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Title: Comparative analysis of universal protein extraction methodologies for screening of lipase activity from agricultural products

Responses to reviewer's comments

: We are very grateful for the thorough review with informative comments and the constructive suggestions by the reviewer. We considered the reviewer's comments with meticulous care. We have prepared answers to the reviewer's comments as sufficiently as possible, and corrections and clarification in our revised manuscript have been made by rewriting related sentences or paragraphs, considering the reviewer's suggestions. The detailed responses are listed below.

- Reviewer #4

The authors analyzed four methods for evaluating the lipase activity presents in the crude extracts of several raw materials. The methods are wide knowing and contributing with low novelty in this field. For improving this work, the authors could consider the below comments.

[Major comments]

1. The authors must include in the manuscript figures where summarize the data obtained.

: We appreciate the reviewer's comment strengthening our manuscript. In the previous manuscript, we demonstrated our main data just using Tables, and it made an intuitive understanding of the results difficult. Therefore, as the reviewer strongly suggested, we have arranged existing

data and included two more figures (Fig. 2 and 3) in the revised manuscript to visualize the overall results of protein extraction yields and specific activities.

2. The authors must explain with detail the analytical methods and the equipment used.

: We appreciate the reviewer's comment enhancing our manuscript. As the reviewer strongly suggested, we have included more description about the analytical methodology and the equipment used in the present study.

3. The authors must indicate how many replicas were made of each sample with the corresponding deviation.

: We appreciate the reviewer's comment and considered it with meticulous care. Indeed, we conducted an enzymatic reaction once per agricultural product in the present study because of the following reason. The present study focused on figuring out the differences between protein extraction processes and providing practical information for screening of lipase activity from agricultural products. For this, it was more critical to reveal a global tendency of each protein extraction methodology on various plant samples than to conduct replication to derive precise results of each experiment. Hence, we used fully 24 kinds of agricultural products and compared the average value of both protein yield and specific activity in accordance with protein extraction methodologies. Additionally, there was a practical limitation to conduct replication in our experimental platform. We screened 87 kinds of agricultural products simultaneously at preliminary screening (Supplementary Data S1) and also comparatively analyzed four kinds of protein extraction methodologies using 24 kinds of samples in terms of both protein extraction yield (Table 1) and specific activity (Table 2 and 3). For these reasons, it was hard to conduct all replication exactly the same conditions. Of course, we certainly revealed a global tendency

and provided practical information based on our present results, although enough replications are required for a further study on a specific source to find single lipase, similar to the reviewer's comment. Therefore, we attentively thought that additional replications of already performed experiments are redundant in the present study because there was no crucial defect in the experimental design, derived results, and following discussion. Nevertheless, as the reviewer carefully commented, we have included the relevant sentences to clarify experimental design and number of experiments.

+ We also modified several descriptions for polishing our manuscript. Once again, we truly appreciate all the reviewer's constructive suggestions.