytica* by means of microarray analysis. *Research in Microbiology*, *163*(5), 378-387. https://doi.org/10.1016/j.resmic.2012.03.002

Mou, Y., Liu, N., Lu, T., Jia, C., Xu, C., & Song, M. (2023). The effects of carbon nitrogen ratio and salinity on the treatment of swine digestion effluent simultaneously producing bioenergy by microalgae biofilm. *Chemosphere*, *339*, Article 139694. https://doi.org/10.1016/j.chemosphere.2023.139694

Mumtaz, S., Mumtaz, S., Ali, S., Tahir, H. M., Kazmi, S. A. R., Mughal, T. A., & Younas, M. (2023). Evaluation of antibacterial activity of vitamin C against human bacterial pathogens. *Brazilian Journal of Biology*, *83*, Article e247165. https://doi.org/10.1590/1519-6984.247165

Music, J., Charlebois, S., Marangoni, A. G., Ghazani, S. M., Burgess, J., Proulx, A., Somogyi, S., & Patelli, Y. (2022). Data deficits and transparency: What led to Canada's 'buttergate'? *Trends in Food Science & Technology*, *123*, 334-342. https://doi.org/10.1016/j.tifs.2022.02.005

Naveira-Pazos, C., Robles-Iglesias, R., Fernández-Blanco, C., Veiga, M. C., & Kennes, C. (2023). State-of-the-art in the accumulation of lipids and other bioproducts from sustainable sources by *Yarrowia lipolytica*.

Reviews in Environmental Science and Bio/Technology, *22*(4), 1131-1158. https://doi.org/10.1007/s11157-023-09670-3

Papanikolaou, S., Chevalot, I., Galiotou-Panayotou, M., Komaitis, M., Marc, I., & Aggelis, G. (2007). Industrial derivative of tallow: A promising renewable substrate for microbial lipid, single-cell protein and lipase production by *Yarrowia lipolytica*. *Electronic Journal of Biotechnology*, *10*(3), 425-435. https://doi.org/10.2225/vol10-issue3-fulltext-8

Pelchat, M. L., Bryant, B., Cuomo, R., Di Salle, F., Fass, R., & Wise, P. (2014). Carbonation. *Nutrition Today*, *49*(6), 308-312. https://doi.org/10.1097/NT.0000000000000010

Pensupa, N., Treebuppachartsakul, T., & Pechprasarn, S. (2023). Machine learning models using data mining for biomass production from *Yarrowia lipolytica* fermentation. *Fermentation*, *9*(3), Article 239. https://doi.org/10.3390/fermentation9030239

Pereira, A. S., Lopes, M., Miranda, S. M., & Belo, I. (2022). Bio-oil production for biodiesel industry by *Yarrowia lipolytica* from volatile fatty acids in two-stage batch culture. *Applied Microbiology and Biotechnology*, *106*(8), 2869-2881. https://doi.org/10.1007/s00253-022-11900-7

Puspitasari, A. D. S., & Habibah, N. A. (2021). Pengaruh zat pengatur tumbuh 2,4-D dan kinetin terhadap pertumbuhan dan morfologi sel gembili (*Dioscorea esculenta*) [Effect of growth regulators 2,4-D and kinetin on the growth and cell morphology of Lesser Yam (*Dioscorea esculenta*)]. *Life Science*, *10*(2), 191-200. https://doi.org/10.15294/lifesci.v10i2.54460

Rakicka, M., Lazar, Z., Dulermo, T., Fickers, P., & Nicaud, J. M. (2015). Lipid production by the oleaginous yeast *Yarrowia lipolytica* using industrial by-products under different culture conditions. *Biotechnology for Biofuels*, *8*(1), Article 104. https://doi.org/10.1186/s13068-015-0286-z

Rayment, G. E., & Higginson, F. R. (1992). *Australian laboratory handbook of soil and water chemical methods*. Inkata Press.

Santoso, U., Kubo, K., Ota, T., Tadokoro, T., & Maekawa, A. (1996). Nutrient composition of kopyor coconuts (*Cocos nucifera* L.). *Food Chemistry*, *57*(2), 299-304. https://doi.org/10.1016/0308-8146(95)00237-5

Simuang, J., Chiewchan, N., & Tansakul, A. (2004). Effects of fat content and temperature on the apparent viscosity of coconut milk. *Journal of Food Engineering*, *64*(2), 193-197. https://doi.org/10.1016/j.jfoodeng.2003.09.032

Singh, V., Haque, S., Niwas, R., Srivastava, A., Pasupuleti, M., & Tripathi, C. K. M. (2017). Strategies for fermentation medium optimisation: An in-depth review. *Frontiers in Microbiology*, *7*, Article 2087. https://doi.org/10.3389/fmicb.2016.02087

Soong, Y. V., Liu, N., Yoon, S., Lawton, C., & Xie, D. (2019). Cellular and metabolic engineering of oleaginous yeast *Yarrowia lipolytica* for bioconversion of hydrophobic substrates into high-value products. *Engineering in Life Sciences*, *19*(6), 423-443. https://doi.org/10.1002/elsc.201800147

Talavera, R. A., Prichard, B. E., Sommer, R. A., Leitao, R. M., Sarabia, C. J., Hazir, S., Paulo, J. A., Gygi, S. P., & Kellogg, D. R. (2024). Cell growth and nutrient availability control the mitotic exit signalling network in budding yeast. *The Journal of Cell Biology*, *223*(8), Article e202305008. https://doi.org/10.1083/jcb.202305008

Udayan, A., Pandey, A. K., Sirohi, R., Sreekumar, N., Sang, B. I., Sim, S. J., Kim, S. H., & Pandey, A. (2023). Production of microalgae with high lipid content and their potential as sources of nutraceuticals. *Phytochemistry Reviews*, *22*(4), 833-860. https://doi.org/10.1007/s11101-021-09784-y

Vasiliadou, I. A., Bellou, S., Daskalaki, A., Hetman, L. T., Chatzikotoula, C., Kompoti, B., Papanikolaou, S., Vayenas, D., Pavlou, S., & Aggelis, G. (2018). Biomodification of fats and oils and scenarios of adding value on renewable fatty materials through microbial fermentations: Modelling and trials with *Yarrowia lipolytica*. *Journal of Cleaner Production*, *200*, 1111-1129. https://doi.org/10.1016/j.jclepro.2018.07.187

Walker, C., Dien, B., Giannone, R. J., Slininger, P., Thompson, S. R., & Trinh, C. T. (2021). Exploring proteomes of robust *Yarrowia lipolytica* isolates cultivated in biomass hydrolysate reveals key processes impacting mixed sugar utilisation, lipid accumulation, and degradation. *mSystems*, *6*(4), Article e00443-21. https://doi.org/10.1128/msystems.00443-21

Wang, K., Shi, T. Q., Lin, L., Wei, P., Ledesma-Amaro, R., & Ji, X. J. (2022). Engineering *Yarrowia lipolytica* to produce tailored chain-length fatty acids and their derivatives. *ACS Synthetic Biology*, *11*(8), 2564-2577. https://doi.org/10.1021/acssynbio.2c00305

Wang, M., Liu, G., Liu, H., Zhang, L., Li, B., Li, X., Liu, D., & Yuan, Y. (2018). Engineering global transcription to tune lipophilic properties in Yarrowia lipolytica. Biotechnology for Biofuels, 11(1), Article 150. https://doi.org/10.1186/s13068-018-1114-z

Yu, X., Dong, T., Zheng, Y., Miao, C., & Chen, S. (2014). Investigations on cell disruption of oleaginous microorganisms: Hydrochloric acid digestion is an effective method for lipid extraction. *European Journal of Lipid Science and Technology*, *117*(5), 730-737. https://doi.org/10.1002/ejlt.201400195

Zhao, Y., Lai, H. S. S., Zhang, G., Lee, G., & Li, W. J. (2014). Rapid determination of cell mass and density using digitally controlled electric field in a microfluidic chip. *Lab on a Chip*, *14*(22), 4426-4434. https://doi.org/10.1039/C4LC00795F