UNIVERSITI PUTRA MALAYSIA

COATING AND PELLETIZATION OF PROBIOTIC *LACTOBACILLUS* STRAINS TO ENHANCE VIABILITY DURING PROCESSING AND STORAGE

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

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Chairman: Professor Ho Yin Wan, PhD

Institute: Bioscience

The use of probiotics is one of several approaches that have potential to be an alternative to antibiotics as growth promoters for improving livestock production. The ability of probiotic microorganisms to remain viable and stable during processing, storage and digestion is very crucial for their positive contribution to the host. However, even with sophisticated formulations under excellent storage conditions, the loss rate is about one log unit of cell reduction per year. In the present study, attempts were made to enhance the viability of three probiotic *Lactobacillus* strains, (*L. reuteri* C 10, *L. gallinarium* I 26 and *L. brevis* I 25), by coating the cells with stearic acid using a fluidized bed granulator, and by pelletization using the extrusion technique. Preliminary studies were carried out to evaluate the tolerance of *L. reuteri* C 10 to high temperatures (58 to 70 ºC) and acidic conditions (pH 4 to 6) because the melting point of stearic acid is 57.23 ºC and pH is 5.5, and input temperatures of the fluidized bed granulator could be as high as 70 ºC. Results of the preliminary study demonstrated that freeze-dried *L. reuteri* C 10 cells incorporated with cryoprotectants (trehalose and sucrose) exhibited higher survivability compared to freeze-dried *L. reuteri* C 10 cells without cryoprotectants when exposed for 30 min

at 64, 66 and 68 ºC. Freeze-dried cells with cryoprotectants were also able to survive for 15 min at 70 ºC, but not freeze-dried cells without cryoprotectants. In acidic conditions (pH 4, 5 and 6), freeze-dried *L. reuteri* C 10 with cryoprotectants also exhibited higher survival rates than those without cyroprotectants. Thus, cells incorporated with cryoprotectants were used for the coating process. The use of a fluidized bed granulator to coat freeze-dried *L. reuteri* C 10 cells resulted in the formation of a big lump consisting of stearic acid on the upper portion, and excessive amount of freeze-dried *Lactobacillus* cells on the lower portion, instead of fine, uniformly-coated granules. The coating process using a fluidized bed granulator was not successful in this study due to rapid solidification of stearic acid inside the tube and spray nozzle because the melting point of stearic acid could not be maintained. Since fluidized bed coating of *L. reuteri* C 10 was not successful, the coating process was not carried out on *L. gallinarium* I 26 and *L. brevis* I 25,

An alternative technique, which was a pelletization technique, was then carried out to pelletize *L. reuteri* C 10, *L. gallinarium* I 26 and *L. brevis* I 25 using the extrusion-spheronization technique and the extrusion-grinding technique. The extrusion-grinding technique was designed and developed in this study. It was a simple technique, very easy to handle and did not require expensive sophisticated equipment as in the extrusion-spheronization technique. The results revealed that the extrusion-grinding technique produced less ($P < 0.001$) cell loss during processing than the extrusion-spheronization technique. A loss of 0.5 to 1.3 log units of viable cells from the granulation to the spheronization or grinding process was observed in both the extrusion-spheronization and extrusion-grinding techniques used. However, the extrusion-grinding technique showed significantly ($P < 0.001$) higher cell viability during the freeze-drying process compared to the extrusion-spheronization technique (approximately 50 % and 77 % of cell loss, respectively). Three formulations (A, B and C, with varied compositions of microcrystalline cellulose, lactose, inulin and skim milk) were used to pelletize *Lactobacillus*

strains in the extrusion-spheronization and extrusion-grinding processes. The results showed that, generally, formulation A was the best ($P < 0.001$) formulation to pelletize *Lactobacillus* cells using both extrusion-spheronization and extrusion-grinding techniques. After 6 months of storage at 30 ºC, higher cell viabilities were exhibited by all three *Lactobacillus* strains in pellets produced by the extrusion-grinding technique than by the extrusion-spheronization technique. However, at 4 ºC, only *L. reuteri* C 10 but not *L. gallinarium* I 26 and *L. brevis* I 25, exhibited higher ($P < 0.001$) cell viability in pellets produced from the extrusion-grinding technique than from the extrusion-spheronization technique throughout 6 months of storage. Formulations A and B were better ($P < 0.001$) than formulation C in maintaining higher cell viability during 6 months of storage at 4 and 30 ºC, regardless of the techniques used. The results for the pellet densities showed that pellets from the extrusion-grinding technique had higher ($P < 0.001$) density compared to those from the extrusion-spheronization technique. Denser pellets (from extrusion-grinding technique) might provide better protection against adverse environmental conditions during storage, especially at 30 ºC (room temperature). In conclusion, the use of fluidized bed granulator to coat *L. reuteri* C 10 with stearic acid was not successful. The simple extrusion-grinding technique designed and developed in this study produced better survival rates of *Lactobacillus* cells during processing and storage than the extrusion-spheronization technique.

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

PENYALUTAN DAN PEMPELETAN PROBIOTIK *LACTOBACILLUS* UNTUK MENINGKATKAN KEBOLEHIDUPAN SEMASA PEMPROSESAN DAN PENYIMPANAN

Oleh

AZIM BIN HARIS

November 2011

Pengerusi: Profesor Ho Yin Wan, PhD

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Penggunaan probiotic ialah satu pendekatan yang berpotensi menjadi satu alternatif terhadap antibiotik sebagai pendorong pertumbuhan untuk meningkatkan pengeluaran ternakan. Keupayaan probiotik mikroorganisma untuk kekal dan stabil semasa pemprosesan, penyimpanan dan penghadaman adalah sangat penting untuk kesan positif pada perumah. Bagaimanapun, walaupun dengan formulasi sempurna di bawah keadaan penyimpanan yang baik, kadar kematian sel ialah satu log unit setiap tahun. Dalam kajian ini, percubaan untuk meningkatkan kebolehhidupan tiga strain probiotik *Lactobacillus* (*L. reuteri* C 10, *L. gallinarium* I 26 dan *L. brevis* I 25), iaitu dengan penyalutan sel dengan asid stearic menggunakan 'fluidized bed granulator', dan pempeletan menggunakan teknik 'extrusion'. Kajian awal telah dijalankan untuk menilai toleransi *L. reuteri* C 10 terhadap suhu tinggi (58 hingga 70 ºC) dan di dalam keadaan berasid (pH 4 hingga 6), kerana suhu takat lebur asid stearik ialah 57.23 ºC dan pH ialah 5.5, dan suhu input 'fluidized bed granulator' mungkin meningkat setinggi 70 ºC. Keputusan kajian awal menunjukkan *L. reuteri* C 10 sel beku-kering dengan 'cryoprotectants' (trehalosa dan sukrosa) mempamerkan kadar kehidupan yang lebih tinggi berbanding dengan *L. reuteri* C 10 sel beku-

kering tanpa 'cryoprotectants' apabila didedahkan selama 30 minit pada suhu 64, 66 dan 68 ºC. Sel beku-kering dengan 'cryoprotectants' juga berupaya untuk hidup selama 15 minit pada suhu 70 ºC, tetapi bukan sel beku-kering tanpa 'cryoprotectants'. Dalam keadaan berasid, (pH 4, 5 dan 6), *L. reuteri* C 10 sel beku-kering dengan 'cryoprotectants' juga mempamerkan kadar kebolehidupan yang lebih tinggi daripada tanpa 'cryoprotectants'. Maka, sel dengan 'cryoprotectant' akan digunakan untuk proses penyalutan. Penggunaan 'fluidized bed granulator' untuk menyalut *L. reuteri* C 10 sel beku-kering menyebabkan pembentukan satu gumpalan besar yang terdiri daripada asid stearik pada bahagian atas, dan beku-kering *Lactobacillus* yang berlebihan pada bahagian bawah, di sebalik membentuk butiran yang seragam dan penyalutan yang sekata. Proses penyalutan menggunakan 'fluidized bed granulator' tidak berjaya dalam kajian ini disebabkan pembekuan asid stearik yang pesat di dalam tiub dan muncung semburan kerana takat lebur asid stearik tidak dapat dikekalkan. Disebabkan penyalutan terhadap *L. reuteri* C 10 tidak berjaya, proses penyalutan tidak dijalankan pada *L. gallinarium* I 26 dan *L. brevis* I 25.

Sebagai teknik alternatif, iaitu teknik pempeletan, ia telah dijalankan untuk mempelet *L. reuteri* C 10, *L. gallinarium* I 26 dan *L. brevis* I 25 menggunakan teknik 'extrusion-spheronization' dan 'extrusion-grinding'. Teknik 'extrusion-grinding' direka dan dicipta dalam kajian ini. Ia merupakan teknik yang mudah, senang untuk digunkan, dan tidak memerlukan kos yang tinggi seperti teknik 'extrusion-spheronization'. Keputusan kajian menunjukkan bahawa teknik 'extrusion-grinding' menghasilkan kurang ($P < 0.001$) kematian semasa pemprosesan berbanding dengan teknik 'extrusion-grinding'. Kematian sebanyak 0.5 sehingga 1.3 log unit sel hidup daripada penggranulan ke 'spheronization' atau pengisaran telah dipamerkan dalam kedua-dua teknik 'extrusion-spheronization' dan 'extrusion-grinding' yang digunakan. Walaubagaimanapun, teknik 'extrusion-grinding' menunjukan secara signifikan ($P < 0.001$)

kebolehhidupan sel yang lebih tinggi semasa pengeringan beku-kering berbanding dengan teknik 'extrusion-spheronization' (anggaran 50 % dan 77 % kematian sel). Tiga formulasi (A, B dan C, dengan pelbagai komposisi 'microcrystalline' selulosa, laktosa, inulin dan susu krim) telah diguna untuk pempeletan sel *Lactobacillus* dalam proses 'extrusion-spheronization' dan 'extrusion-grinding'. Keputusan menunjukkan bahawa, secara umumnya, formulasi A adalah terbaik ($P < 0.001$) untuk pempeletan sel *Lactobacillus* menggunakan kedua-dua teknik 'extrusion-spheronization' dan 'extrusion-grinding'. Selepas 6 bulan penyimpanan di 30 ºC, kebolehidupan sel yang lebih tinggi dipamerakan oleh ketiga-tiga palet *Lactobacillus* yang dihasilkan oleh teknik 'extrusion-grinding' berbanding dengan teknik 'extrusion-spheronization'. Walaubagaimanapun, di 4 ºC, hanya *L. reuteri* C 10 tetapi bukan *L. gallinarium* I 26 dan *L. brevis* I 25 menunjukkan lebih tinggi ($P < 0.001$) kebolehidupan sel dalam pelet yang dihasilkan oleh teknik 'extrusion-grinding' berbanding dengan teknik 'extrusion-spheronization' semasa 6 bulan penyimpanan. Formulasi A dan B adalah lebih baik ($P < 0.001$) daripada formulasi C dalam mengekalkan kebolehidupan yang lebih tinggi semasa 6 bulan penyimpanan di 4 dan 30 ºC, tanpa mengira teknik yang digunakan. Keputusan ketumpatan palet menunjukkan pelet yang dihasilkan oleh teknik 'extrusion-grinding' mempunyai ketumpatan yang lebih tinggi ($P < 0.001$) berbanding dengan yang dihasilkan oleh teknik 'extrusion-spheronization'. Pelet berketumpatan tinggi (dari 'extrusion-grinding') mungkin memberi perlindungan yang lebih baik terhadap keadaan persekitaran yang kurang baik semasa penyimpanan, terutama di 30 ºC (suhu bilik). Konklusinya, penggunaan 'fluidized bed granulator' untuk menyalut *L. reuteri* C 10 sel beku-kering tidak berjaya. Teknik 'extrusion-grinding' yang direka dan dicipta dalam kajian ini menghasilkan kebolehhidupan *Lactobacillus* sel yang lebih tinggi semasa pemprosesan dan penyimpanan daripada teknik 'extrusion-spheronization'.

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I certify that a Thesis Examination Committee has met on 30/11/2011 to conduct the final examination of Azim Bin Haris on his thesis entitled "Coating and Pelletization of Probiotic *Lactobacillus* Strains to Enhance Viability During Processing and Storage" 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 the 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 at any other institution.

AZIM BIN HARIS

Date: 30 November 2011

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