

Improving Citrus Peel Essential Oil Production Using *Aspergillus Niger* as A Biological Pretreatment

Peningkatan Produksi Minyak Atsiri dari Kulit Jeruk Melalui Perlakuan Awal Biologis Menggunakan *Aspergillus Niger*

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Abstract

*Orange peel represents a promising renewable feedstock for essential oil production; however, the rigid lignocellulosic matrix restricts the efficiency of conventional extraction techniques. This study investigates the effectiveness of biological pretreatment in enhancing essential oil yield from orange peel through fungal fermentation. Pretreatment was conducted using *Aspergillus niger* to modify the lignocellulosic structure, followed by essential oil extraction and statistical evaluation of process parameters. The results demonstrate that biological pretreatment significantly increases essential oil yield compared with untreated samples. Optimal conditions were achieved at an inoculum concentration of 2.5%, glucose supplementation of 2.5 g, and a fermentation duration of 2 days. The improved extraction performance is attributed to enzymatic degradation of structural components, which enhances oil release. These findings highlight the potential of *Aspergillus niger* – based biological pretreatment as an efficient and environmentally sustainable strategy for valorizing orange peel waste in essential oil production.*

Keywords: *Aspergillus niger, Biological Pretreatment, Essential Oil, Orange peel, process optimization*

Abstrak

Kulit jeruk merupakan bahan baku terbarukan yang berpotensi tinggi untuk produksi minyak atsiri; namun, keberadaan matriks lignoselulosa yang kaku membatasi efisiensi metode ekstraksi konvensional. Penelitian ini bertujuan untuk mengkaji efektivitas perlakuan awal biologis dalam meningkatkan rendemen minyak atsiri dari kulit jeruk melalui proses fermentasi. Perlakuan awal dilakukan menggunakan *Aspergillus niger* untuk memodifikasi struktur lignoselulosa, kemudian dilanjutkan dengan proses ekstraksi minyak atsiri dan analisis statistik terhadap parameter proses. Hasil penelitian menunjukkan bahwa perlakuan awal biologis mampu meningkatkan rendemen minyak atsiri secara signifikan dibandingkan dengan bahan tanpa perlakuan. Kondisi optimum diperoleh pada konsentrasi inokulum sebesar 2,5%, penambahan glukosa sebanyak 2,5 g, dan waktu fermentasi selama 2 hari. Peningkatan kinerja ekstraksi dikaitkan dengan degradasi enzimatik komponen struktural yang mempermudah pelepasan minyak atsiri. Temuan ini menunjukkan bahwa perlakuan awal biologis berbasis *Aspergillus niger* merupakan strategi yang efisien dan ramah lingkungan untuk meningkatkan pemanfaatan limbah kulit jeruk dalam produksi minyak atsiri.

Kata Kunci: *Aspergillus niger, Perlakuan Awal Biologi, Minyak Atsiri, Kulit Jeruk, Proses Optimasi*

INTRODUCTION:

In West Kalimantan, Pontianak Orange (*Citrus nobilis* var. *macrocarpa*) can be found all year round, and the cultivation of oranges leads to the generation of a byproduct: orange peels. Orange peels can be converted to more valuable products, like essential oils, value which is lost by the processes we currently must use to separate the oils from the peels. For this reason, new and low-cost methods that enhance oil extraction and recovery from orange peels would be more ecologically sound. One method is to use the fungus *Aspergillus niger*, which is known to have a rare ability to secrete enzymes that break down lignocellulose. The orange peels' lignocellulosic structure means that certain essential oils cannot be extracted from peels. Methods that extract oils from plant tissues without using the oils' end products require more process steps. In addition to oil extraction processes, orange peels have to be treated first. In addition, pretreatment can be physical, chemical, and biological, but only biological pretreatment, as opposed to chemical or physical pretreatment methods, is free of toxic chemical byproducts, is more socially acceptable, more readily available, simpler and process is more ecological.

Biological pretreatment using *Aspergillus niger* has received considerable attention due to its generally recognized as safe (GRAS) status and efficient enzyme secretion system (Shet et al., 2018). As an aerobic fungus, *Aspergillus niger* produces extracellular enzymes, particularly pectinases and cellulases, which play a crucial role in degrading plant cell wall polysaccharides (Pandey et al., 2019; de Souza et al., 2020). These enzymes hydrolyze the α -1,4-glycosidic bonds in pectin located in the middle lamella, thereby weakening the structural integrity of plant tissue.

The enzymatic breakdown of pectin and cellulose relaxes the rigid cell wall matrix and facilitates the disruption of oil glands (*schizogenic cavities*) in citrus peels. This targeted degradation primarily affects the structural "glue" components between cells without altering the chemical constituents of the essential oil (Martins et al., 2020). Therefore, *Aspergillus niger* represents a promising biological approach to enhance essential oil recovery through increasing cell wall permeability while maintaining oil quality.

Despite this fact, most of the prior research has been centered around the

assessment of one or possibly two process variables in isolation, such as fermentation time or the type of microorganism, overlooking the impact of various interacting process variables, concurrently. Moreover, the biological pretreatment process optimization has most often employed the response surface methodology (RSM), whereas the use of three-way factorial ANOVA to evaluate the impact of the most prominent three variables interacting has been most limited in, orange peel fermentation systems (Kwanga et al., 2022; Boum et al., 2021).

In addition, the evaluation of the quality of essential oils post pretreatment to international standards, such as ISO 3140:2019, has been limited (Ferreira et al., 2018; ISO, 2019).

MATERIAL AND METHODS:

Materials and Sample Preparation

Orange peel (OP) was obtained from agricultural orange waste in West Kalimantan, Indonesia. The peel was washed with clean water to remove adhering impurities and subsequently air-dried at ambient temperature (approximately 27°C) overnight to reduce surface moisture.

The dried OP, subjected to size reduction to approximately 2-5mm. Then the dried OP was stored in a thin-wall container to prevent contamination.

For control experiments (without pretreatment) fresh OP was directly subjected to steam distillation under identical extraction conditions.

Biological Pretreatment

A fermentation process was carried out with *Aspergillus niger* under solid-state fermentation (SSF) conditions as the microorganism for biological pretreatment. 100g of dried OP was placed in thinwall plastic bowl previously cleaned using 70% of ethanol. Then, dried OP was then inoculated with *Aspergillus niger* at varying inoculum concentration (2.5%, 5% & 7.5%). The sugar was added as a supplemented carbon source for the support of microbial growth during the fermentation process with 4 variation (0g, 2.5g, 5g and 7.5g). Fermentation was conducted under static conditions at ambient temperature (27°C) for 2-4 days.

Essential Oil Extraction

Following the pretreatment process, the fermented OP was subjected to essential oil extraction using steam distillation. A

water to substrate ratio of 2:1 (v/w) was applied during distillation.

of main effects and interaction effects among the three variables.

The distillation process was continued until the extraction yield reached a plateau, which occurred at approximately 40 minutes. The condensed distillate was and the essential oil layer was separated manually.

Essential Oil Quality Analysis

Essential oil yield was calculated based on the volume of essential oil obtained per 100g of raw material using the following equation:

Qualitative analysis determined if biological pretreatment had any impact on the quality of the essential oil. The chemical composition of essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS), while the organoleptic properties were evaluated according to ISO 3140:2019.

$$Yield (\%) = \frac{Volume\ of\ Oil\ (ml)}{100g\ raw\ material} \times 100$$

Experimental Design and Statistical Analysis

A three factors factorial experimental design was applied to evaluate the effects of:

- Inoculum concentration (α)
- Sugar addition (β)
- Fermentation time (γ)

Each factor consisted of multiple levels according to the treatment combinations described in the study.

All experiments were conducted in triplicate for each treatment combination. Data were analyzed using three-way factorial analysis of variance (ANOVA) at 95% confidence level ($\alpha = 0.05$) to determine the significance

RESULTS AND DISCUSSION

Overall Effect of Biological Pretreatment

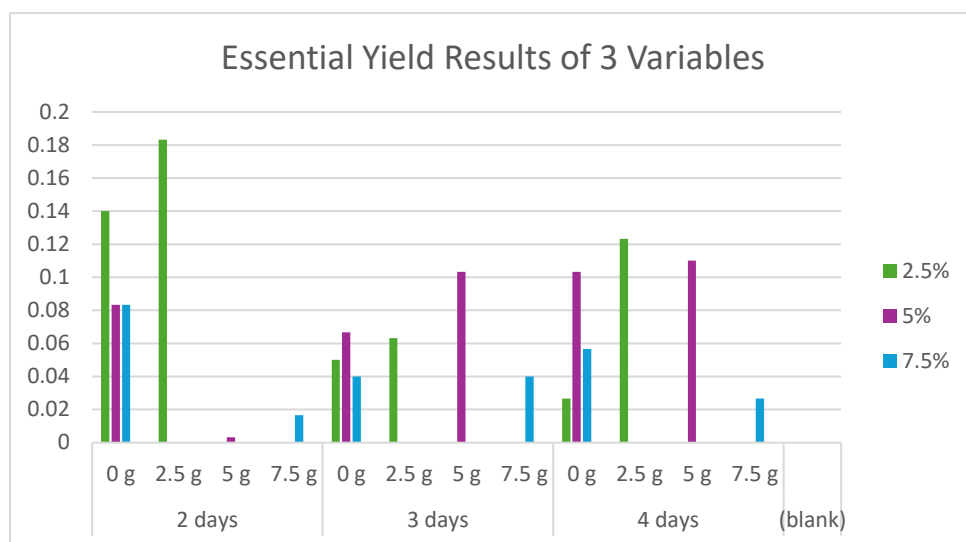


Figure 1 Essential Yield Results of 3 variables

The results demonstrated that biological pretreatment using *Aspergillus niger* significantly enhanced the essential oil yield from OP. Compared to the non-pretreated condition (0.06ml/100g), the optimized fermentation treatment increased the yield to 0.183 ml/100g, representing an approximately 205% increase (about a three-fold improvement). This, substantial enhancement highlights the effectiveness of solid-state fermentation in improving oil recovery.

Variations in inoculum concentration, glucose supplementation, and fermentation time affected extraction performance, both individually and through interactions between these parameters. The significant interaction effects observed in the ANOVA indicate that yield improvement does not depend on a single parameter alone, but rather on a synergistic combination of process variables.

Comparison of Essential Oil Yield with Previous Studies

Table 1 Yield Comparison with Previous Studies

Experiment	Pre Treatment with Microorganism	Extraction Methods	Raw Materials (g)	Volume (ml)	Yield (v/100g Raw Materials)
<i>Saulie et al.</i> (2023) – <i>Citrus sinensis</i>	No	Hydrodistillation	300	0.233	0.078%
<i>Sabang et al.</i> (2023) – <i>Citrus Reticulata</i>	No	Steam Distillation	200	0.115	0.058%
<i>Sabang et al.</i> (2023) – <i>Citrus Amblycarpa</i>	No	Steam Distillation	200	1.01	0.505%
This Experiment - <i>Citrus nobilis</i> var. <i>microcarpa</i>	<i>Aspergillus niger</i>	Steam Distillation	100	0.183	0.183%

The highest essential oil yield obtained in this experiment (0.183ml/100g, 0.18%) was higher than those reported for *Citrus sinensis* (0.233ml/300g, 0.078%) and *Citrus reticulata* (0.115ml/200g, 0.058%) extracted without microbial pretreatment using hydro distillation and steam distillation methods, respectively. This improvement suggesting that solid-state fermentation using *Aspergillus niger* effectively enhances oil recovery from citrus peel.

However, the yield in this experiment was lower than *Citrus amblycarpha* (1.01ml/200g, 0.505%). This difference may be attributed to species-specific variations in oil gland density, peel thickness, and intrinsic essential oil composition.

The enhanced yield observed in this study can be mechanistically explained by the enzymatic activity of *Aspergillus niger*, which is known to produce cellulases, pectinases, and hemi cellulases. These enzymes degrade structural polysaccharide in

citrus peel cell walls, facilitating rupture of oil glands during steam distillation. The significant interaction effects observed in the ANOVA further indicate that oil recovery is

influenced by synergistic interactions among inoculum concentration, sugar adding and fermentation time

Three Way ANOVA Result

Table 2 Results Yield of Essential Oil with interaction of 3 factors

Faktor	df	SS	MS	FCount	p-value
α (Inoculum)	2	0.04501	0.02250	11.73	0.0001
β (Sugar)	1	0.00007	0.00007	0.03	0.8532
γ (Fermentation)	2	0.00541	0.00271	1.41	0.2572
$\alpha\beta$	2	0.01703	0.00852	4.44	0.0189
$\alpha\gamma$	4	0.04579	0.01145	5.97	0.0009
$\beta\gamma$	2	0.02866	0.01433	7.47	0.0019
$\alpha\beta\gamma$	4	0.04785	0.0120	6.24	0.0006
Error	36	0.0691	0.00192	-	

From **Table 1**, we can see the effects of inoculum concentration (α), glucose addition (β), fermentation time (γ), and their interactions on essential oil yield were evaluated using three-way factorial ANOVA at a 95% confidence level ($\alpha = 0.05$).

Three-way ANOVA analysis revealed that inoculum concentration significantly affected essential oil yield ($F_{Count} = 11.73$, $p = 0.0001$). In contrast, sugar addition ($F_{Count} = 0.03$, $p = 0.8532$) and fermentation time ($F_{Count} = 1.41$, $p = 0.2572$) did not show significant individual effects.

Interaction Effects among Process Variables

Significant interaction effects were observed between inoculum concentration and sugar addition ($F_{Count} = 4.44$, $p = 0.0189$), inoculum concentration and fermentation time ($F_{Count} = 5.97$, $p = 0.0009$), sugar addition and fermentation time ($F_{Count} = 7.47$, $p = 0.0019$), as well as the three-way interaction ($F_{Count} = 6.24$, $p = 0.00006$).

These findings indicate that essential oil yield is strongly influenced by the combined interaction of process variables rather than by individual factors alone.

Optimal Pretreatment Condition

After considering the experimental results and subsequent three-way ANOVA

analysis, the best pretreatment parameters for essential oil yield optimization were found to be an inoculum concentration of 2.5%; glucose 2.5 g; and a fermentation period of 2 days. This method is seen as innovative and sustainable in optimizing the use of waste OP's.

The *Aspergillus niger* biological pretreatment during this period of fermentation structurally modified the *lignocellulosic* components of the OP, which, in conjunction with subsequent steam distillation, made available the essential oil.

The increase in essential oil yield after biological pretreatment can be explained by the enzymatic action of *Aspergillus niger* on the cell wall structure of the OP. Pectinase is crucial in the degradation of the middle lamella pectin substances which hold plant cells together. Loosening of the cell matrix is initiated by the degradation which paves the way for the

separation of cell walls and the breaking of the structural cells surrounding the oil sacs. Oil sacs are also known as oil glands. The action of cellulase and hemicellulose also raises the degradation of cellulose and hemicellulose, which increases the wall's porosity and reduces the mass transfer resistance. All of these enzymes act together to improve the ease of accessing the oil glands of the peel tissue, which helps to release essential oil during the steam distillation process.

Aspergillus niger pretreatment optimally demonstrates that both moderate inoculum inclusion and fermentation period are important to attain the maximum yield. Primary and marginal fermentation periods, in conjunction with fermentation nutrient deficiencies, did not yield higher results and were probably a result of substrate depletion, enzyme suppression, or a shift in the metabolic activity of the microorganisms.

Essential Oil Quality Analysis

Table 3 Results of GC-MS for sample MAKJ01 (With Pretreatment)

Peak	RT (min)	Compound	Chemical Class	Area (%)
1	2.96	α -Pinene	Monoterpene hydrocarbon	5.70
2	4.01	Limonene	Monoterpene hydrocarbon	85.56
3	11.15	Minor oxygenated terpene	Oxygenated compound	1.28
4	15.17	Minor oxygenated compound	Oxygenated compound	7.46

Table 4 Results of GC-MS for sample MAKJ02 (Without Pretreatment)

Peak	RT (min)	Compound	Chemical Class	Area (%)
1	2.967	α -Pinene	Monoterpene	7.22
2-8	3.1-3.9	Trace Compounds	Minor Volatil	2.5 (Total)
9	4.057	Limonene	Monoterpene	88.39
10-23	4.9-10.4	Unidentified oxygenated compound	Oxygenat	1.9 (Total)

GC-MS analysis showed that limonene was the dominant compound in both samples, accounting for 85.56% (MAJK01) and 88.39% (MAJK02) of the total chromatographic area. According to ISO 3140, limonene is the main constituent of citrus peel essential oils and typically accounts for more than 70% of the total composition. Therefore, the limonene levels obtained in this study are within the acceptable composition range, indicating

compliance with international quality standards. The presence of α -pinene (5–7%) and minor oxygenated compounds in small proportions further reflects the typical citrus monoterpene profile.

Importantly, no abnormal peaks or excessive oxygenated degradation products were detected in the chromatograms. This indicates that the biological pretreatment using *Aspergillus niger* did not cause

oxidative damage or thermal decomposition during steam distillation. Instead, fermentation increased oil yield while maintaining the chemical integrity required by ISO 3140:2019 specifications.

From an organoleptic perspective, the dominance of limonene strongly correlates with the fresh, sweet, and citrus-like aroma typical of high-quality orange essential oils. The sample with a higher limonene content (MAJK02) exhibited a more intense citrus aroma, consistent with the known sensory contribution of monoterpene hydrocarbons. The absence of any off-odor or fermentation aroma further indicates that microbial pretreatment did not negatively impact the quality of the volatile aromas.

Visually, the oil exhibited a clear to pale yellow appearance without turbidity, sediment, or discoloration. This observation aligns with ISO 3140:2019 quality expectations for orange essential oils and supports the GC–MS findings that no significant degradation or contamination occurred. Overall, both the chemical composition and organoleptic characteristics confirm that solid-state fermentation improves oil recovery while maintaining international quality standards.

CONCLUSIONS

Biological pretreatment using *Aspergillus niger* has proven effective in increasing essential oil yield from OP. A three-way factorial ANOVA analysis showed that inoculum concentration significantly affected essential oil yield, while glucose addition and fermentation time showed a significant effect primarily through interaction between the variables. The strong interaction effect between the three variables highlights the importance of simultaneous process optimization rather than evaluating a single variable.

Optimal pretreatment conditions were achieved at an inoculum concentration of 2.5%, glucose addition of 2.5%, and a fermentation time of 2 days. Under these conditions, biological pretreatment effectively disrupted the lignocellulose structure of OP, thereby increasing oil recovery during steam distillation. As per the quality evaluation, which included an analysis from GC–MS, the essential oil produced was within the standard quality parameters which demonstrated that the pretreatment process did not negatively influence any characteristics of the oil. In conclusion, the use of *Aspergillus niger* as a biological pretreatment method is straight

forward, sustainable, and economical, improving the conversion of OP waste into value-added products in the form of essential oils.

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REFERENCES

Bailey, J. E., & Ollis, D. F. (1986). *Biochemical engineering fundamentals* (2nd ed.). McGraw-Hill.

Boum, J. P., Ndoye, F. O., & Tchiégang, C. (2021). *Chemical composition and antimicrobial activity of essential oils from Citrus sinensis peels*. International Journal of Food Science.

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). *Ultrasound assisted extraction of food and natural products*. *Ultrasonics Sonochemistry*, **34**, 540–560.

Farhat, A., Ginies, C., Romdhane, M., & Chemat, F. (2017). *Eco-friendly and cleaner process for isolation of essential oil using microwave energy: Experimental and theoretical study*. *Journal of Chromatography A*, 1218, 5077–5085.

Hasanah, Uswatun. (2021). *Skrining Fitokimia dan Uji Aktivitas Antioksidan Minyak Atsiri dari Kulit Jeruk Manis (Citrus sinensis L.)*. *Jurnal Kimia dan Pendidikan*

ISO. (2019). *ISO 3140:2019 – Essential oil of sweet orange (Citrus sinensis)*. *International Organization for Standardization*.

Kurniawan, Hery. (2017). *Ekstraksi dan Karakterisasi Minyak Atsiri Kulit Jeruk dengan Metode Distilasi Air*. *Jurnal Teknologi Pertanian*.

Kwanga, J. K., et al. (2022). *Biological pretreatment strategies to enhance*

- essential oil yield*. Journal of Cleaner Production, 335, 130260
- Laurita, Risma. (2016). *Aktivitas Antijamur Minyak Atsiri Jeruk terhadap Pertumbuhan Jamur Kontaminan*. Indonesian Journal of Pharmacy.
- Liu, Y., Cao, J., Li, X., & Zhang, L. (2021). *Chemical composition and biological activities of essential oils from the peels of various Citrus species*. Molecules, 26(10).
- Martins, M., Martins, A., Ferreira, I. C., & Figueiredo, I. (2020). *Aspergillus niger as a cell factory for the production of industrial enzymes using agro-industrial wastes*. Frontiers in Bioengineering and Biotechnology.
- Montgomery, D. C. (2017). *Design and analysis of experiments* (9th ed.). Wiley.
- Nurhadianty, R., et al. (2017). *Pretreatment of citrus peel using microbial fermentation to enhance essential oil yield*. Indonesian Journal of Chemistry, 17(3), 452–460.
- Nurhadianty, R., et al. (2020). *Optimization of biological pretreatment on citrus peel essential oil extraction*. Journal of Applied Research on Medicinal and Aromatic Plants, 18, 100244
- Pandey, Ashok., Larroche, Christian., & Soccol, Carlos Ricardo. (2019). *Enzyme production by Aspergillus niger and its industrial applications*. Book: Biology, Cultivation and Applications.
- Putra, A. S., Sembiring, L., & Wahyuningsih, M. S. H. (2018). *Synchronization of hyphal growth and enzyme production in Aspergillus niger*. Journal of Microbial Biotechnology.
- Revian, R., Saulie, Diffa Aprilydia., Kulla, Periskila Dina Kali., Zulwanis., & Marniati. (2026). *Volatile profiles and phytochemical screening of Citrus microcarpa and its potential as an antibacterial agent*. Journal of Healthcare Technology and Medicine.
- Ruijter, G. J. G., & Visser, J. (1997). *Carbon catabolite repression in Aspergillus niger*. Microbiology, 143(9), 2991-2998.
- Saulie, D. A., Kulla, P. D. K., Zulwanis, & Marniati. (2024). *Skrining Fitokimia Minyak Atsiri Kulit Jeruk Manis (Citrus sinensis) dan Batang Serai*

- (*Cymbopogon citratus*). Journal of Healthcare Technology and Medicine, 10(1), 357-364.
- Sawamura, M. (2000). *Citrus essential oils: Flavor and fragrance*. Wiley.
- Shet, A. R., Desai, S. V., & Achappa, S. (2018). *Pectinolytic Enzymes: Classification, Production, Purification and Applications. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 4(3), 337-348.
- de Souza, L. P., et al. (2020). *Enzymatic pretreatment to improve essential oil extraction from plant matrices. Biotechnology Reports*, 26, e00444.
- Suardhika, I. M. (2018). *Perbandingan pengaruh lama pengeringan terhadap rendemen minyak atsiri kulit jeruk manis (Citrus sinensis) dengan destilasi uap dan identifikasi linalool dengan KLT-Spektrofotodensitometri. Jurnal Farmasi Udayana*, 7(2), 77-83.
- Supriyatman. (2023). *Determination of essential oil yield from extraction. Jurnal Health Technology and Medicine*, 9(1), 28-33..
- Tutuarima, T., & Handayani, S. (2019). *Pengaruh fermentasi terhadap rendemen minyak atsiri limbah pertanian. Jurnal Rekayasa Proses*, 13(2), 89-96.
- Tri Wijayanti, Y., Sumiyati, S., Tira, D. S., & Khalid Fredy Saputra, M. (2024). *Health education for the community in the prevention of hypertension and stroke. Jurnal Pengabdian Masyarakat Edukasi Indonesia*, 1(3), 77-84
- Yuniantari, N. L. P., Susilowati, E., & Anjarsari, D. N. (2025). *Beta myrcene dan dl-limonene sebagai komponen utama ekstrak kulit jeruk siam (Citrus nobilis) dari ekstraksi dengan metode sokhletasi. Jurnal Crystal: Publikasi Penelitian Kimia dan Terapannya*, 7(2), 176-182.