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

Study of probiotic approach to reduce food process-induced toxicants using an in vitro digestion model

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	1/1/2018	NA	NA
Project Completion Date	30/6/2019	31/3/2020	16/5/2019
Duration *(in month)*	18	27	16/5/2019
Deadline for Submission of Completion Report	30/6/2020	31/3/2021	16/5/2019

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To investigate the efficacy of probiotics in reducing concentrations of (i) ethyl carbamate or (ii) acrylamide in selected food samples;
2. To evaluate the bioaccessibility of (i) ethyl carbamate and (ii) acrylamide with and without the addition of the selected probiotic strains using an in vitro digestion model under simulated gastrointestinal digestion conditions;
3. To evaluate the risk assessment of (i) ethyl carbamate and (ii) acrylamide before and after probiotic treatments of the ethyl carbamate-contaminated alcoholic drinks and fermented bread or acrylamide-contaminated potato chips and 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 bacteria (*Lactobacillus* and *Bifidobacterium*) in reducing concentration 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 from *lactobacillus* and *Bifidobacterium* were added in food samples and incubated at 37ºC for 4 hours. The content of acrylamide and ethyl carbamate in food samples 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 different types of alcoholic beverages (sake, yellow wine, brandy, plum wine) were used for ethyl carbamate reduction assay. The efficacy of probiotics 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 most effective two probiotic strains were identified from experiments (Objective 1) and further used in experiment for evaluating the bioaccessibility of ethyl carbamate or acrylamide by adding probiotic bacteria using in vitro gastrointestinal digestion model (**Objective 2)**. The bioaccessibility of ethyl carbamate or acrylamide from selected food samples were evaluated with the simulated fed conditions. When the digestion process is completed, the supernatant was collected for quantification of ethyl carbamate or acrylamide as mentioned above. The content of ethyl carbamate or acrylamide with or without probiotics were determined at the end of digestion process to compare the bioaccessibility under simulated gastrointestinal digestion conditions.

Objective 3 (100% achieved):
The risk assessment of ethyl carbamate and acrylamide before and after probiotic treatments of 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 probiotics in reducing concentrations of (i) ethyl carbamate or (ii) acrylamide in selected food samples;	√	100%
2. To evaluate the bioaccessibility of (i) ethyl carbamate and (ii) acrylamide with and without the addition of the selected probiotic strains using an in vitro digestion model under simulated gastrointestinal digestion conditions;	√	100%
3. To evaluate the risk assessment of (i) ethyl carbamate and (ii) acrylamide before and after probiotic treatments of the ethyl carbamate-contaminated alcoholic drinks and fermented bread or acrylamide-contaminated potato chips and 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)

1. The efficacy of probiotics in reducing ethyl carbamate and acrylamide in selected food samples

The efficacy of selected probiotic bacteria to reduce ethyl carbamate and acrylamide was assessed. For acrylamide reduction, the tested bacteria exhibited acrylamide reduction ability which was found to be probiotic strain-, acrylamide concentration-, probiotic concentration-, incubation time- and pH-dependent. Both *L. acidophilus* and *B. longum* showed acrylamide reduction ability in selected potato chips and soda cracker samples. A noticeable reduction of acrylamide was observed (9.45–22.15%) in two different food matrices. *L. acidophilus* showed higher acrylamide reduction percentage than *B. longum* (21.41 %) in biscuit sample while *B. longum* showed higher acrylamide reduction percentage (22.15%) in potato chip sample. Both strains exhibited the lowest reduction percentage at pH 2.5–3.0. When the pH values were increased, higher acrylamide reduction percentage were found. The results indicated that acrylamide reduction in food matrices could be influenced by probiotic strains, the type of food sample and pH condition. For ethyl carbamate reduction, the result revealed that probiotic strain, ethyl carbamate concentration and pH were important factors affecting the ethyl carbamate reduction ability. Among the selected probiotic strains, *L. rhamnosus* (19.81-54.98%) and *B. longum* (19.25-51.34%) showed the higher reduction rate in ethyl carbamate chemical solutions (100-700ng/mL). *B. longum* could significantly reduce ethyl carbamate in sake with 46.34% reduction while *L. rhamnosus* had 30.60% reduction. Both *L. rhamnosus* (42.09%) and *B. longum* (47.38%) showed comparable reduction ability in brandy. *B. longum* also demonstrated notable reduction in yellow wine (68.08%). The findings showed that *B. longum* was the most effective in reducing ethyl carbamate in selected alcoholic drinks ranging from 46.34-68.08%.

2. Bioaccessibility of ethyl carbamate and acrylamide with selected probiotic strains using an in vitro digestion model

The bioaccessibility of acrylamide was lower with probiotic incubation after simulated digestion. Both *L. acidophilus* and *B. longum* strains showed ability to reduce acrylamide ranged from 14.46 to 21.29% at the end of intestinal digestion.

In ethyl carbamate reduction, *B. longum* was the most effective in decreasing bio-accessibility of ethyl carbamate in selected alcoholic beverages after in vitro digestion. *B .longum* displayed a remarkable ethyl carbamate reduction ability in yellow wine (76.37%) and brandy (51.56%) under simulated digestion model. Additional evaluation on reduction ability of live and dead probiotic cells was conducted and demonstrated the binding mechanism. Higher reduction percentage of acrylamide and ethyl carbamate was obtained by ultrasound-treated (5 min) probiotic cells. This may be explained by more binding sites in ultrasound-treated cell wall of probiotic bacteria exposed to the toxicants for binding.

3. Risk assessment of ethyl carbamate and acrylamide before and after probiotic treatments

Margin of exposure (MOE) approach was used for risk assessment of toxicants after probiotic treatment. Significant reductions of ethyl carbamate and acrylamide in selected food were obtained with the incorporation of probiotic bacteria which lead to increase the ratio of MOE for risk assessment. The higher the MOE, the lower the health concern. The results are presented in the manuscript under preparation (Choi et al., 2021) [Annex 1].

The above results of acrylamide part were presented in "10th International Conference on Food Safety and Regulatory Measures" held in Zurich, Switzerland in May 2019 [Annex 2] and "8th Asia-Oceania Mass Spectrometry Conference (AOMSC) 2020" held in Macau in Jan, 2020 [Annex 3]. Financial support from RGC 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 bacteria in reducing process-induced toxicants. The effectiveness of reducing acrylamide or ethyl carbamate could be further increased by probiotic formulas.

- 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.

- The current approach can also be applied in other types of food toxicants or contaminants. For example, the natural plant toxins could be reduced when probiotic bacteria are incorporated in human diet.

7. Layman's Summary

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

Acrylamide and ethyl carbamate are process-induced food toxicants formed during processing which raising the public health concerns nowadays as both are classified as probably carcinogenic to human (Group 2A). Hence, various mitigation strategies are developed to reduce the formation of acrylamide or ethyl carbamate during processing. Most of strategies are pre-processing control and focused on physical and chemical treatments during food processing. However, the current strategy is a new alternative post-processing approach of using probiotic bacteria in reducing the potential toxicants in food.

The results demonstrated the ability of selected probiotic strains (*Lactobacillus* and *Bifidobacterium*) to reduce acrylamide and ethyl carbamate at different extents depending on type and concentration of probiotic strains, concentration of toxicants, pH conditions and type of food models. Positive impacts of probiotic effects on the fraction of toxicants released from the food in the gastrointestinal condition via in vitro digestion model were obtained. The findings suggest the beneficial effects of probiotic to reduce the content of potential carcinogens and mutagens by lowering the dietary ethyl carbamate or acrylamide in the selected food. This explores the potential industrial applications of probiotic bacteria in food development and the incorporation of probiotic bacteria in human diet for reducing acrylamide or ethyl carbamate.

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)*						
			√	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 [Annex 1]	**NA**	**Yes [Annex 1]**	**Yes**	**No**

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)*
May/ 2019/ Zurich	Study of the efficacy of probiotic bacteria to reduce process-induced toxicant - acrylamide	10th International Conference on Food Safety and Regulatory Measures [Annex 2]	NA	Yes	Yes	Yes
Jan/ 2020/ Macau	Examining the potential effect of probiotic bacteria in reducing acrylamide (Oral presentation)	8th Asia-Oceania Mass Spectrometry Conference (AOMSC) 2020 [Annex 3]	NA	Yes	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 Final year Project" in THEi. The students were involved in different parts of sample preparation and acrylamide/ ethyl carbamate determination. Undergraduate students have chance to do research in this area and gain an insight into chemical contaminant analysis and the potential applications of probiotic bacteria. Students presented poster in "THEi Student Applied Research Presentations SARP 2020" [Annex 4a,b]. The new knowledge of this innovative idea from the present research project has been incorporated into the STEM joint project (International STEM Students Forum 2020).

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

12. Other Impact

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

Invited Speaker

- "Examining the potential effect of probiotic bacteria in reducing acrylamide", presented at 8th Asia-Oceania Mass Spectrometry Conference (AOMSC) 2020, Macau, 5-7 Jan 2020.

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 a collaborative project between Technological and Higher Education Institute of Hong Kong and Manchester Metropolitan University (UK) for further application of probiotics in new food product development in STEM project.
- Two students from THEi BSc (Hons) in Food Science and Safety were inspired by their Final Year project and the project-based learning in the module of Advanced Topics in Food Science to explore the innovative application of probiotic bacteria to develop healthy food products. They joined the International STEM Students' Forum (ISSF) 2020 STEM project and presented their collaborative project "The Role of Probiotic Bacteria and Its Application in Healthy Product Development" with two students from Nutritional Sciences/Sport, Exercise Nutrition in Manchester Metropolitan University (UK) in the first digital ISSF 2020 on 16 May 2020 [Annex 9a,b]. The Forum was successfully held and there are over 6 000 views from students, teachers and industry partners across the globe with having around 3 000 registrants on the webinar platform. In addition, a Food Science and Safety programme Yr-4 student and the PI were interviewed by RTHK programme to share the research concept and experience with the general public in June 2020 [Annex 10a,b].

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	1*	2	NA	NA	Type	No.
					Student thesis	6
					Media interview	2

**Manuscript is under preparation to be submitted to LWT*

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