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Research Article

Enhanced Cell Harvesting for Lipid Production by *Lipomyces maratuensis* InaCC Y720 Using Flocculation Induction

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ABSTRACT

Lipomyces maratuensis InaCC Y720 is a yeast from Indonesia that is known to be able to produce lipids on a production medium that has been optimized in the previous study. One of the efforts to increase the harvesting of yeast cells needed on a production scale larger than the laboratory scale can be done by flocculation induction. This study showed that flocculation induction significantly increased the cell harvest of *Lipomyces maratuensis* InaCC Y720, with the biomass obtained increasing up to 2.7 times compared to the method without flocculation induction. The combination of pH 9 adjustment and the addition of Ca^{2+} ions proved to be the optimal conditions to induce flocculation, resulting in cell aggregations that appeared adherent based on scanning electron microscopy (SEM). These findings suggest that the flocculation induction not only improves cell harvesting efficiency but also has the potential to be applied in industrial-scale lipid production as a more effective harvesting method. Although the main composition of the fatty acids produced remained consistent, there were differences in the percentage of each fatty acid between harvesting methods with and without flocculation induction. However, to maximize the utilization of lipids from flocculated cells, further research is needed to develop more efficient extraction methods. Thus, this study provides a solid foundation for further exploration in the optimization of lipid production using oleaginous yeast.

Key words: Biomass, flocculation, lipids, Lipomyces maratuensis, SEM

INTRODUCTION

The need for vegetable oils is currently met by plants such as rapeseed (Brassica napus) (84%), sunflower seeds (13%), oil palm (1%), soybeans, and others (2%). These plants have a high oleic acid content, but the production of vegetable oils requires a large cost and energy (Sitepu *et al*., 2014). Although these plants are rich in oleic acid, the production of vegetable oil is costly and energy-intensive. Alternatively, microbial oil production offers various advantages, such as a short life cycle, does not require a large area of land, is not affected by climate, simpler care and maintenance, low contamination of viruses and bacteria, does not require a lot of workers, and production is easily increased (Li *et al*., 2008).

Lipids are generally stored in the form of triacylglycerols (TAGs) intracellularly by yeast (Sitepu *et al*., 2019). TAGs of oleaginous yeasts have a similar character to the fatty acid composition of vegetable oils. Published oleaginous yeasts include *Lipomyces, Rhodosporidium*, *Cryptococcus,* and *Yarrowia* (Sitepu *et al*., 2019). However, most of these potential strains were isolated outside Indonesia. The discovery of a new oleaginous yeast isolate, *Lipomyces maratuensis* InaCC Y720, in Indonesia by Yamazaki *et al*. (2017) opened new opportunities in microbial-based lipid production. This strain is able to produce lipids up to 1.6 g/L in three days using a production medium that has been optimized in previous studies (Audinah, 2022).

Despite its great potential, the application of *L. maratuensis* InaCC Y720 on an industrial scale is still constrained by inefficient cell harvesting methods. Harvesting of yeast cells is generally done by mechanical methods such as centrifugation and filtration, which require high energy and intensive equipment maintenance (Sander & Murthy, 2010). Therefore, the flocculation method is considered a cheaper alternative because it does not require special tools in its application. Factors that can affect cell flocculation include pH and the presence of cations such as Ca^{2+}, Mg^{2+}, and Mn^{2+} (Soares, 2011). However, these flocculant factors have different effects on different yeast strains (Louhasakul *et al*., 2018). Therefore, further research is needed to determine the optimal flocculation condition for *L. maratuensis* InaCC Y270 to improve cell harvesting efficiency and support future industrial applications.

MATERIALS AND METHODS

Materials

The isolate used was *L. maratuensis* InaCC Y720, a yeast from the InaCC collection that was first isolated from soil on

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Maratua Island, Indonesia. The materials used consist of glucose, yeast extract, NH_4Cl, $FeCl_3$, $ZnSO_4$, NaOH, HCl, $CaCl_2$, $MgCl_2$, and $MnCl_2$.

Methods

Maintenance and multiplication of microorganisms

The *L. maratuensis* InaCC Y720 strain was collected from the Indonesian Culture Collection. The yeast was stored in Potato Dextrose Agar (PDA) medium in the refrigerator. Before storage, the strain is subcultured using the streaking method and rejuvenated every three months. Cell biomass multiplication for inoculum was carried out by growing the yeast in 100 mL of Potato Dextrose Broth (PDB) medium in a 250 mL Erlenmeyer flask for 48 hr at 30°C and 200 r.p.m agitation.

Flocculation factor screening

The effect of flocculation factors was carried out on the cell suspension of *L. maratuensis* InaCC Y720. Inoculation cells from PDB (10% v/v) were inoculated into 1 L medium containing 50 g/L glucose, 4.5 g/L yeast extract, 1.5 g/L $MgSO_4$, 1 g/L NH_4Cl, 0.016 g/L $FeCl_3$, 0.0002 g/L $ZnSO_4$, 0.0002 g/L $Co(NO_3)_2$, and 0.0001 g/L $MnSO_4$ (Audinah, 2022). Incubation was carried out in a jacketed glass reactor at 28°C and 200 rpm for 72 hr.

The cell suspension obtained from the growth was then put into a centrifuge tube, and the pH of the flocculation factor was adjusted and added to the flocculation factor (Table 1). The experiment was designed using a full factorial design with 28 factor variations (Table 2). The mixture was vortexed for 30 sec before being allowed to settle. Absorbance was measured at 0 and 15 min after the vortex was turned off. The settling cell obtained was measured for absorbance at OD600, and the flocculation efficiency was calculated using the following equation (Louhasakul *et al*., 2018).

$$Flocculation\ \ efficiency\ \ (\%) = \left(1 - \frac{A}{B}\right) \times 100$$

A = absorbance after flocculation
B = absorbance before flocculation

Table 1. Flocculation factors used

Chemical compound	Concentration	Flocculation factor	Reference
$CaCl_2$	5 mM	Ca^{2+}	(Miki *et al*., 1982)
$MgCl_2$	5 mM	Mg^{2+}	(Miki *et al*., 1982)
$MnCl_2$	5 mM	Mn^{2+}	(Miki *et al*., 1982)

Table 2. Variations of the flocculation factor

Running	pH	Cation	Running	pH	Cation
1	pH 6	Mn^{2+}	15	pH 6	Ca^{2+}
2	pH 7	Ca^{2+}	16	pH 3	Mg^{2+}
3	pH 5	Mn^{2+}	17	pH 8	Mg^{2+}
4	pH 9	without cation	18	pH 9	Mg^{2+}
5	pH 4	without cation	19	pH 7	Mn^{2+}
6	pH 9	Ca^{2+}	20	pH 6	Mg^{2+}
7	pH 8	Mn^{2+}	21	pH 7	without cation
8	pH 8	without cation	22	pH 6	without cation
9	pH 9	Mn^{2+}	23	pH 4	Mg^{2+}
10	pH 5	Mg^{2+}	24	pH 3	Ca^{2+}
11	pH 3	Mn^{2+}	25	pH 4	Mn^{2+}
12	pH 4	Ca^{2+}	26	pH 3	without cation
13	pH 5	Ca^{2+}	27	pH 5	without cation
14	pH 8	Ca^{2+}	28	pH 7	Mg^{2+}

Use of selected flocculation factors on lipid production

Incubation of *L. maratuensis* InaCC Y720 on lipid production was performed as described in the flocculation factor screening methodology. The control was left without flocculant addition, while the flocculation was supplemented with flocculant. The flocculation efficiency was measured using the previously described method and calculated using Equation 1. The biomass obtained was then measured for dry weight, and lipid content was determined. In addition, the microscopic appearance of flocculated colonies was observed using Scanning Electron Microscopy (SEM) and compared with the microscopic appearance of colonies without flocculant.

Measurement of biomass and lipid content

Biomass measurement was performed by centrifuging 35 mL of culture at 4000 rpm for 10 min. The pellet was washed with distilled water twice, centrifuged again, and then dried at 60°C. Biomass was measured until a constant weight was obtained.

Lipid extraction was performed by the gravimetric method based on Bligh & Dyer (1959) with modifications. A total of 35 mL of sample was centrifuged at 5000 rpm for 10 min, then washed using 35 mL of distilled water, and repeated twice. 10 mL

of 4M HCl was added and incubated for 2 hr at room temperature. The hydrolysate was then mixed and stirred with 20 mL of chloroform: 10 mL methanol (2:1 (v/v) ratio) for 2 hr at room temperature. The sample was centrifuged at 5000 r.p.m for 10 min until two layers were formed. The lower layer containing lipids was collected using a pipette, and the solvent was evaporated using a mini water bath in a fume hood. The dried biomass was then weighed. Lipid percentage was calculated by comparing lipid weight (g) to dry biomass weight using the following equation.

$$Lipid\ \ percentage\left(\%\right) = \frac{lipid\ \ weight\ \ \left({}^{g}/_{L}\right)}{dry\ \ biomass\ \ weight\ \ \left({}^{g}/_{L}\right)} \times 100$$

Fatty acid profile assay

Lipids produced by the yeast were acid transesterified by adding 500 µL of BF3-MeOH into fatty acid methyl esters (FAME). FAME preparations in GC vials were analyzed using Gas Chromatography-Mass Spectrophotometry (Thermo Scientific ISQ LT Single Quadrupole), TG-WAXM column (0.25 mm diameter, 0.25 µm film, 30 m length). Fatty acids were identified using Chromeleon™ Chromatography Data System (CDS) software.

SEM (Scanning Electron Microscopy) observation

Cells that have been separated from the production medium through centrifugation were observed using an electron microscope. Cells were first coated with Au using a current of 20 mA for 60 sec (Au ion sputter, Hitachi MC1000, Japan). The configuration used in SEM (Hitachi SU3500, Japan) is an accelerating voltage of 3 kV with 30% image contrast. Observations were made at magnification and 10,000×.

Data analysis

Data on flocculation efficiency obtained in the flocculation factor testing stage, as well as biomass and lipids produced in the application of flocculation factors to lipid production, were statistically analyzed using Design Expert 13.

RESULTS AND DISCUSSION

Flocculation factor screening

Screening of flocculation factors resulted in 22 combination variants that affect the occurrence of flocculation in lipid production by *L. maratuensis* Y720. The desirability of the best flocculation factor combination obtained shows the highest value of 0.995 (Table 3). This value is the value closest to the value of 1; thus, this best combination of flocculation factors is considered to me*et al*l the criteria set well.

Table 3. Screening results of flocculation factors

pH	Cation	% Flocculation	Desirability
pH 9	**Ca^{2+}**	**56.031**	**0.995**
pH 9	Mn^{2+}	55.774	0.990
pH 8	Mn^{2+}	51.132	0.908
pH 4	Mn^{2+}	5.185	0.092
pH 7	Mg^{2+}	5.134	0.091
pH 6	no cation	4.700	0.083
pH 8	Mg^{2+}	4.516	0.080
pH 7	no cation	4.512	0.080
pH 8	Ca^{2+}	4.130	0.073
pH 6	Mg^{2+}	3.727	0.066
pH 9	Mg^{2+}	3.711	0.066
pH 5	no cation	2.868	0.051
pH 5	Ca^{2+}	2.718	0.048
pH 7	Ca^{2+}	2.593	0.046
pH 9	no cation	2.566	0.046
pH 6	Mn^{2+}	2.321	0.041
pH 7	Mn^{2+}	1.808	0.032
pH 3	Ca^{2+}	1.684	0.030
pH 5	Mn^{2+}	1.230	0.022
pH 3	Mg^{2+}	1.177	0.021
pH 8	no cation	0.783	0.014
pH 3	no cation	0.310	0.006

The data reveal that the highest flocculation percentage was achieved with the combination of pH adjusted to 9 and the addition of 5 mM Ca^{2+} (Figure 1). The ANOVA test of flocculation factor screening results (Table 4) indicates a *p*-value <0.0001, demonstrating a significant difference between the combination of flocculation factors on affecting flocculation production.

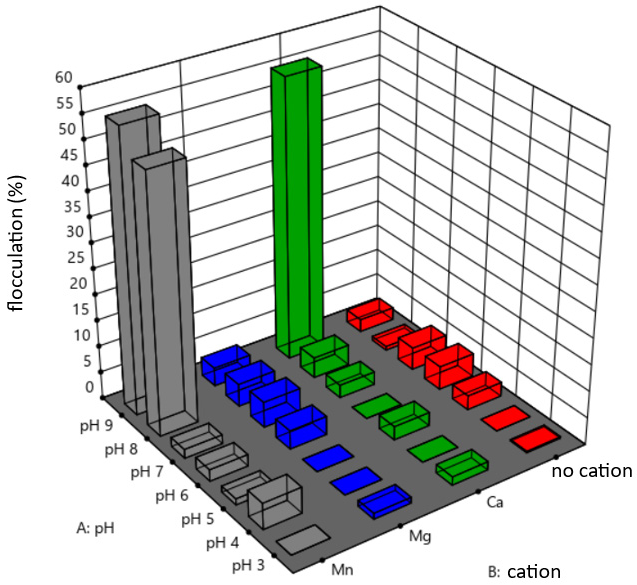

Fig. 1. Distribution of the results of the percentage of flocculation.

Table 4. Analysis of variance of flocculation factor screening results

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	22088.26	27	818.08	9049.13	< 0.0001	Significant
A-pH	8384.96	6	1397.49	15458.20	< 0.0001	
B-kation	2973.48	3	991.16	10963.60	< 0.0001	
AB	10729.82	18	596.10	6593.70	< 0.0001	

A research conducted by Kedong *et al*. (2010) showed that the level of flocculation increased significantly with the addition of Ca^{2+}. In addition, according to research by Stratford (1989), calcium ions can induce flocculation at a wider range of pH values compared to other ions, and calcium-induced flocculation will only be inhibited at very low pH. Yeast such as *Saccharomyces cerevisiae* NCYC 1195 exhibits flocculation ability that is affected by calcium chloride ($CaCl_2$) concentration and pH. Calcium ions can form bonds with mannose on the surface of the yeast cell wall, while acidity affects the specific charge on proteins in the cell wall. This flocculation ability allows *S. cerevisiae* cells to settle and separate from the fermentation medium (Pienasthika *et al*., 2020). This study shows that under the conditions of testing the combination of flocculation factors, a flocculation rate of 56.031% was produced, which was influenced by the addition of Ca^{2+} and pH 9 in the cell harvesting process during lipid production by *L. maratuensis* InaCC Y720.

Biomass flocculation

In the harvesting of lipid production (Figure 2) by *L. maratuensis* InaCC Y720 without pH 9 adjustment and addition of 5 mM Ca^{2+}, no flocculation was observed. Conversely, when a combination of selected flocculation factors was applied, flocculation was evident. The biomass flocculated significantly within the first 10 min after agitation stopped.

Flocculation measurements were recorded every 10 min from min 0 to min 60 since agitation stopped (Figure 3). The settling behavior in the absence of flocculation induction showed no significant differences. However, with flocculation induction through pH adjustment and calcium addition, settling became significant at the 10th min. The fact that no flocculation was observed without pH adjustment and calcium addition emphasizes the important role of environmental parameters in the microbial flocculation process. The introduction of these factors, in particular the adjusted pH and the presence of calcium, played a major role in facilitating cell aggregation, which was evident from the significant increase in flocculation percentage after stirring was stopped. Although no further significant differences were observed in the following min until the end of the observation period, these results suggest that certain environmental factors can significantly accelerate the flocculation process at an early stage after agitation.

Flocculation that occurred in the control and treatment was a combination of pH 9 adjustment and the addition of 5 mM Ca^{2+} (Table 5) at min 0 was not significantly different. However, from the 10th to the 60th min, a significant difference in flocculation was noted between the control and treatment. In general, the protein that plays a role in flocculation, namely lectins, is secreted from mannoproteins that are inserted into the cell wall as part of α-mannan. The presence of lectins in the wall involves genes that code for the synthesis of lectin proteins, in the yeast *Saccharomyces cerevisiae,* called FLO (Stratford, 1994). Calcium ions added as a factor that induces flocculation allow lectins to reach their active conformation as receptors so that they are able to recognize and interact with mannan (Speers *et al*., 2006). In addition, Jin *et al*. (2001) stated that high pH is also able to change ionization on cell surface proteins, including conformational changes in lectin molecules.

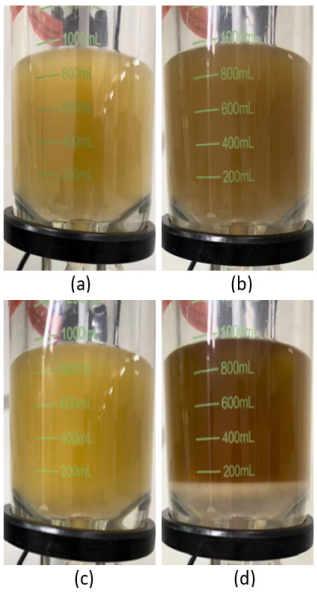

Fig. 2. Settling of *L. maratuensis* Y720 cells. The top pictures are at 0 min after agitation stopped with (a) no flocculation induction (control), and (b) flocculation induction (pH 9 & 5 mM Ca^{2+} addition). Bottom pictures are at 10th min after agitation with (c) no flocculation induction (control), and (d) flocculation induction (pH 9 and 5 mM Ca^{2+} addition)

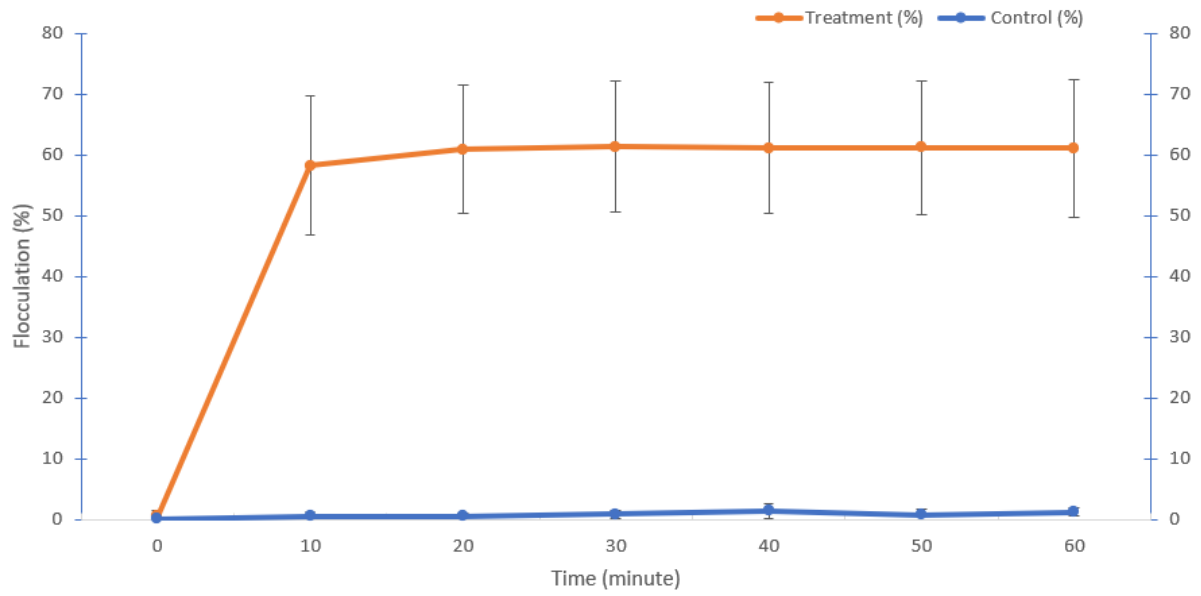

Fig. 3. Control and flocculation percentage graph.

Table 5. Percentage flocculation of control and treatment

Min	% Flocculation	
	Control	Treatment
0	0.17 ± 0.2[a]	0.56 ± 0.9[a]
10	0.58 ± 0.7[a]	58.26 ± 11.5[b]
20	0.57 ± 0.5[a]	60.95 ± 10.6[b]
30	0.88 ± 0.6[a]	61.38 ± 10.8[b]
40	1.41 ± 1.3[a]	61.14 ± 10.8[b]
50	0.84 ± 0.8[a]	61.21 ± 10.9[b]
60	1.28 ± 0.6[a]	61.19 ± 11.3[b]

* The same notation indicates no significant difference

As stated by Soares (2011), yeast flocculation seems to be very effective for producing large-scale products such as renewable fuels. One example is bioethanol, which, despite having minimal added value, is very sensitive to production costs. In the bioethanol industry, batch processes are often used, especially in facilities with smaller production capacities, where cell separation is usually done by centrifugation. Recent data have shown that pH and the presence of certain cations have a significant impact on flocculation efficiency. These factors not only facilitate cell harvesting but can also improve lipid production processes in biotechnology applications, providing further optimization potential in the production of fuels and related products.

Biomass and lipid production

In 200 mL of control production at the bottom of the reactor tube, 0.310 g of dry biomass was collected. In contrast, the biomass yield for production with flocculation, achieved by adjusting the pH to 9 and adding 5 mM Ca^{2+}, was 0.825 g. The harvested production without flocculation factor and with flocculation factor obtained 0.032 g/200 mL (10.323%) and 0.040 g/200 mL (4.848%) lipids, respectively (Table 6).

The application of flocculation factors, pH adjustment to 9, and the addition of Ca^{2+} resulted in a biomass yield that was 2.7 times higher compared to harvesting without these flocculation factors. However, the lipid percentage extracted from the biomass with flocculation was lower than that from the control or non-flocculated biomass.

Table 6. Biomass and lipid production results for control and treatment

	Control	Treatment
Biomass (g/200mL)	0.310 ± 0.020	0.825 ± 0.154
Lipid (g/200mL)	0.032 ± 0.011	0.040 ± 0.014
Lipid (%)	10.323 ± 4.318	4.848 ± 0.823

Increased flocculation is positively correlated with the hydrophobicity of the cell surface (Jin *et al*., 2001). Consequently, the cell wall degradation process, which uses 4 M HCl as a pretreatment for lipid extraction, may be less effective due to the cell wall's hydrophobic nature. The hydrophobicity of *L. maratuensis* InaCC Y720's cell surface impedes the optimal action of HCl, preventing adequate cell wall damage. This observation aligns with findings from Wilcocks & Smart (1995), who reported that acid washing does not significantly impact strains with a highly flocculent phenotype.

The low percentage of total lipid observed in the harvest with induced flocculation is probably due to the addition of Ca^{2+} ions. Calcium is known to contribute to the stability of the cell wall by regulating the synthesis and composition of key polysaccharides like β-glucan and mannan. In Saccharomyces cerevisiae and many ascomycete yeasts, the cell wall is predominantly made up of mannoproteins, β-glucans, and chitin (Verstrepen & Klis, 2006).

Scanning electron microscopy of cells

SEM observations (Figure 4) revealed that the control cells appeared relatively detached and did not adhere to one another. Additionally, the presence of debris around the control cells obscured the clarity of the visual images of *L. maratuensis*. Stewart *et al*. (2013) in their research found that the centrifugation process can cause cell damage. This is due to the results of cell observations in this study, which are less clearly visible due to the presence of object debris, which is strongly suspected to be damaged cells. However, in the observation of the sample of flocculated cells, the cells look dense and appear attached as a result of the intercellular bonds formed from flocculation, so as to prevent cell damage.

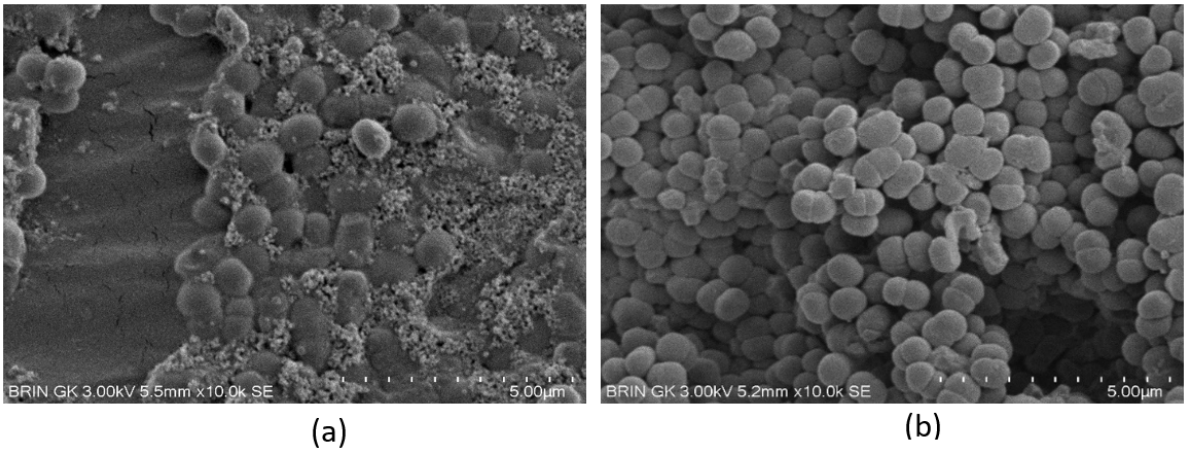

Fig. 4. SEM of (a) control yeast cells and (b) flocculated yeast cells at a magnification of 10.000x

Stewart (2017) observed that the hydrodynamic shear induced by centrifugation led to the release of cell surface components, such as mannoproteins. The centrifugal force within the centrifuge generates various reactive forces among the liquids, solids, and disks, including shear stress on the yeast cell surface, which can result in destabilization of the yeast cells.

Fatty acid analysis of *L. maratuensis* InaCC Y720

Fatty acids identified from lipid production by *L. maratuensis* InaCC Y720 in control and flocculation consisted of the same types of oleic acid, palmitic acid, stearic acid, and linoleic acid (Table 7). However, there is a difference in the percentage of fatty acid acquisition between the control and flocculation. The exact percentage difference in the same type of fatty acid between the control and flocculated samples remains uncertain. The variation in percentage of lipid content is attributed to the hydrophobic nature of the flocculated cells, which prevents HCl from effectively lysing them. Hydrophobic surfaces tend to resist contact with acids, thus blocking of HCL from the cell and reducing the ability of acids to solubilize lipids. In contrast, HCl faces no such obstruction in the control cells. Consequently, the partial destruction of the flocculated cells impedes the dissolution of fatty acids in the solvent during the lipid extraction process.

Table 7. Fatty acids identified from lipid production by *L. maratuensis* InaCC Y720

Chemical Compound	Formula	Molecular Weight (g/mol)	Rel. Area (%)		Synonym
			Control	Flocculation	
9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296	38.21	23.21	Oleic acid
Hexadecanoic acid, methyl ester	C17H34O2	270	21.19	22.28	Palmitic acid
Methyl stearate	C19H38O2	298	8.35	9.27	Stearic acid
Methyl 9-cis,11-trans- octadecadienoate	C19H34O2	294	2.78	1.48	Linoleic acid

CONCLUSION

Induction of flocculation by adjusting the pH to 9 and adding 5 mM Ca^{2+} improved the cell harvesting efficiency of *L. maratuensis* InaCC Y720. The flocculation factor applied at the stage of yeast cell harvesting did not affect the type of fatty acids as a result of lipid production from *L. maratuensis* InaCC Y720, but caused differences in total lipids obtained. Observations using SEM showed that flocculation caused the cells to adhere to each other more tightly and form solid aggregates, making it easier to separate the biomass from the liquid medium. This finding has the potential to improve lipid extraction efficiency for industrial-scale production by reducing biomass harvesting costs. Further studies can explore the optimization of flocculation conditions to increase lipid yield as well as its application on an industrial scale.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Audinah, L. 2022. Optimasi Medium untuk Produksi Lipid oleh *Lipomyces maratuensis* InaCC Y720 menggunakan Desain Eksperimen Statistikal (M.Sc.). Institut Teknologi Bandung.

Bligh, E.G. & Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37(8): 911-917. https://doi.org/10.1139/o59-099

Jin, Y.L., Ritcey, L.L., Speers, R.A. & Dolphin, P.J. 2001. Effect of cell surface hydrophobicity, charge, and zymolectin density on the flocculation of *Saccharomyces cerevisiae*. Journal of the American Society of Brewing Chemists, 59(1): 1-9. https://doi.org/10.1094/ASBCJ-59-0001

Kedong, M., Minato, W., Kenji, S. & Yoshihito, S. 2010. Flocculation phenomenon of a mutant flocculent Saccharomyces cerevisiae strain: Effects of metal ions, sugars, temperature, pH, protein-denaturants and enzyme treatments. African Journal of Biotechnology, 9(7): 1037-1045. https://doi.org/10.5897/AJB09.1541

Li, Q., Du, W. & Liu, D. 2008. Perspectives of microbial oils for biodiesel production. Applied Microbiology and Biotechnology, 80(5): 749-756. https://doi.org/10.1007/s00253-008-1625-9

Louhasakul, Y., Cheirsilp, B., Maneerat, S. & Prasertsan, P. 2018. Potential use of flocculating oleaginous yeasts for bioconversion of industrial wastes into biodiesel feedstocks. Renewable Energy, 136: 1311-1319. https://doi.org/10.1016/j.renene.2018.10.002

Miki, B.L., Poon, N.H., James, A.P. & Seligy, V.L. 1982. Possible mechanism for flocculation interactions governed by gene FLO1 in *Saccharomyces cerevisiae*. Journal of Bacteriology, 150(2): 878-889. https://doi.org/10.1128/jb.150.2.878-889.1982

Pienasthika, M., Brahmanti, A.A., Purwatiningrum, I. & Wardani, A.K. 2020. A study of the effect of calcium chloride ($CaCl_2$) and pH on the flocculation ability of *Saccharomyces cerevisiae* (NCYC 1195). In: IOP Conference Series: Earth and Environmental Science. IOP Publishing, Bristol. pp. 012082. https://doi.org/10.1088/1755-1315/475/1/012082

Sander, K. & Murthy, G.S. 2010. Life cycle analysis of algae biodiesel. The International Journal of Life Cycle Assessment, 15(7):

704-714. https://doi.org/10.1007/s11367-010-0194-1

Sitepu, I.R., Garay, A.L., Cajka, T., Fiehn, O. & Boundy-Mills, K.L. 2019. Laboratory screening protocol to identify novel oleaginous yeasts. In: Methods in Molecular Biology. A. Sigurdsson (Ed.). Springer, New York. pp. 33-50. https://doi.org/10.1007/978-1-4939-9484-7_2

Sitepu, I.R., Garay, L.A., Sestric, R., Levin, D., Block, D.E., German, J.B. & Boundy-Mills, K.L. 2014. Oleaginous yeasts for biodiesel: current and future trends in biology and production. Biotechnology Advances, 32(7): 1336-1360. https://doi.org/10.1016/j.biotechadv.2014.08.003

Soares, E.V. 2011. Flocculation in *Saccharomyces cerevisiae*: A review. Journal of Applied Microbiology, 110(1): 1-18. https://doi.org/10.1111/j.1365-2672.2010.04897.x

Speers, R.A., Wan, Y.Q., Jin, Y.L. & Stewart, R.J. 2006. Effects of fermentation parameters and cell wall properties on yeast flocculation. Journal of the Institute of Brewing, 112(3): 246-254. https://doi.org/10.1002/j.2050-0416.2006.tb00720.x

Stewart, G.G. 2017. Harvesting and cropping yeast: Flocculation and centrifugation. In: Brewing and Distilling Yeasts. G.G. Stewart (Ed.). Springer International Publishing, Cham. pp. 259-308. https://doi.org/10.1007/978-3-319-69126-8_13

Stewart, G.G., Hill, A.E. & Russell, I. 2013. 125th Anniversary Review: Developments in brewing and distilling yeast strains. Journal of the Institute of Brewing, 119(4): 202-220. https://doi.org/10.1002/jib.104

Stratford, M. 1989. Yeast flocculation: calcium specificity. Yeast, 5(6): 487-496. https://doi.org/10.1002/yea.320050608

Stratford, M. 1994. Genetic aspects of yeast flocculation: in role of FLO genes in the flocculation of. Colloids and Surfaces B: Biointerfaces, 2(1-3): 151-158.

Verstrepen, K.J. & Klis, F.M. 2006. Flocculation, adhesion and biofilm formation in yeasts. Molecular Microbiology, 60(1): 5-15. https://doi.org/10.1111/j.1365-2958.2006.05072.x

Wilcocks, K.L. & Smart, K.A. 1995. The importance of surface charge and hydrophobicity for the flocculation of chain-forming brewing yeast strains and resistance of these parameters to acid washing. FEMS Microbiology Letters, 134(2-3): 293-297. https://doi.org/10.1111/j.1574-6968.1995.tb07953.x

Yamazaki, A., Kanti, A. & Kawasaki, H. 2017. Three novel lipomycetaceous yeasts, *Lipomyces maratuensis* sp. nov., *Lipomyces tropicalis* sp. nov., and *Lipomyces kalimantanensis* f.a., sp. nov. isolated from soil from the Maratua and Kalimantan Islands, Indonesia. Mycoscience, 58(6): 413-423. https://doi.org/10.1016/j.myc.2017.06.002