1	Exploring the production of α -Galactosidase and its utilization in
2	legume-based products.
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4	2024/04/17
5	Outline
6	1. Introduction
7 8 9	 Biochemical characterization and insights into the potency of the acidic <i>Aspergillus niger</i> NRC114 purified α-galactosidase in removing raffinose family oligosaccharides from soymilk yogurt
10 11	 Removal of raffinose family oligosaccharides from soymilk by α-galactosidase immobilized on Sepabeads EC-EA and Sepabeads EC-HA
12	4. Conclusion
13	Abstract
 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 	α-Galactosidase, a hydrolytic enzyme with significant potential in medicine and food industries, especially for removing raffinose family oligosaccharides (RFOs) from legume products, was explored in this study. Purified α-Galactosidase from <i>Aspergillus</i> <i>niger</i> NRC114 exhibited a purification fold of 123 and a molecular weight of 64 kDa, with optimal pH and temperature at pH 3.5 and 60°C, respectively, demonstrating acid and thermal stability. Addition of K ⁺ , Mg ²⁺ , Co ²⁺ , and Zn ²⁺ enhanced enzyme activity, with K_m (Michaelis constant) and V_{max} values of 0.401 µM and 14.65 µmol min ⁻¹ . Enzyme-treated soy yogurt showed increased total phenolics and flavonoids over storage time, indicating high acceptability. Immobilization of α-Galactosidase on Sepabeads EC-EA and Sepabeads EC-HA via direct covalent coupling and glutaraldehyde-mediated adsorption/cross-linking methods achieved activity yields of 63% and 55%, respectively. Optimal temperatures for immobilized enzymes were 60°C, with slightly higher temperatures for cross-linked enzymes. The covalently immobilized enzyme had an optimal pH of 6.0, while the cross-linked enzyme had an optimal pH of 5.0. α-Galactosidase immobilized on these carriers showed effective hydrolysis of stachyose and raffinose, with the immobilized enzyme reusable up to 18 times when using raffinose as a substrate, and Mn ²⁺ addition enhancing enzyme
32 33	galactosidase exhibited excellent stability and potential for application in various industries beyond legume product processing.

Reference 1 Celem, E. B. a., & Önal, S. i. (2022). Removal of raffinose family oligosaccharides 2 from soymilk by α-galactosidase immobilized on sepabeads EC-EA and 3 sepabeads EC-HA. ACS Food Science & Technology, 2(8), 1266-1275. 4 Elango, D., Rajendran, K., Van der Laan, L., Sebastiar, S., Raigne, J., Thaiparambil, 5 N. A., El Haddad, N., Raja, B., Wang, W., & Ferela, A. (2022). Raffinose 6 family oligosaccharides: friend or foe for human and plant health Frontiers in 7 8 Plant Science, 13, 829118. Katrolia, P., Jia, H., Yan, Q., Song, S., Jiang, Z., & Xu, H. (2012). Characterization of 9 a protease-resistant α -galactosidase from the thermophilic fungus *Rhizomucor* 10 11 *miehei* and its application in removal of raffinose family oligosaccharides. Bioresource Technology, 110, 578-586. 12 Othman, A. M., Elshafei, A. M., Elsayed, M. A., Ibrahim, G. E., Hassan, M. M., & 13 Mehanna, N. S. (2023). Biochemical characterization and insights into the 14 potency of the acidic Aspergillus niger NRC114 purified a-galactosidase in 15 removing raffinose family oligosaccharides from soymilk yogurt. BMC 16 17 *Biotechnology*, 23(1), 3. Potumarthi, R., & Mangamoori, L. N. (2015). Purification and characterisation of 18 19 intracellular alpha-galactosidases from Acinetobacter sp. 3 Biotech, 5(6), 925-932. 20 Ye, F., Geng, X., Xu, L., Chang, M., Feng, C., & Meng, J. (2018). Purification and 21 22 characterization of a novel protease-resistant GH27 α-galactosidase from Hericium erinaceus. International Journal of Biological Macromolecules, 120, 23 24 2165-2174..

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