RGC Ref. No.:
UGC/FDS25/M06/19
(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

Study of potential synergistic effect of probiotic formulas in reducing acrylamide and ethyl carbamate in selected foods

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr CHOI Siu Mei/ Assistant Professor	Department of Food and Health Sciences/ Faculty of Science and Technology/ THEi
Co-Investigator(s)	Dr CHU, Ivan Keung/ Associate Professor	Department of Chemistry, The University of Hong Kong
Others	NA	NA

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	1/1/2020	NA	NA
Project Completion Date	31/12/2021	30/6/2022	17/6/2021
Duration *(in month)*	24	30	17/6/2021
Deadline for Submission of Completion Report	31/12/2022	30/6/2023	17/6/2021

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To investigate the efficacy of probiotic strains in reducing the concentration of (i) ethyl carbamate or (ii) acrylamide in selected food samples;
2. To investigate the potential synergistic effects of various probiotic formulas in reducing the concentration of (i) ethyl carbamate or (ii) acrylamide in selected food samples;
3. To evaluate the bioaccessibility of (i) ethyl carbamate and (ii) acrylamide with and without addition of probiotic formulas using an in vitro digestion model under simulated gastrointestinal digestion conditions;
4. To study the risk assessment of (i) ethyl carbamate and (ii) acrylamide before and after treating with probiotic formulas in the ethyl carbamate-contaminated alcoholic drinks/fermented bread or acrylamide-contaminated potato chips/snack type biscuits.

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change: NA

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 (100% achieved):

The efficacy of selected probiotic strains in reducing the concentrations of acrylamide and ethyl carbamate in some selected food samples were investigated. The efficacy of selected probiotic bacteria to reduce ethyl carbamate and acrylamide was first assessed in chemical standard solutions and followed by reduction assay in selected food samples. Five probiotic strains *Lactobacillus bulgaricus, Lactobacillus paracasei, Lactobacillus plantarum, Bifidobacterium lactis* and *Streptococcus thermophilus* were added in food samples and incubated at 37ºC for 4 hours. The content of acrylamide and ethyl carbamate in food samples with or without incubation of with probiotic strains were extracted, clean-up by solid phase extraction and then quantified by using liquid chromatography mass spectrometry (LC-MS). Biscuits and potato chips are food models for acrylamide reduction assay while sake, yellow wine and plum wine were used for ethyl carbamate reduction assay. The efficacy of the single probiotic strains in reducing concentration of ethyl carbamate or acrylamide were obtained by comparing their content before and after incubation with different strains of probiotics. The results obtained were used to identify the strain of probiotics with capability of reducing ethyl carbamate or acrylamide in these selected food products.

Objective 2 (100% achieved):

The potential synergistic effects of various probiotic formulas in reducing the concentrations of acrylamide and ethyl carbamate in some selected food samples were studied. The potential synergistic effects of various probiotic formulas were investigated based on the reduction ability of individual probiotic obtained from previous experiment (Objective 1). The efficacy of different probiotic formulas to reduce ethyl carbamate and acrylamide was first assessed in chemical standard solutions. Probiotic formulas with higher reduction ability were selected and incubated in selected food samples. The content of acrylamide and ethyl carbamate in food samples with or without incubation with probiotic formula were extracted, clean-up by solid phase extraction and then analyzed by mass spectrometry to determine whether the extent of reducing toxicants by probiotic formulas can be enhanced when comparing with individual probiotics.

Objective 3 (100% achieved):

The selected probiotic formulas were identified from experiments (Objective 2) and further evaluated their potential synergistic effect in selected food samples under *in vitro* gastrointestinal digestion condition. The content of ethyl carbamate or acrylamide with or without probiotic formulas were obtained at the end of digestion process to compare the bioaccessibility under simulated gastrointestinal digestion conditions.

Objective 4 (100% achieved):

The risk assessment of ethyl carbamate and acrylamide before and after treating with probiotic formulas in the ethyl carbamate-contaminated alcoholic drinks and acrylamide-contaminated potato chips and snack type biscuits was evaluated. By comparing the concentrations of ethyl carbamate or acrylamide and the consumption data in HK population, the dietary exposure to ethyl carbamate or acrylamide (ng/kg bw/day) and margins of exposure (MOE) data were estimated.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To investigate the efficacy of probiotic strains in reducing the concentrations of (i) ethyl carbamate or (ii) acrylamide in selected food samples;	√	100%
2. To investigate the potential synergistic effects of various probiotic formulas in reducing the concentrations of (i) ethyl carbamate or (ii) acrylamide in selected food samples;	√	100%
3. To evaluate the bioaccessibility of (i) ethyl carbamate and (ii) acrylamide with and without the addition of probiotic formulas using an in vitro digestion model under simulated gastrointestinal digestion conditions	√	100%
4. To study the risk assessment of (i) ethyl carbamate and (ii) acrylamide before and after treating with probiotic formulas in the ethyl carbamate-contaminated alcoholic drinks/ fermented bread or acrylamide-contaminated potato chips/ snack type biscuits.	√	100%

6. Research Outcome

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

In this study, the potential synergistic effects of probiotic formulas in reducing acrylamide and ethyl carbamate were studied. Different probiotic strains were firstly examined to evaluate their abilities to reduce acrylamide or ethyl carbamate for selection to form several probiotic formulas for further investigation (Objectives 2-4). LC-MS was used to analyze concentration of acrylamide and ethyl carbamate of the tested samples. The selected probiotic strains were *Lactobacillus bulgaricus, Lactobacillus paracasei, Lactobacillus plantarum, Bifidobacterium lactis* and *Streptococcus thermophilus)*. It was found that *Lactobacillus plantarum* (at cell concentration of 10^8 CFU/mL) showed the highest acrylamide reduction percentage (43–51%) when exposed to different concentrations of AA standard chemical solutions (350, 750, and 1250 ng/mL). The tested strains were further analysed in selected food samples. The efficacy of probiotic strains to reduce acrylamide was studied in potato chips and biscuits while the ability of probiotic strains to reduce ethyl carbamate was investigated in different alcoholic beverages (plum wine, yellow wine, brandy and sake). The results showed that the acrylamide or ethyl carbamate content in food samples were reduced after incubation with different probiotic strains. The results demonstrated that the reducing capacity of selected probiotic strains was different under different food matrix, probably caused by different food composition and processing treatment. This demonstrated that the tested probiotic strains exhibited acrylamide or ethyl carbamate reduction abilities which were probiotic strain-, contaminant concentration-, probiotic concentration-, incubation time- and pH-dependent [Annex 1].

The potential synergistic effect of probiotic formulas to reduce acrylamide was examined based on the results of acrylamide reducing abilities from single probiotic strains. Due to the highest acrylamide reduction ability, *Lactobacillus plantarum* was selected and combined with the other strains to form various probiotic formulas. The result demonstrated a synergistic acrylamide reduction effect by the probiotic formula: *Lactobacillus plantarum* + *Lactobacillus bulgaricus*, which also showed the highest acrylamide reduction ability among the tested formulas. The acrylamide reduction percentage by *Lactobacillus bulgaricus* was significantly increased after combining with *Lactobacillus plantarum*. A further study was conducted by incubating the selected probiotic formulas with potato chips and biscuit samples followed by an in vitro digestion model. The findings demonstrated acrylamide content of the selected food samples was significantly reduced and the percentage of acrylamide reduction in biscuits was higher than that in potato chips by tested probiotic formulas: *Lactobacillus plantarum* + *Lactobacillus bulgaricus* and *Lactobacillus plantarum* + *Streptococcus thermophilus* (10^8 CFU/mL). This study first indicated the synergistic effect of probiotic formulas on acrylamide reduction and its effect was also highly strain-dependent [Annex 2].

The potential synergistic effect of probiotic formulas to reduce ethyl carbamate in selected wine samples (yellow wine, sake and plum wine) and an *in-vitro* digestion model were investigated. Synergic effect of the probiotic formula was only observed in yellow wine which the average reduction percentage of ethyl carbamate by *Lactobacillus bulgaricus* + *Lactobacillus plantarum* was 12.9% and was significantly higher ($p<0.05$) than that by single strain *Lactobacillus plantarum* (9.26%) and *Lactobacillus paracasei* (7.5%). Single strains of probiotic and probiotic formula showed significant increase in ethyl carbamate reduction percentage in both sake and yellow wine in simulated digestion condition, which was 18.3-23.1% for sake and 15.5-21.6% for yellow wine. However, not all the tested probiotic

formulas showed the synergic effect under selected testing conditions. This may be affected by pH and composition of tested wine samples and the characteristics of probiotics (related to reduce toxicants whether by physical binding or enzymatic degradation). Additional evaluation on ethyl carbamate by other probiotic strains was conducted. The probiotic formula: *L. helveticus* demonstrated synergistic effect to reduce ethyl carbamate significantly in sake (54.5%) and yellow wine (26.9%).

Margin of exposure (MOE) approach was used for risk assessment of toxicants after probiotic treatment. Significant reductions of acrylamide in selected food were obtained with the incorporation of selected probiotic strains or probiotic formulas which lead to an increase in the ratio of MOE for risk assessment. The higher the MOE, the lower the health concern [Annex 1].

The above results were presented in various conferences in poster or oral presentation [Annex 1-7]. Financial support from RGC funding was acknowledged.

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

- The potential synergistic effects of various probiotic formulas in reducing toxicants can be further investigated and enhanced the potential applications of probiotic strains in reducing process-induced toxicants. The effectiveness of reducing acrylamide or ethyl carbamate could be further increased by probiotic formulas. More probiotic strains can be further studied and combined to form optimal probiotic formulas for investigating the toxicants reduction ability.

- Both active and inactive probiotic cell could be used to investigate the toxin-reduction ability in food. Further in-depth investigation of both live and dead probiotic cell (via heat or ultrasound treatment) in reducing toxicants in food can increase the industrial application of probiotic bacteria in reducing acrylamide and ethyl carbamate in real-world practice.

- Further application of probiotics in food product development can be investigated to explore the potential functions on food safety management by reducing the potential hazards. The current approach can also be applied to other types of food toxicants or chemical contaminants. The beneficial impact of food choices combination in a meal to reduce bioaccessibility of food toxicants could be further investigated.

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)

This study looked at how probiotics can help reduce harmful contaminants in food. Acrylamide and ethyl carbamate are common process-induced food toxicants formed during processing which raising the public health concerns nowadays as both are classified as probably carcinogenic to human. These findings demonstrate the potential of probiotic formulas to reduce acrylamide in potato chips and biscuits and reduce ethyl carbamate in wine like sake and yellow wine. *Lactobacillus plantarum* was the most effective at reducing acrylamide, while *Lactobacillus bulgaricus* and *Lactobacillus plantarum* together were most effective at reducing ethyl carbamate in yellow wine. Different probiotic formulas added to food and simulated digestive condition were studied showing the reduced levels of these

harmful contaminants. The reduction of contaminants was dependent on the type of probiotic strain, the concentration of the contaminant, the concentration of the probiotic, the incubation time, and the pH levels. The study also found that combining certain probiotic strains could have a synergistic effect, making them even more effective at reducing contaminants. Based on the results, it suggested that incorporating probiotics in food or diet could lower the health risk associated with the contaminants and further research is needed to optimize their efficacy in different food matrices.

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)*						
2022	√			Siu Mei Choi*, Yang Ling, Chang Yuxuan, Ivan K Chu, Nai-ping Dong	Study of the Efficacy of Probiotic Bacteria to Reduce Acrylamide in Food and In Vitro Digestion, *Foods* 2022, *11*(9), 1263	**No**	**Yes** [Annex 1]	**Yes**	**No**
2023	√			Siu Mei Choi*, Hongyu Lin, Weiying Xie, Ivan K Chu	Study of Potential Synergistic Effect of Probiotic Formulas on Acrylamide Reduction, *Int. J. Mol. Sci.* 2023, *24*(5), 4693	**No**	**Yes** [Annex 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)*
Jan/ 2020/ Macau	Examining the potential effect of probiotic bacteria in reducing acrylamide (Oral presentation)	8th Asia-Oceania Mass Spectrometry Conference (AOMSC) 2020	Yes (Sept 2020 progress report)	Yes [Annex 3]	Yes	Yes

Jun/ 2022/ Rome (Virtual)	Study of Potential Synergistic Effect of Probiotic Formulas in Food Toxin Reduction (Oral presentation)	2nd International Nutrition Research Conference 2022, online	NA	Yes [Annex 4]	Yes	Yes
Dec/ 2022/ Hong Kong (Virtual)	Evaluation of the efficacy of probiotic bacteria in reducing food toxicants (Oral presentation)	Probiotics & Prebiotics in Health and Disease Symposium 2022	NA	Yes [Annex 5]	Yes	Yes
May 2022/2023 / Osaka	Study of Potential Synergistic Effect of Probiotic Formulas in Reduction Acrylamide (Poster presentation)	The 9th Annual World Congress of Food and Nutrition-2022/23)/ 11th World Gene Convention-2023 (WGC-2023)	NA	Yes [Annex 6]	Yes	Yes
May 2022/2023 / Osaka	Evaluation of the efficacy of probiotic strains in reducing ethyl carbamate (Oral presentation)	The 9th Annual World Congress of Food and Nutrition-2022/23)/ 11th World Gene Convention-2023 (WGC-2023)	NA	Yes [Annex 7]	Yes	Yes

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

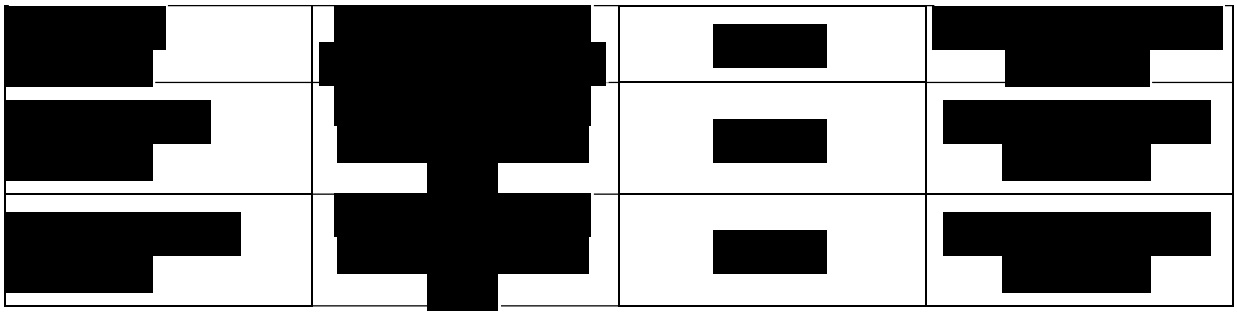
(Please elaborate)

The research experience and new knowledge from this project are shared to students in the modules of "SFS 5413 Food toxicology" and "SFS 5423 Advanced Topics in Food Science" as well as the "SFS 5410/5420 Final year Project" in THEi. The students were involved in different parts of sample extraction and acrylamide/ ethyl carbamate determination after probiotic incubation. Undergraduate students also had chances to do research in this area and gain an insight into food toxicant analysis and the potential applications of probiotic strains and probiotic formulas. Students presented poster in "THEi Student Applied Research Presentations SARP 2021". The new knowledge of this innovative idea from the present research project has been incorporated into some industrial collaboration projects related to probiotic applications.

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
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]



12. Other Impact

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

Collaboration with other research institutions

- This research project is a collaborative work between Technological and Higher Education Institute of Hong Kong and The University of Hong Kong.
- The innovative idea from this research project also created an industrial collaborative project for further application of probiotics in food product development.

Teaching Enhancement

- Trained undergraduate students and student helpers to conduct sample preparation with probiotic incubation, extraction using SPE techniques and LC-MS alongside the research staff during the sample extraction and testing.
- Incorporated the project proposal, literature review and latest probiotic applications in the module "Final Year Project" under PI's supervision.

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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	5	NA	NA	Type	No.
					Student thesis	8

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	NA