BAB V
KESIMPULAN DAN SARAN

V.1 Kesimpulan

Berdasarkan hasil penelitian, dapat disimpulkan bahwa ampas kopi masih memiliki komposisi kimia yag potensial sebagai substrat fermentasi, dengan kandungan hemiselulosa, selulosa, dan lignin yang tinggi serta kandungan senyawa fenolik dan aktivitas antioksidan yang signifikan. Fermentasi ampas kopi dengan *Aspergillus niger* dan penambahan natrium nitrat ($NaNO_3$) sebagai sumber nitrogen menunjukkan pengaruh yang signifikan terhadap peningkatan senyawa metabolit sekunder. Perlakuan penambahan $NaNO_3$ dengan konsentrasi 1% menghasilkan peningkatan tertinggi pada total senyawa fenolik hingga 1340 mg GAE/L dan aktivitas antioksidan sebesar 77% dengan persentase peningkatan sebesar 103% dan 43% dibandingkan sebelum fermentasi. Sebaliknya, parameter metabolit primer seperti kadar protein dan glukosa tidak menunjukkan perubahan signifikan pada tiap variasi konsentrasi. Hal ini mengindikasikan bahwa variasi konsentrasi $NaNO_3$ lebih berperan dalam regulasi metabolisme sekunder. Dengan demikian, fermentasi ampas kopi berpotensi meningkatkan kandungan senyawa bioaktif, serta mendukung pemanfaatan limbah agrikultur dan dapat dikembangkan menjadi produk fungsional untuk mendukung ekonomi sirkular.

V.2 Saran

Analisis senyawa fenolik dapat dikembangkan lebih lanjut untuk karakterisasi jenis-jenis senyawa fenolik tertentu, guna mengidentifikasi peningkatan pada senyawa fenolik spesifik yang dihasilkan. Hasil terbaik dapat dianalisa konsentrasi IC_{50} untuk menguji efektivitas antioksidan.

DAFTAR PUSTAKA

[1] *United States Department of Agriculture, Foreign Agricultural Service,* (2024). "*Coffee: World Markets and Trade*".

[2] Rodriguez, S., Ruizl, G.P., Chinchay, E.H. & Rafael, D.F., 2023. Bio-based carrageenan composite coatings for food packaging application. *Proceedings of the 21st LACCEI International Multi-Conference for Engineering, Education and Technology*, vol. 2023-July. Buenos Aires, Argentina, 19–21 July. Available through Latin American and Caribbean Consortium of Engineering Institutions.doi:10.18687/LACCEI2023.1.1.849.

[3] Brzezińska, R.; Górska, A.; Wirkowska-Wojdyła, M.; Piasecka, I. *Response Surface Methodology as a Tool for Optimization of Extraction Process of Bioactive Compounds from Spent Coffee Grounds.* Appl. Sci. 2023, 13, 7634. https://doi.org/10.3390/app13137634

[4] Zengin, G., Sinan, K. I., Mahomoodally, M. F., Angeloni, S., Mustafa, A. M., Vittori, S., Maggi, F., & Caprioli, G. (2020). Chemical composition, antioxidant and enzyme inhibitory properties of different extracts obtained from spent coffee ground and coffee silverskin. *Foods*, *9*(6). https://doi.org/10.3390/FOODS9060713

[5] Zengin, G., Sinan, K. I., Mahomoodally, M. F., Angeloni, S., Mustafa, A. M., Vittori, S., Maggi, F., & Caprioli, G. (2020). Chemical composition, antioxidant and enzyme inhibitory properties of different extracts obtained from spent coffee ground and coffee silverskin. *Foods*, *9*(6). https://doi.org/10.3390/FOODS9060713

[6] Andrade, C., Perestrelo, R., & Câmara, J. S. (2022). Bioactive Compounds and Antioxidant Activity from Spent Coffee Grounds as a Powerful Approach for Its Valorization. *Molecules*, *27*(21). https://doi.org/10.3390/molecules27217504

[7] Zainol, M.K., (2020) Antioxidative Properties and Proximate Analysis of Spent Coffee Ground (SCG) Extracted using Ultrasonic-Methanol Assisted Technique as a Potential Functional Food Ingredient. Food Research 4 (3) : 636 - 644

[8] Ansori, N.A., et al., (2021). Antioxidant Activites of Different Spent Coffee Ground (SCG) Extracted using Ultrasonic-Ethanol Extraction Mehod, 33-42.

[9] Sanchez, R.D.V., et al., (2023). Antioxidant activity of the aqueous extract from spent coffee grounds fermented with *Pleurotus ostreatus*. Scientia Fungorum. Vol 54: e1433

[10] Milic, M.D., et al. The development of a combined enzxymatic and microbial fermentation as a viable technology for the spent coffee ground full untilization. *Biomass Conv. Bioref.* 14, 6767-6759 (2023). https://doi.org/10.1007/s13399-021-01605-8.

[11] Devanathan, G., Shanmugan, A., & Balasubramanian, T. et al. (2007) Cellulase production by Aspergillus niger isolated from coastal mangrove debris. Trends in Applied Sciences Research, 2(1), pp.23–27.

[12] Iriondo-DeHond, M., Martín, Á., Miguel, E. and del Castillo, M.D., 2020. *Applications of compounds from coffee processing by-products*. Biomolecules, 10(9), p.1219. https://doi.org/10.3390/biom10091219

[13] Zuorro, A. and Lavecchia, R., 2012. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. *Journal of Cleaner Production*, 34, pp.49–56. https://doi.org/10.1016/j.jclepro.2011.12.003

[14] Abduh, M.Y., Merari, G., Angkasa, M.D., Pangastuti, W.I.R., Rahmawati, A., Taufik, I., Tan, M.I. and Rosmiati, M., 2024. Effects of fermentations using *Aspergillus* spp. towards extraction yield and bioactivity of coffee pulp extract. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-024-06274-x

[15] Kovalcik, A., Obruca, S. and Marova, I., 2018. Valorization of spent coffee grounds: A review. *Food and Bioproducts Processing*, 110, pp.104–119. https://doi.org/10.1016/j.fbp.2018.05.002

[16] Selma, M.V., Espín, J.C. and Tomás-Barberán, F.A., 2009. Interaction between phenolics and gut microbiota: role in human health. Journal of Agricultural and Food Chemistry, 57(15), pp.6485–6501. https://doi.org/10.1021/jf902107d

[17] Abduh, M.Y., Merari, G., Angkasa, M.D., Pangastuti, W.I.R., Rahmawati, A., Taufik, I., Tan, M.I. and Rosmiati, M., 2024. Effects of fermentations using Aspergillus spp. towards extraction yield and bioactivity of coffee pulp extract. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-024-06274-x

[18] Scheller, H.V. & Ulvskov, P. (2010) 'Hemicelluloses', Annual Review of Plant Biology, 61, pp.263–289. doi:10.1146/annurev-arplant-042809-112315.

[19] Cory, H., Passarelli, S., Szeto, J., Tamez, M. and Mattei, J., 2018. *The role of polyphenols in human health and food systems: a mini-review*. Frontiers in Nutrition, 5, p.87. https://doi.org/10.3389/fnut.2018.00087

[20] Martins, S., Mussatto, S.I., Martínez-Avila, G., Montañez-Saenz, J., Aguilar, C.N. and Teixeira, J.A., 2011. B*ioactive phenolic compounds: production and extraction by solid-state fermentation. Biotechnology Advances*, 37(5), p.107461. https://doi.org/10.1016/j.biotechadv.2011.01.008

[21] Kumar, N. and Goel, N., 2019. Phenolic acids: natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24, p.e00370. https://doi.org/10.1016/j.btre.2019.e00370

[22] Zhou, Y., Zheng, J., Li, Y., Xu, D.-P., Li, S., Chen, Y.-M. and Li, H.-B., 2016. Natural polyphenols for prevention and treatment of cancer. *Nutrients*, 8(8), p.515. https://doi.org/10.3390/nu8080515

[23] Brand-Williams, W., Cuvelier, M.E. and Berset, C., 1995. *Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology*, 28, pp.25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

[24] Chaves, N., Santiago, A. and Alías, J.C., 2020. Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used. *Antioxidants*, 9(1), p.76. https://doi.org/10.3390/antiox9010076

[25] Gutiérrez-Correa, M., Ludeña, Y. and Villena, G.K., 2012. Recent advances on filamentous fungal biofilms for industrial uses. *Applied Biochemistry and Biotechnology*, 167(5), pp.1235–1253. https://doi.org/10.1007/s12010-012-9555-5

[26] Chang, J.H., Wang, Y.L. & Chu, J. (2018) A Study of the Optimal Condition for the Growth of Aspergillus niger BCRC 31494, poster, Minghsin University of Science and Technology.

[27] Koutrotsios, G., Kalogeropoulos, N., Stathopoulos, P., Kaliora, A.C. and Zervakis, G.I., 2017. Bioactive compounds and antioxidant activity exhibit high intraspecific variability in *Pleurotus ostreatus* mushrooms and correlate well with cultivation performance parameters. *World Journal of Microbiology and Biotechnology*, 33, Article No. 98. https://doi.org/10.1007/s11274-017-2262-1

[28] Demain, A. L. & Fang, A., 2000. *The Natural Functions of Secondary Metabolites.* Advances in Biochemical Engineering/Biotechnology, 69, pp.1–39.doi:10.1007/3-540-44964-7_1

[29] Horton, H.R., Moran, L.A., Ochs, R.S., Rawn, J.D., and Scrimgeour, K.G., 2002. *Principles of Biochemistry*. 3rd ed. Upper Saddle River, NJ: Prentice Hall.
[30] Santos-Sánchez, N.F., Salas-Coronado, R., Hernández-Carlos, B., and Villanueva-Cañongo, C., 2019. Shikimic acid pathway in biosynthesis of phenolic compounds. *IntechOpen*. Available at: https://doi.org/10.5772/intechopen.83815 [Accessed 17 June 2025].
[31] Ifedibalu, I.M., Ejiofor, C., and Igbokwe, M.-G.C., 2021. Flavonoids: Understanding their biosynthetic pathways in plants and health benefits. *IntechOpen*. Available at: https://doi.org/10.5772/intechopen.96715
[32] Gupta, V.K., Mach, R.L. & Sreenivasaprasad, S. (eds.), 2015. *Fungal Biomolecules: Sources, Applications and Recent Developments*. Chichester: John Wiley & Sons. Chapter 16: Sharma, B.S. & Jha, D.K., 'Role of nitrogen sources in regulation of fungal secondary metabolism', pp. 213–224. https://doi.org/10.1002/9781118958308.ch16
[33] Chandra, P. and Arora, D.S., 2016. Production of bioactive phenolic compounds and antioxidant activity by solid-state fermentation of lignocellulosic residues. *Asian Journal of Biological and Life Sciences*, 1(1), pp.8–15. https://doi.org/10.3923/ajbkr.2016.8.15
[34] Butar Butar, Ruth Aurellia. (2023). Peningkatan Perolehan Ekstraksi Senyawa Polifenol dari Dedak Padi Menggunakan Pelarut Metanol dengan Perlakuan Awal Fermentasi Substrat Padat Menggunakan Aspergillus niger [Skripsi, Institut Teknologi Bandung]. ITB Digital Library. https://digilib.itb.ac.id/gdl/view/74484
[35] Simanjuntak, Elen Etha Yulinar. (2023). Ekstraksi Senyawa Fenolik dari Kulit Jeruk Keprok Menggunakan Pelarut Metanol dengan Perlakuan Awal Fermentasi Substrat Padat oleh Jamur Aspergillus niger [Skripsi, Institut

Teknologi Bandung]. ITB Digital Library. https://digilib.itb.ac.id/gdl/view/74975

[36] Ningrum, A. S., Rochmah, N. N., & Kumala, M. T. (2023). Pengaruh Fermentasi Bakteri Asam Laktat Pada Buah Pandan Laut (*Pandanus tectorius*) Terhadap Kandungan Fenolik Totalnya. Cilacap: Program Studi Farmasi, Universitas Al Irsyad Cilacap.

[37] Pangastuti, W. I. R. (2022). Fermentasi substrat padat pulp kopi (*Coffea arabica*) oleh *Aspergillus oryzae* untuk meningkatkan perolehan ekstraksi asam fenolik sebagai agen antioksidan dan anti-inflamasi [Skripsi, Institut Teknologi Bandung]. ITB Digital Library. https://digilib.itb.ac.id/gdl/view/65775

[38] Cheng, K.-C., et al. (2013). "Enhancements of isoflavone aglycones, total phenolic content, and antioxidant Capacity of black soybean by solid-state fermentation with Rhizopus spp." Eur. Food Res. Technol. 236: 1107-1113.

[39] Cai, S., et al. (2011). "Comparative study of the effects of solid-state fermentation with three filamentous fungi on the total phenolics content (TPC), flavonoids, and antioxidant activities of subfractions from oats (Avena sativa L.)." J. Agric. Food. Chem. 60: 507-513.

[40] Rochín-Medina, J. J., Ramírez, K., Rangel-Peraza, J. G., & Bustos-Terrones, Y. A. (2018). Increase of content and bioactivity of total phenolic compounds from spent coffee grounds through solid state fermentation by *Bacillus clausii*. *BMC Chemistry*, 12, 30. https://doi.org/10.1186/s13065-018-0419-z

[41] Ballesteros, L. F.. Teixeira, J. A. and Mussatto, S. 1. 2014. Chemical, functional, and structural properties of spent coffee grounds and coffee Silverskin. *Food Bioprocess Technol*. 713493-3503.

[42] Silva, C.N.d.; Silva, R.M.d.; Lemes, A.C.; Ribeiro, B.D. Recovery of Phenolic Compounds by Deep Eutectic Solvents in Orange By-Products and Spent Coffee Grounds. Sustainability 2024, 16, 7403. https://doi.org/10.3390/su16177403https://doi.org/10.3390/su16177403
[43] Choi, Y.J., Kim, J.K., Lee, J.Y. and Lee, H.G., 2023. Enzyme-Assisted Extraction of Water-Soluble Protein from Spent Coffee Grounds. *Proceedings*, 26(1), p.43. Available at: https://www.mdpi.com/2673-9976/26/1/43
[44] Ho, H. M., Tran, H. L. N., Tran, T. T. T., Nguyen A. T. V., & Trinh, L. T. P. (2022). Evaluating the extraction of oil and sugars from spent coffee grounds. The Journal of Agriculture and Development 21(1), 28-39.
[45] Gougouli, M., & Koutsoumanis, K. P. (2010). "Modeling growth of *Aspergillus niger* as a function of temperature and water activity." *International Journal of Food Microbiology*, 140(1), 25–32. https://doi.org/10.1016/j.ijfoodmicro.2010.02.010
[46] Ransedokken, Y., Asplund, J., Morgado, L.N., Kauserud, H., Mundra, S., Ohlson, M., Halvorsen, R. and Nybakken, L. (2024). 'Synchronic shifts in phenolic compounds and fungal communities during litter decomposition in boreal forests', *Forest Ecology and Management*, 560, 121696. Available at: https://doi.org/10.1016/j.foreco.2024.121696
[47] Bai, Z., Zhang, H., Zhang, Y., He, Y., Li, X. and Xu, H., 2003. Elevated temperature effects on the oxidant/antioxidant balance in submerged batch cultures of the filamentous fungus *Aspergillus niger* B1-D. *Process Biochemistry*, 38(8), pp.1133–1138. https://doi.org/10.1016/S0032-9592(02)00238-3
[48] Xu, J., Jia, J., Xu, H. & Yuan, Q., 2021. Solid-state fermentation of citrus peel with *Aspergillus niger* to enhance phenolic content and antioxidant activity. *Journal of the Science of Food and Agriculture*, 101(7), pp.2925–

2932. doi:10.1002/jsfa.10913. [49] Madigan, M.T., Bender, K.S., Buckley, D.H., Sattley, W.M. and Stahl, D.A., 2021. *Brock Biology of Microorganisms*. 16th ed. New York: Pearson.

[50] Curtin, C., O'Keeffe, G., O'Connor, C. & Fitzpatrick, D.A., 2018. *The fungus Aspergillus niger consumes sugars in a sequential manner that is not mediated by the carbon catabolite repressor CreA*. Fungal Genetics and Biology, 110, pp. 14–23.

[51] Hang, X., Lee, B.H. & Woodams, E.E., 1991. Single-cell protein production by *Aspergillus niger* on alkali-treated corn cobs. *Journal of Industrial Microbiology*, 8(4), pp.239–241. doi:10.1007/BF01575835.

[52] Dinarvand, M., Rezaee, M. & Foroughi, M., 2013. Optimizing culture conditions for production of intra- and extracellular inulinase and invertase from Aspergillus niger ATCC 20611 by response surface methodology. *Brazilian Journal of Microbiology*, 48(3), pp.427–441. doi:10.1016/j.bjm.2016.10.026.

[53] Tudzynski, B., 2014. *Nitrogen regulation of fungal secondary metabolism in fungi*. *Frontiers in Microbiology*, **5**, p.656. doi:10.3389/fmicb.2014.00656

[54] Alifah, N., & Hidayat , A. R. (2025). Utilization of coffee grounds waste as organic fertilizer to improve soil quality and plant productivity. *EQA - International Journal of Environmental Quality*, *68*, 79–86. https://doi.org/10.6092/issn.2281-4485/21303

[55] Nguyen, T. T. T., et al. (2018). "Enhancement of antioxidant activity and phenolic content in rice bran fermented by Rhizopus oryzae and Monascus purpureus."

[56] Dordevic, D., Dordevic, S., Abdullah, F. A. A., Mader, T., Medimorec, N., & Tremlova, B. (2023). “Development of edible/biodegradable

packaging based on κ-carrageenan with spent coffee grounds as active additives." *Foods*, 12(13).
[57] Ahmed, N.S., Al-Shamary, E. I., (2021). *Optimization of Phenolic Compound Production by Local Aspergillus niger B1b Isolate.* IOP Conf. Ser.: Earth Environ. Sci. 761 012119.
[58] Association of Official Analytical Chemist [AOAC]. 2005. Official Methods of Analysis (18 Edn). Association of Official Analytical Chemist Inc. Mayland. USA.
[59] A.M. Mansora, J.S. Lima, F.N. Anib, H. Hashima and H. Shin, "Characteristics of Cellulose, Hemicellulose and Lignin of MD2 Pineapple Biomass," vol. 27, 2019, doi: 10.3303/CET1972014
[60] L. Hotni, Y. Elvi, and Yelmida, "Isolasi Lignin dari Jerami padi dengan Metode Klason" *Jurnal Fakultas Teknik,* vol. 2, no. 2, 2015.
[61] M. Arsyad, "Effect of Alkali Treatment on The Coconut Fiber Surface." *ARPN Journal of Engineering and Applied Sciences,* vol. 12, no. 6, 2017.
[62] Miller, GL. Use of dinitrosalicylic acid ragent for determination of reducing sugar. Analytical Chemistry. 1959:31 (3) : 426-426
[63] Zaguri M, Kandel S, Rinehart SA, Torsekar VR, Hawlena D. Protein quantification in ecological studies: A literature review and empirical comparisons of standard methodologies. *Methods Ecol Evol*. 2021; 12: 1240–1251. https://doi.org/10.1111/2041-210X.13601
[64] Flavonoid Content and Antioxidant Activity of Various Organic Crude Extracts of Licuala Spinosa Leaves from Sabah, Malaysia." ASM Sc. J, 11 Special Issue 3, 2018 for SANREM, 53-58
[65] I. Bouhzam *et al.*, "Extraction of Bioactive Compounds from Spent Coffee Grounds Using Ethanol and Acetone Aqueous Solutions," *Foods*, vol. 12, no. 24, 2023, doi: 10.3390/foods12244400.

[66] Blois, M.S. 1958. Antioxidant determination by the use of a stable free radical. Nat., 181: 1199-1200.