Dear Editors and Reviewers:

Thank you for your letter and comments relating to our manuscript entitled "Characterization of an α-amylase from the honey bee chalk brood pathogen *Ascosphaera apis*" (ID: jof-2687324). The comments were very valuable in addition to being helpful for revising and improving our manuscript, as well as the important guiding significance to our research. We have read the comments carefully and made corrections accordingly. Revised parts are marked in blue in the manuscript. The main corrections in the paper and our responses to the reviewer's comments are given below. We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in *Journal of Fungi*.

Responses to the comments of the reviewers:

Reviewer 2 (Round 2)

Comments 1: Although the authors mention that they added the Pareto graphs, they are not in the manuscript.

Response1: We have added the Pareto chart in the supplementary material and mentioned it in the revised manuscript.

The PBD modeling with Pareto chart revealed maltose (X1), $CaCl_2$ (X4), and pH (X8) as the key factors affecting amylase production (Table 1 and Figure S1).

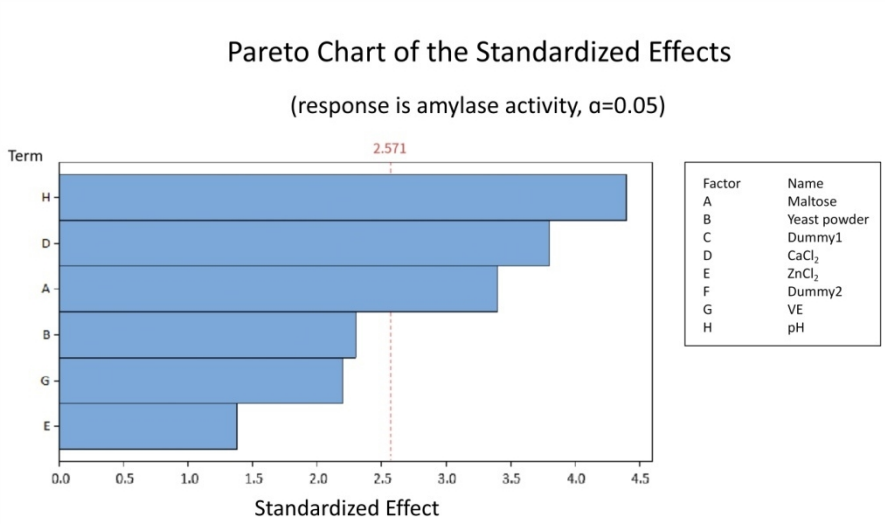
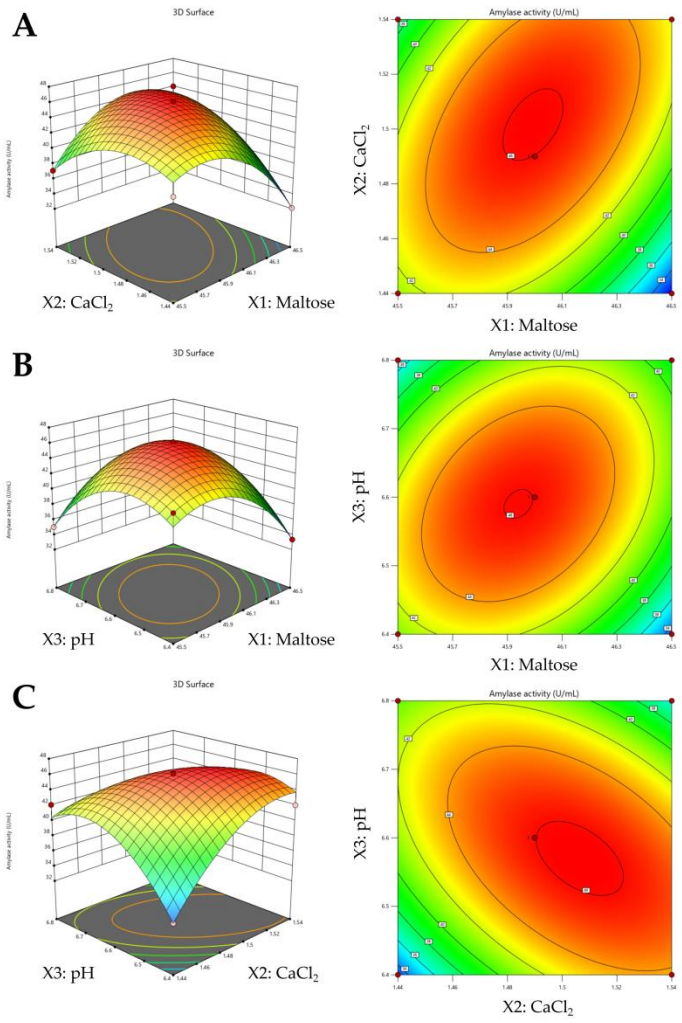


Figure S1. Pareto chart of the standardized effects for amylase activity.

Comments 2: Replace with colors that make the result clear in Fig. 1.

Response 2: We have changed the color scale of Fig.1.



Comments 3: Add the HPLC methodology in Material and Methods.

Response 3: We have added the HPLC methodology in Material and Methods.

The concentrations of hydrolysis products were analyzed by ZORBAX carbohydrate analysis column chromatography (5 μm, 4.6 × 250 mm, Agilent, USA). The injection volume of diluted and filtered hydrolysate was 10 μL and the column temperature was set to 30 °C. The mobile phase was acetonitrile/water (7:3, v:v) mixed solvent with a flow rate of 1.0 mL/min.

Comments 4: Add a space in "ion exchange" in Table 3.

Response4: We have added the space.

Table 3. Purification and activity of amylase from *A. apis*.

Fraction	Total activity(U)	Total protein(mg)	Specific activity(U/mg)	Purification (fold)	Yield (%)
Crude enzyme	2082.62	80.68	25.81	1	100

50% ammonium sulfate	1008.77	9.97	101.18	3.92	24.96
Ion exchange chromatography	692.05	2.14	323.39	12.53	7.12
Gel filtration chromatography	597.55	1.61	371.15	14.38	2.51

We tried our best to improve the manuscript and made some changes marked in blue in revised paper which will not influence the content and framework of the paper. We appreciate for Editors/Reviewers' warm work earnestly and hope the revision will meet with your approval. Once again, thank you very much for your comments and suggestions.

Kind regards,

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