RGC Ref. No.:
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(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Optimization of antioxidant activities of polysaccharides or polysaccharide-protein Complexes from medicinal mushrooms by modulation of ultrasound-assisted extraction effect on the molecular structures and physicochemical properties

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	**Name / Post**	**Unit / Department / Institution**
Principal Investigator	TAM Wan Ting Emily / Senior Lecturer	Department of Applied Science, Hong Kong Metropolitan University
Co-Investigator(s)	CHEUNG Yi Ching / Teaching Fellow	Faculty of Science, The Hong Kong Polytechnic University

3. Project Duration

	Original	**Revised**	**Date of RGC / Institution Approval** *(must be quoted)*
Project Start Date	1 January 2020	NA	NA
Project Completion Date	31 December 2022	31 December 2023	10 February 2023
Duration *(in month)*	36	48	10 February 2023
Deadline for Submission of Completion Report	31 December 2023	31 December 2024	10 February 2023

4.4 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. *Examination the US effect on the chemical composition, molecular properties and antioxidant activities of PS (and PSPs) isolated from selected edible/medicinal mushroom fruit bodies by various conditions of UAE*
2. *Modulation of the relative US effect on the molecular structures and physicochemical properties with US powers and various extraction conditions*
3. *Optimization of antioxidant activities of the PS (and PSPs) with various molecular structures and physicochemical properties attained by different UAE conditions*

5.2 Revised objectives

5.3 Realisation of the objectives
(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

Objective 1 – The effects of various ultrasound assisted extraction (UAE) parameters, including ultrasound power intensity, temperature, mushroom particle size, and solid-to-liquid ratio, on the chemical composition, molecular properties and antioxidant activities of polysaccharides (PS) and polysaccharide-protein complexes (PSPs) extracted from *Lentinula edodes* and *Grifola frondosa* were evaluated using a one-factor-at-a-time approach. Objective 1 has been fully achieved.

Objective 2 – Statistical models for *L. edodes* and *G. frondosa* relating the ultrasound (US) effect on the molecular structures and physicochemical properties of the extracts to US powers and various extraction conditions were developed. This was achieved using a

three-level, three-factor Box–Behnken design and response surface methodology. Objective 2 has been fully achieve.

Objective 3 – The optimized antioxidant activities of the PS and PSPs in *L. edodes* and *G. frondosa* were predicted using design-Expert software. Objective 3 has been fully achieved.

Project scope adjustment – Due to the reduction in the supporting staff budget from the originally proposed mount, this project focused exclusively on studying *G. frondosa* and *L. edodes*. As noted by the RGC in the project revision, this limitation precluded the extension of the study to *Cordyceps sinensis*, *Ganoderma lucidum* and *Coriolus versicolor*. Despite this adjustment, the same objectives and deliverables were attained.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. *Examination the US effect on the chemical composition, molecular properties and antioxidant activites of PS (and PSPs) isolated from selected edible/medicinal mushroom fruit bodies by various conditions of UAE*	✓	100%
2. *Modulation of the relative US effect on the molecular structures and physicochemical properties with US powers and various extraction conditions*	✓	100%
3. *Optimization of antioxidant activities of the PS (and PSPs) with various molecular structures and physicochemical properties attained by different UAE conditions*	✓	100%

6. Research Outcome

6.1 Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

The study demonstrated that UAE parameters significantly affect the polysaccharide and protein compositions in PSP extracts from *L. edodes* and *G. frondosa*.

For *L. edodes*, a UAE power intensity of 6.21 W/cm² and 11.34 W/cm² resulted in PSPs with a higher protein content relative to polysaccharide content. However, when the intensity was increased to 21.62 W/cm², the protein content in the PSPs was significantly affected, resulting in similar protein and polysaccharide percentages. Regarding temperature, UAE at 40°C and 55°C produced PSPs with a higher protein content relative to polysaccharide content. At 70°C, the protein content was significantly reduced, resulting in PSPs with a higher polysaccharide content than protein. For mushroom particle size, all three different particle sizes used in UAE yielded PSP extracts with a higher protein percentage than polysaccharide content. Notably, a comparatively high protein-to-polysaccharide ratio in PSPs was observed when the particle size was <600 µm. Regarding the solid-to-liquid ratio, a more concentrated solid-to-liquid ratio in UAE extracts both polysaccharides and proteins most efficiently. However, the solid-to-liquid ratio did not significantly affect the protein-to-polysaccharide ratio in PSPs.

For *G. frondosa*, at a UAE power intensity of 6.21 W/cm², the PSPs contained significantly more protein than polysaccharides. At 11.34 W/cm², both polysaccharides and proteins were extracted more efficiently, but the protein-to-polysaccharide ratio in PSPs was reduced. This ratio further decreased when the intensity was raised to 21.62 W/cm². Regarding temperature, at both 40°C and 55°C, polysaccharide and protein contents in PSPs exhibited a gradual increase over time, but a high protein-to-polysaccharide ratio was not observed. At 70°C, PSPs contained a higher polysaccharide content than protein. For particle size, a high protein-to-polysaccharide ratio in PSPs was not observed across different mushroom particle sizes, indicating that mushroom particle size does not significantly affect the molecular composition of PSPs. Similarly, the solid-to-liquid ratio did not significantly affect the protein-to-polysaccharide ratio in PSPs.

Second-order polynomial models correlating UAE process factors with antioxidant activities were successfully developed and used to predict the optimal UAE conditions for maximizing antioxidant activities in PSPs. The models exhibiting high F-values and low p-values, indicating strong model fit. The model demonstrated a high degree of correlation between observed and predicted values and were species-dependent. Optimal UAE conditions for *L. edodes* were identified as a temperature of 55°C, ultrasound power intensity of 6.21 W/cm², and mushroom particle size of <600 µm, resulting in the highest antioxidant activities. For *G. frondosa*, optimal conditions were a temperature of 70°C, ultrasound power intensity of 6.21 W/cm², and mushroom particle size of 600–850 µm. A higher protein-to-polysaccharide ratio in PSPs was significantly correlated with enhanced antioxidant activity for both mushrooms. However, statistically significant models correlating UAE process factors with molecular weight in PSPs could not be built. This study provides valuable insights into optimizing UAE conditions for extracting bioactive compounds from medicinal mushrooms, which can guide future research and practical applications aimed at enhancing the health benefits of mushroom extracts.

As of October 2024, two manuscripts arising from this research project have been submitted to peer-reviewed journals and are under revision. The PI also presented the research findings at two international conferences. Additionally, two bachelor's degree theses were generated directly from this project, and two undergraduates were trained. One undergraduate extended this study to an MPhil degree for the investigation of using UAE in the extraction of bioactive compounds in Chinese medicinal herbs.

6.2 Potential for further development of the research and the proposed course of action *(Maximum half a page)*

High-intensity ultrasound is regarded as an innovative, versatile, and promising means for processing food and medicinal products. In recent years, the application of ultrasound in polysaccharide extraction has become more common in the industry due to its high extraction efficiency. This research project provides new and interesting results on the ultrasound-assisted extraction (UAE) processes and the relationships between efficiency, product quality, and process conditions for the bioactivities of medicinal products.

The models developed in this project are useful for further development, improvement, and application of ultrasonic processes on medicinal mushrooms. By leveraging high-power ultrasound, we can achieve more effective and targeted extraction processes, ultimately leading to higher quality nutraceutical and pharmaceutical products. The developed models not only offer a robust tool for optimizing extraction conditions but also pave the way for future innovations in the field. This work underscores the potential of ultrasound-assisted extraction as a versatile and powerful technique, with broad applications extending beyond mushrooms to various medicinal herbs and plants, thereby contributing to the advancement of natural product-based health solutions.

7. Layman's Summary

(Describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

The research and commercial interest in polysaccharides and polysaccharide-protein complexes from medicinal mushrooms for nutraceutical and pharmaceutical uses are increasing. This research project aims to apply high-power ultrasound to facilitate the extraction and processing of bioactive polysaccharides and polysaccharide-protein complexes from several important medicinal mushrooms, optimizing their bioactivity effects.

The study revealed that different ultrasound conditions can significantly affect the composition and bioactivities of mushroom extracts. We developed statistical models to relate various ultrasound conditions to the antioxidant activities of two important medicinal mushrooms, shiitake (*Lentinula edodes*) and maitake (*Grifola frondosa*). These models can be used to predict and optimize the nutraceutical values of mushroom extracts by manipulating the ultrasound extraction conditions.

The models developed in this project are useful for further development, improvement, and application of ultrasonic processes on medicinal mushrooms. Additionally, they can be extended to the extraction of bioactive compounds from various medicinal herbs and plants, thereby contributing to the advancement of natural product-based health solutions.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				**Author(s)** *(denote the corresponding author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
2025				Tam EW*, Cheung YC, Lung WC, Hui CC, Lam WW, Ng SS, Chow FW*	Modulating ultrasound-assisted extraction of polysaccharide-protein complexes from β-glucan-rich medicinal mushrooms for enhanced antioxidant activity. *Carbohydrate Polymer technologies and Applications* 2025; 10:100817.	No	Yes [Attachment 1]	Yes	Yes
2025				Hui CC, Lee FW, Lung WC, Fan KC, Wong IT, Siu GK, Chow YL, Chan PL, Ng SM, Shi L, Seto SW, Chow FW*, Tam EW*.	Ultrasound-assisted extraction: unlocking the antibacterial potential of *Coptis Chinese* Franch. against ESBL-producing *Enterobacterales*. *Molecules* 2025; *30*(22), 4331.	No	Yes [Attachment 2]	Yes	Yes

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered
(Please attach a copy of each conference abstract)

Month / Year / Place	**Title**	**Conference Name**	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
July 2023 Hamburg Germany	Modulation of ultrasound-assisted extraction effect on the molecular composition of fungi	FEMS 2023 Congress	No	Yes [Attachment 3]	Yes	Yes
April 2023 HKSAR China	Modulation of ultrasound-assisted extraction effect on the molecular composition of fungi	International Conference on Conservation and Sustainable Development of coastal Wetland	No	Yes [Attachment 4]	Yes	Yes

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

Two student helpers and two final-year project students were recruited to support this project. Students participated in samples preparation, UAE extraction and measurements of protein and polysaccharides contents. One of the final year project students extended this study by using UAE in the extraction of bioactive compounds from Chinese medicinal herbs in her Mphil study.

11. Student(s) Trained
(Please attach a copy of the title page of the thesis)

Name	**Degree Registered for**	**Date of Registration**	**Date of Thesis Submission / Graduation**
	Bachelor of Science (Honours) in Food Science and Safety	September 2016	May 2020
	Bachelor of Science (Honours) in Food Science and Safety	September 2020	May 2022

12. Other Impact
(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

The study is a collaborative effort between Hong Kong Metropolitan University, The Hong Kong Polytechnic University, and THEi. This collaboration benefits the academic community by pooling resources and expertise from these esteemed institutions. In addition, the new knowledge acquired through this study has been integrated into teaching and learning practices. Specifically, the application of ultrasound technology for the extraction of bioactive compounds has been incorporated into two undergraduate courses: "Food Processing" and "Functional Food & Nutraceuticals". This integration ensures that students are exposed to cutting-edge techniques and advancements in the field, enhancing their Educational experience and better preparing them for future careers.

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	**Conference Papers**	**Scholarly Books, Monographs and Chapters**	**Patents Awarded**	**Other Research Outputs (please specify)**	
No. of outputs arising directly from this research project	2	2	NA	NA	Type	No.
					NA	

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	**Reasons**
NA	