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Enzymatic Activity of Polyphenol Oxidase: A Laboratory Experiment in Flexible Learning

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ABSTRACT

Polyphenol oxidases (PPO) are enzymes that catalyze the browning of fruits and vegetables when oxygen in the air reacts with the present phenolic compounds. This study demonstrates the enzymatic browning of food samples and the inhibition of its activity by common household materials. Fresh food samples were tested in different treatments of acidic and ionic solutions, and syrups. Observations from the students' work showed that changes in pH, surface area for the site of reaction, and ionic conditions affect enzymatic browning. The deviation from the optimum working pH, introduction of ionic interaction, and alteration of the surface area led to the interruption of the interaction within the enzyme structure and between its active site and the substrate thereby inhibiting the enzyme function. Results of the experiment can also serve as a basis for further studies on the development of methods and products to inhibit PPO action and maintain the sensory value and nutritional quality of foods. Furthermore, experiments of similar nature can be crafted as practical activities and alternative teaching techniques designed for students to apply chemistry concepts and laboratory fundamentals to the conduct of an experiment suited for the flexible learning set-up.

Keywords: Polyphenol oxidase, PPO, Enzymatic browning, Food chemistry, Laboratory experiment, Flexible learning.

INTRODUCTION

Enzymatic browning is a common post-harvest processing and storage reaction in fruits, vegetables, and food products especially those rich in phenolic compounds such as apple[1], avocado[2], jackfruit[3], potato[4], mung bean sprout[5], sweet potato[6], and even fresh wet noodles[7]. This undesired browning causes the deterioration in appearance, shelf-life, and nutritional quality of foods leading to increased food waste and significant income losses[8]. This process is the result of the oxidation of phenolic compounds catalyzed by polyphenol oxidase (PPO), a copper-containing enzyme present in the thylakoid lumen of plants as membrane-bound and soluble forms[9]. To manage the negative impacts of PPO activity in the food industry, the mechanism of its action and various inhibition techniques are continuously explored[10].

Awareness of this concern and knowledge of the chemistry principle behind is an important learning integration, especially for students in the chemistry and food science fields. Students taking these science courses are expected to gain the skills in conducting experiments, analyzing data, interpreting results, and developing discipline, interpersonal skills, positive attitude, and proper work ethics in performing individual tasks or group assignments[11]. Hence, competence in the laboratory is an essential part of the chemistry curriculum. However, restrictions due to the COVID-19 pandemic brought limitations and challenges to the delivery of laboratory classes in higher education. In response, remote learning was implemented adapting hybrid or blended learning approach. At-home laboratories became the alternative designed with easy-to-follow procedures using household materials as safer reagents that can be disposed of as household wastes[12]. Various simple at-home laboratory experiments include the preparation of local plant extracts as natural acid-base indicators[13,14], identification of the presence of non-reducing sugars using povidone-iodine[15], malting, and brewing at home activity[16] and kinetic analysis of tyrosinase from banana[17]. Although in-person experiments offer the best first-hand laboratory skills, flexible learning set-up provided learning opportunities during the COVID-19 lockdown which promoted the student's independence in designing experiments, and made way for cheaper, less toxic, and basic alternatives to the traditional conduct of laboratory classes.

This conducted activity was designed for a flexible learning modality in a chemistry course offered in the BS Food Technology program. The purpose of this qualitative experiment is to demonstrate the activity and inhibition of polyphenol oxidase which causes the undesirable browning of phenolic-rich foods. This aimed to allow the students to design and conduct a remote experiment with the application of the discussed chemistry principle and practice laboratory skills.

MATERIALS AND METHODS

Materials

Reagents and equipment used in this study are household materials commonly found in the kitchen and pharmacy. Test samples studied are fruits and vegetables that are available from the local market. Distilled water is used for the preparation of the samples and solutions in the experiment.

Preparation of Treatment Solutions

Five treatments were used in this experiment and prepared as follows. For treatment A, ascorbic acid tablets were pulverized and about 10 g was dissolved in 150 mL hot water. The solution is cooled down and labeled as treatment A. For treatment B, 10 g of sugar was weighed and dissolved in 50 mL of distilled water. The solution is heated until it thickens. About 10 g of salt was weighed and mixed with 20 mL distilled water until completely dissolved. This is treatment C. washed calamansi fruits were squeezed and enough juice was collected to cover the test samples. For the use of vinegar, make sure to transfer enough volume that will cover the samples, and this is labeled as treatment D. Lastly, generous amounts of distilled water were transferred into the reaction cup as treatment E. A separate reaction cup was allotted for the control without any treatment solution.

Table 1: Treatments for the inhibition of PPO

Treatments	Solution
A	ascorbic acid
B	sugar syrup
C	salt
D	calamansi/vinegar
E	distilled water
F	control

Preparation of Test Samples

Selected fruits and vegetables including apple, banana, sweet potato, eggplant and potato were washed and peeled for the experiment. Samples were cut into cubes and immediately submerged into the corresponding treatment solutions. After 2 min the samples were removed from the soaking treatments. The samples were then allowed to stand in the open air for 45 minute. Changes in the surface and appearance of the samples were observed.

RESULTS AND DISCUSSION

Observations and results collated from the students' outputs showed the activity of PPO in apples *(Malus domestica)*, potatoes *(Solanum tuberosum L.)*, eggplant *(Solanum melongena)*, sweet potato *(Ipomoea batatas)*, and banana *(Musa acuminata × balbisiana)* as evident in the browning response of control samples after exposure to air. This browning occurs on the cut or damaged tissues of samples when phytomelanins are produced from the polymerization of PPO-generated quinones[18]. Several studies have quantified the PPO activity of apple (762, 666.67 U)[1], potato (52,857.14 U)[19], eggplant (2, 894 U)[20], sweet potato (43, 400 U) and banana (141, 600 U)[21].

Table 2: Documentation of the enzymatic browning in the treated and control food samples after exposure to air

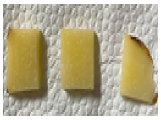
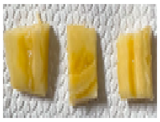
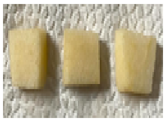
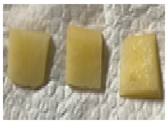
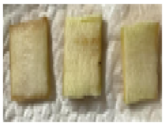
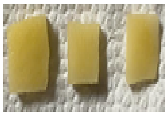
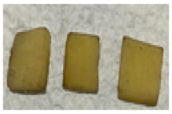
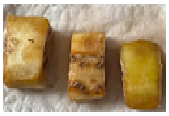
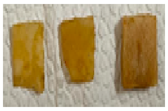
Treatment	Apple	Potato	Eggplant	Sweet Potato	Banana
A					
B					
C					
D					
E					
F					

Table 3: Students' observation on the enzymatic browning in the treated and control food samples after exposure to air

Treatment	Apple	Potato	Eggplant	Sweet Potato	Banana
A (ascorbic acid)	minimal browning	minimal browning	minimal browning	minimal browning	minimal browning
B (sugar syrup)	minimal browning	minimal browning	minimal browning	minimal browning	minimal browning
C (salt)	minimal browning	minimal browning	minimal browning	minimal browning	minimal browning
D (calamnsi/ vinegar)	minimal browning	minimal browning	minimal browning	minimal browning	minimal browning
E (distilled water)	major browning	major browning	major browning	major browning	major browning
F (control)	major browning	major browning	major browning	major browning	major browning

Response of the PPO in the treated samples after exposure to air shows the potential inhibition of the enzymatic activity of PPO by the different soaking solutions (Table 2 and 3). Least enzymatic browning was observed in treatments A, B, C, and D while more visible browning was seen in treatment E. acidulants such as ascorbic acid, acetic acid in vinegar, and citric acid in calamansi juice lower the pH of foods beyond the optimum pH of PPO causing the inhibition of its activity thereby functioning as anti-browning agents[22]. Citric acid and ascorbic acid are also noncompetitive inhibitors of PPO by acting as chelating agents to Cu^{2+}, an important cofactor of PPO[23]. Sugar including sucrose and trehalose has been studied as a component in a dipping solution for fresh-cut produce which acts as surface barrier preventing the interaction of the atmosphere with the phenolic groups in fruits[24]. On the other hand, salt is also used as dipping solution and as anti-browning agent owing to its ability to form halide-copper ion complex resulting in decreased PPO activity[25]. Soaking the samples in distilled water did not prevent the browning of the fruits and vegetables hence, the visible coloration of the samples.

In the conduct of the lab at-home experiment, the students were able to perform a lecture-guided experiment. Chemistry principles were discussed prior to the conduct hence, scientific concepts were applied by the students. Skills meant to be learned in a physical classroom were also developed in the students as they carried out the activity. Planning of the experiment delivery, preparation of the samples and solutions, careful execution, data collection, and analysis were all done by the students in the alternative activity proving that knowledge and skills can still be gained in remote learning set-up. This study can serve as a guide in conducting similar teaching techniques as well as in developing qualitative at-home laboratory experiments that involve more practical and affordable materials and less toxic reagents as substitutes to lab-scale set-ups. This study can be adapted and it is recommended that the use of common local samples as well as exploration of other PPO inhibition methods from natural and indigenous sources be conducted. It is also recommended that analysis of the student and faculty responses in the conduct of this activity be included. Moreover, the development of anti-browning products from natural and more accessible sources for the prevention of unwanted enzymatic browning of foods in the post-harvest and storage phases can be employed.

CONCLUSION

Limitations in the conduct of in-person classes due to the COVID-19 pandemic indeed affected the academic curriculum which led to the development of alternative teaching techniques. In this study, the activity of PPO and its inhibition was demonstrated in the performance of the experiment. Laboratory skills and discussed chemistry principles were applied and practiced by the students. With the transition to the new normal and flexible learning modality, this study can be utilized to demonstrate enzyme activity and inhibition being a simple, less toxic, and affordable laboratory experiment.

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Conflict of Interest

The author declares that there is no conflict of interest concerning the publication of this work.

REFERENCES

1. Guo, S.; Zhang, L.; Zhang, L.; Zhao, M.; & Meng, X. Inhibition kinetics of oligochitosan as an uncompetitive inhibitor on fuji apple polyphenol oxidase., *Journal of Food Biochemistry*., **2018**, *42*(5). https://doi.org/10.1111/jfbc.12585.
2. Fuentes Campo, A.; Sancho, M. I.; Melo, G.; Dávila, Y. A., & Gasull, E. *In vitro* and in vivo inhibition of Hass avocado polyphenol oxidase enzymatic browning by paeonol, β-cyclodextrin, and paeonol:ββ-cyclodextrin inclusion complex. *Journal of Bioscience and Bioengineering*., **2019**, *127*(6), 703–709. https://doi.org/10.1016/j.jbiosc.2018.11.009.
3. Tao, Y. M.; Yao, L. Y.; Qin, Q. Y., & Shen, W. Purification and characterization of polyphenol oxidase from jackfruit (Artocarpus heterophyllus) bulbs. *Journal of Agricultural and Food Chemistry*., **2013**, *61*(51), 12662–12669. https://doi.org/10.1021/jf403828e4.
4. Cerit.; Pfaff, A.; Ercal, N., & Demirkol, O. Postharvest application of thiol compounds affects surface browning and antioxidant activity of fresh-cut potatoes. *Journal of Food Biochemistry*., **2020**, *44*(10). https://doi.org/10.1111/jfbc.13378.
5. Sikora, M., & Świeca, M. Effect of ascorbic acid postharvest treatment on enzymatic browning, phenolics and antioxidant capacity of stored mung bean sprouts. *Food Chemistry.,* **2018**, *239*, 1160–1166. https://doi.org/10.1016/j.foodchem.2017.07.067.

6. Li, F.; Purification, kinetic parameters, and isoforms of polyphenol oxidase from "Xushu 22" sweet potato skin. *Journal of Food Biochemistry.*, **2020**, *44*(11). https://doi.org/10.1111/jfbc.13452.
7. Wu, X. T.; Guo, X. N., & Zhu, K. X. Inhibition of l-cysteine on the browning of fresh wet noodles. *Foods.*, **2021**, *10*(6). https://doi.org/10.3390/foods10061156.
8. Jia, S.; Jiang, S.; Chen, Y.; Wei, Y., & Shao, X. Comparison of Inhibitory Effects of Cinnamic Acid, β-Cyclodextrin, L-Cysteine, and Ascorbic Acid on Soluble and Membrane-Bound Polyphenol Oxidase in Peach Fruit. *Foods.*, **2023**, *12*(1). https://doi.org/10.3390/foods12010167.
9. Han, Q. Y.; Liu, F.; Li, M.; Wang, K. L., & Ni, Y. Y. Comparison of biochemical properties of membrane-bound and soluble polyphenol oxidase from Granny Smith apple (Malus×domestica Borkh.). *Food Chemistry.,* **2019**, *289*, 657–663. https://doi.org/10.1016/j.foodchem.2019.02.064.
10. Hong, X.; Luo, X.; Wang, L.; Gong, D., & Zhang, G. New Insights into the Inhibition of Hesperetin on Polyphenol Oxidase: Inhibitory Kinetics, Binding Characteristics, Conformational Change, and Computational Simulation. *Foods.*, **2023**, *12*(4), 905. https://doi.org/10.3390/foods12040905.
11. Guidote, A. Jr. M. Teaching college chemistry in the time of COVID-19 pandemic: A personal account of teaching in the old normal vs. the new normal. *KIMIKA.*, **2020**, *31*(1), 70–75. https://doi.org/10.26534/kimika.v31i1.70-75.
12. Yeerum, C.; Issarangkura Na Ayutthaya, P.; Kesonkan, K.; Kiwfo, K.; Suteerapataranon, S., Panitsupakamol, P.; Paengnakorn, P.; Chinwong, D.; Chinwong, S.; Saenjum, C.; Vongboot, M., & Grudpan, K. Lab-at-Home: Hands-On Green Analytical Chemistry Laboratory for New Normal Experimentation. *Sustainability (Switzerland).,* **2022**, *14*(6). https://doi.org/10.3390/su14063314.
13. Reyes, D.; Jayson, L. L.; Grace, M. B.; Ronald, J. T., & Jr, S. Nueva Ecija 3121 Philippines 5 DepEd-Carranglan National High School. *Brgy. FC Otic.*, **2022**, *1*(2). https://www.researchgate.net/publication/367046065.
14. Sanchez, J. M.; Fernandez, M. J.; Abgao, J. M.; Sarona, H.; Asenjo, S. B.; Guiroy, B.; Oponda, A. J., & Vale, X. Experimenting on Natural Acid-Base Indicators: A Home-based Chemistry Activity during the COVID-19 Pandemic as evaluated by Teachers. *KIMIKA.*, **2021**, *32*(1). https://doi.org/10.26534/kimika.v32i1.34-45.
15. Velarde, V.; Casado-Barragán, F.; Thamar, M.; Rands, V. F., & Gonzalez, A. A. Home-based laboratory experiences during COVID-19 pandemic in undergraduate biochemistry students. *Frontiers in Education.*, **2022**, *7*. https://doi.org/10.3389/feduc.2022.965438.
16. Cash, K. J. Malting in the Lab and at Home: The Forgotten Step on the Path to Beer. *Journal of Chemical Education.*, **2021**, *98*(4). https://doi.org/10.1021/acs.jchemed.0c01279.
17. Barton, C.; Gasaway, K. C.; Islam, R.; Aziz, T.; Krewall, J.; Punthrangkul, D., & Willian, K. R. Implementation of an At-Home, First-Semester Biochemistry Lab Course: A Module Based on Banana Tyrosinase. *Journal of Chemical Education.*, **2022**, *99*(4), 1571–1578. https://doi.org/10.1021/acs.jchemed.1c00675.
18. Omiadze, N. T.; Mchedlishvili, N. I., & Abutidze, M. O. Phenoloxidases of perennial plants: Hydroxylase activity, isolation and physiological role. *Annals of Agrarian Science.*, **2018**, *16*(2). https://doi.org/10.1016/j.aasci.2018.03.009.
19. Öztürk, C.; Bayrak, S.; Demir, Y.; Aksoy, M.; Alım, Z.; Özdemir, H., & rfan Küfrevioglu, Ö. Some indazoles as alternative inhibitors for potato polyphenol oxidase. *Biotechnology and Applied Biochemistry.*, **2022**, *69*(5). https://doi.org/10.1002/bab.2283.
20. Murniati, A.; Shardi, S.; Fauzi, I.; Hardian, A.; Ibrahim, R. M.; Buchari, B.; Gandasasmita, S., Nurachman, Z., & Jasmansyah. Immobilization of crude polyphenol oxidase purple eggplant extract on chitosanmembrane for removal of phenol wastewater. *European Chemical Bulletin.*, **2022**, *11*(10). https://doi.org/10.31838/ecb/2022.11.10.016.

21. Lim, W. Y.; Cheng, Y. W.; Lian, L. Bin, Chan, E. W. C., & Wong, C. W. Inhibitory effect of Malaysian coastal plants on banana (Musa acuminata colla “Lakatan”), ginger (Zingiber officinale Roscoe) and sweet potato (Ipomoea batatas) polyphenol oxidase. *Journal of Food Science and Technology*., **2021**, *58*(11). https://doi.org/10.1007/s13197-020-04886-5
22. O. Y. A.; W. I. A., & Al-lehebe, Nashwan. I. A. Inhibition of enzymatic browning in fruit and vegetable, review. *Samarra Journal of Pure and Applied Science*., **2021**, *3*(1). https://doi.org/10.54153/sjpas.2021.v3i1.242.
23. Altunkaya, A., & Gökmen, V. Effect of various anti-browning agents on phenolic compounds profile of fresh lettuce (*L. sativa*). *Food Chemistry.,* **2009**, *117*(1). https://doi.org/10.1016/j.foodchem.2009.03.085.
24. Albanese, D.; Cinquanta, L., & Di Matteo, M. Effects of an innovative dipping treatment on the cold storage of minimally processed Annurca apples. *Food Chemistry*., **2007**, *105*(3). https://doi.org/10.1016/j.foodchem.2007.05.009.
25. Nogales-Delgado, S. Polyphenoloxidase (Ppo): Effect, current determination and inhibition treatments in fresh-cut produce. *In Applied Sciences (Switzerland)*, **2021**, *11*(17). MDPI AG. https://doi.org/10.3390/app11177813.