UNIVERSITI PUTRA MALAYSIA

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By

TEOH PECK LIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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OPTIMIZATION OF FREEZE-DRYING PROTECTANTS BY RESPONSE SURFACE METHODOLOGY AND ENCAPSULATION FOR *BIFIDOBACTERIUM PSEUDOCATENULATUM* G4 AND *LACTOBACILLUS ACIDOPHILUS* LA-5 SURVIVAL ENHANCEMENT

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Chair: Prof. Mohd Yazid Abd. Manap, PhD

Faculty: Food Science and Technology

Probiotic can easily lose their cell viability during processing, storage, as well as during gastrointestinal transit. Therefore, the main objective of this study was to optimize a suitable protective media combination (skim milk, sucrose, trehalose and inulin) for freeze-drying of *Bifidobacterium pseudocatenulatum* G4 using Response Surface Methodology (RSM) and to encapsulate both *B. pseudocatenulatum* G4 and *Lactobacillus acidophilus* LA-5 for enhanced survival during heat exposure, high sodium concentration and gastrointestinal transit.

The identity of *B. pseudocatenulatum* G4 was confirmed by genus-specific PCR using the specific gene-targeted primers (Lm26 and Lm3). The morphology of the probiotic was also examined by microscopic method, in particular, by using the Gram-staining method and scanning electron microscope.

Analysis of variance (ANOVA) showed that skim milk, sucrose and inulin concentration had significant ($P < 0.05$) effect on the viability of *B. pseudocatenulatum* G4 after freeze-drying. On the other hand, concentration of

trehalose did not have any significant effect ($P > 0.05$) on viability of the freeze-dried cells. Optimization model indicated that a combination of 15.04% (w/v) skim milk, 2.17% (w/v) sucrose, 1.87% (w/v) trehalose and 0.36% (w/v) inulin resulted in high survival (9.86 log cfu/mL) of *B. pseudocatenulatum* G4. Experimental verification showed that the experimental values were adequately close to the predicted values, with no significant difference ($P > 0.05$) in terms of survival level of *B. pseudocatenulatum* G4 after freeze-drying, thus verifying the accuracy and validity of final reduced model.

The next part of the research focused on encapsulation of *B. pseudocatenulatum* G4 and *L. acidophilus* LA-5 using the extrusion technique. The encapsulated probiotic were further coated with chitosan to enhance protection. The encapsulated probiotic were compared with free cells in terms of thermotolerance, sodium tolerance, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) resistant properties. Encapsulated probiotic survived significantly ($P < 0.05$) better than free cells during heat exposure (55, 60 and 65 °C) up to 30 min. Encapsulated probiotic also showed higher viability when they were exposed to high sodium concentrations (1% w/v, 2% w/v and 3% w/v) for 3 h. The survival of free cells decreased with increasing sodium concentration and prolonging incubation period. However, the encapsulated cells did not show this similar trend of cell reduction.

Similarly, encapsulation of probiotic resulted in significantly ($P < 0.05$) higher survival of cells during exposure to SGF and SIF. Free cells were not detectable after 1 h exposure to SGF, whereas the encapsulated probiotic decreased from about 9 to 5 log cfu/mL. Only approximately 3.21-4.24 log cfu/mL of encapsulated cells

remained after sequential incubation in SIF. Chitosan coated alginate-starch capsules provided the best protection for both *L. acidophilus* LA-5 and *B. pseudocatenulatum* G4 during SGF and SIF exposure.

Encapsulation using extrusion technique produced homogenous spherical shaped capsules. The extrusion technique tends to produce capsules with large particle size (~3.6 mm). Scanning electron micrographs showed that probiotic cells were not visible at the surface of freshly prepared capsules which had not been subjected to any treatment. All the capsules were found to retain their shape with a little shrinkage of the capsules during SGF exposure. There was drastic decrease in mechanical strength of the probiotic capsules when they were subjected to SIF, which favoured the release of probiotic cells. This ensured proper colonization of probiotic cells in the colon.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN MEDIA PELINDUNG PENGERINGAN SEJUK-BEKU DENGAN RESPONSE SURFACE METHODOLOGY DAN PENGKAPSULAN TERHADAP PENINGKATAN KEUPAYAAN HIDUP *BIFIDOBACTERIUM PSEUDOCATENULATUM* G4 DAN *LACTOBACILLUS ACIDOPHILUS* LA-5

Oleh

TEOH PECK LIAN

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Pengerusi: Prof. Mohd Yazid Abd. Manap, PhD

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Daya hidup probiotik mudah menurun semasa pemprosesan, penyimpanan, serta transit gastrointestinal. Oleh itu, objektif utama kajian ini adalah untuk mengoptimumkan media campur pelindung yang sesuai untuk pengeringan sejuk-beku *Bifidobacterium pseudocatenulatum* G4 serta pengkapsulan kedua-dua *B. pseudocatenulatum* G4 dan *Lactobacillus acidophilus* LA-5 untuk meningkat upaya hidup apabila terdedah kepada haba, kepekatan natrium tinggi dan transit gastrointestinal.

Pencaman *B. pseudocatenulatum* G4 disahkan dengan PCR menggunakan primer (Lm26 dan LM3). Morfologi probiotik diperhati dengan kaedah mikroskopik, khususnya, dengan kaedah perwarnaan-Gram dan mikroskop elektron.

Kajian ini bertujuan untuk mengoptimumkan kombinasi media pelindung (susu skim, sukrosa, trehalosa dan inulin) untuk mengekalkan bilangan sel hidup probiotik, *B.*

pseudocatenulatum G4 yang tinggi semasa pengeringan sejuk-beku. 'Response Surface Methodology' (RSM) telah digunakan. Analisis Varians (ANOVA) menunjukkan bahawa kepekatan susu skim, sukrosa dan inulin mempunyai kesan yang signifikan ($P < 0.05$) terhadap keupayaan hidup *B. pseudocatenulatum* G4 semasa pengeringan sejuk-beku. Manakala, kepekatan trehalosa tidak mempunyai kesan signifikan ($P > 0.05$) terhadap keupayaan hidup sel sejuk-beku kering. Model optimum menunjukkan bahawa kombinasi 15.04% (w/v) susu skim, 2.17% (w/v) sukrosa, 1.87% (v/v) trehalosa dan 0.36% (w/v) inulin menghasilkan bilangan sel hidup *B. pseudocatenulatum* G4 yang tinggi (9.86 log cfu/mL). Pengesahan eksperimen telah dilakukan. Nilai-nilai eksperimen didapati agak hampir dengan nilai-nilai jangkaan, dengan tidak ada perbezaan signifikan ($P > 0.05$) bagi bilangan sel hidup *B. pseudocatenulatum* G4 sejuk-beku kering.

Seterusnya, pengkapsulan *B. pseudocatenulatum* G4 dan *L. acidophilus* LA-5 dengan menggunakan teknik penyemperitan telah dijalankan. Kapsul probiotik yang dihasilkan selanjutnya dilapisi chitosan untuk meningkatkan perlindungan terhadap sel. Bilangan sel hidup probiotik yang dikapsul telah dibandingkan dengan sel tidak dikapsul dari segi toleransi haba, toleransi natrium serta toleransi terhadap pendedahan jus gaster simulasi (SGF) dan lelahan usus simulasi (SIF). Probiotik yang telah dikapsul mempunyai daya keupayaan hidup yang lebih signifikan ($P <0.05$) berbanding dengan sel yang tidak dikapsul apabila didedahkan pada suhu tinggi (55, 60 dan 65 ° C) sehingga 30 min. Selain itu, probiotik yang dikapsul juga menunjukkan bilangan sel hidup yang lebih tinggi apabila terdedah kepada kepekatan natrium (1% (w/v), 2% (w/v) dan 3% (w/v)) selama 3 j. Bilangan sel hidup bagi sel yang tidak dikapsul makin berkurangan dengan peningkatan

kepekatan natrium dan peningkatan masa inkubasi. Namun, sel yang dikapsul tidak mempunyai trend pengurangan bilangan sel hidup yang serupa dengan sel yang tidak dikapsul.

Pengkapsulan probiotik menghasilkan bilangan sel hidup yang lebih tinggi ($P < 0.05$) daripada sel yang tidak dikapsul semasa pendedahan kepada SGF dan SIF. Sel yang tidak dikapsul tidak dapat dikesan selepas 1 j pendedahan terhadap SGF, sedangkan bilangan probiotik yang dikapsul menurun daripada 9-5 log cfu/mL. Hanya lebih kurang 3.21-4.24 log cfu/mL sel yang dikapsul tinggal setelah inkubasi di SIF. Chitosan-alginate-kanji kapsul didapati memberikan perlindungan yang terbaik untuk kedua-dua *L. acidophilus* LA-5 dan *B. pseudocatenulatum* G4 semasa didedahkan kepada SGF dan SIF.

Pengkapsulan menggunakan teknik penyemperitan mampu menghasilkan kapsul homogen berbentuk bulat. Teknik penyemperitan lebih cenderung untuk menghasilkan kapsul bersaiz besar (~ 3.6 mm). Mikrograf mikroskop elektron menunjukkan bahawa sel-sel probiotik tidak kelihatan pada permukaan kapsul yang baru dihasilkan. Semua kapsul mengekalkan bentuk masing-masing dengan penyusutan sedikit semasa didedahkan kepada SGF. Pengurangan drastik dalam kekuatan mekanikal kapsul probiotik diperhatikan apabila kapsul-kapsul didedahkan kepada SIF. Keadaan sedemikian amat diingini kerana ia menyokong pembebasan sel probiotik dan memastikan penjajahan sel probiotik di dalam usus besar.

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I certify that a Thesis Committee has met on 20 June 2011 to conduct the final examination of Teoh Peck Lian on her thesis entitled "Optimization of freeze-drying protectants by response surface methodology and encapsulation for *Bifidobacterium pseudocatenulatum* G4 and *Lactobacillus acidophilus* LA-5 survival enhancement" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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TEOH PECK LIAN

Date: 20 June 2011

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