



**UNIVERSITI PUTRA MALAYSIA**

**MICROBIAL GROWTH, ACIDIFICATION PROPERTIES AND  
FORMATION OF METABOLITES IN FERMENTED MILK PRODUCTS  
USING VARIOUS STARTER CULTURE COMBINATIONS**

**AREZOU AGHLARA**

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OF METABOLITES IN FERMENTED MILK PRODUCTS USING VARIOUS  
STARTER CULTURE COMBINATIONS**

**By**

**AREZOU AGHLARA**

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirement for the Degree of Doctor of Philosophy

January 2008



Dedicated to  
my parents and my two brothers,  
who have always stayed beside me despite the distance,  
who received less of my attention while this project was being done,  
for their unwavering support and encouragement during this time.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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**January 2008**

**Chairman : Professor Mohd Yazid Abdul Manap, PhD**

**Faculty : Food Science and Technology**

The present study investigated the microbial growth, acidification properties and changes in key metabolite compounds during fermentation of sterilized reconstituted skim milk. Fermentation was performed by using single and mixed cultures of *Lactobacillus acidophilus* LA5 (probiotic strain; A), *L. delbrueckii* subsp. *bulgaricus* LB12 (B), *Streptococcus thermophilus* TH4 (T) and *Kluyveromyces marxianus* subsp. *marxianus* LAF4 (M). Fermentation time, lag time and final titratable acidity ranged from 225 to 721 min, 2 to 31 min and 0.76 to 1.25 % (w/v) lactic acid, respectively. At the end of fermentation, the highest counts of *L. acidophilus* LA5, *L. delbrueckii* subsp. *bulgaricus* LB12, *S. thermophilus* TH4 and *K. marxianus* subsp. *marxianus* LAF4 were 8.42, 8.56, 8.91 and 8.69 log<sub>10</sub> cfu mL<sup>-1</sup>, respectively. In all single and mixed cultures containing *L. acidophilus* LA5, the viable probiotic cell count met the minimum proposed effective level required to observe a positive health effect.



In this study, product fermented with traditional yoghurt culture (mixed culture of *L. delbrueckii* subsp. *bulgaricus* LB12 and *S. thermophilus* TH4) showed the highest titratable acidity and the lowest pH during entire storage period. The most stable product in terms of the changing in titratable acidity during storage period was prepared with single culture of *S. thermophilus* TH4. However, this strain was found likely the responsible of post-acidification when incorporated into the mixed cultures. Viable counts of all lactic acid bacteria and yeast in single and mixed cultures always remained higher than  $7 \log_{10} \text{ cfu mL}^{-1}$ , however, fluctuation in their counts was observed during 4 weeks of refrigerated storage.

Changes in lactose and total glucose and galactose were monitored during fermentation and cold storage by using high performance liquid chromatography (HPLC) coupled to refractive index (RI) detector. Lactose was utilized by all starter culture combinations during fermentation and storage time. The consumption of lactose during fermentation and storage was significantly ( $P < 0.05$ ) higher in single culture of *K. marxianus* subsp. *marxianus* LAF4. The data obtained in this study showed that the changes in total content of glucose and galactose was not paralleled with the changes in lactose content during fermentation and storage.

Changes in concentration of citric, orotic, pyruvic, succinic, lactic, formic, acetic, uric, propionic, butyric and hippuric acids was carried out using HPLC coupled to ultraviolet (UV) detector. In general, variable amount of each organic acid was formed with different starter culture used in this study during fermentation process and storage.

However, lactic acid was found to be the most abundant organic acid in majority of the products at the end of fermentation and throughout the storage time. The final concentration of lactic acid at the end of fermentation was ranged between 1167 and 8895 mg L<sup>-1</sup>. The most prominent organic acid in products fermented with single culture of *L. delbrueckii* subsp. *bulgaricus* LB12 and mixed culture of *L. delbrueckii* subsp. *bulgaricus* LB12 and *K. marxianus* subsp. *marxianus* was acetic and citric acid, respectively, followed by lactic acid.

Forty volatile compounds were detected using gas chromatography coupled to time-of-flight mass spectrometer (GC-TOFMS). The representative of the alcohols (i.e. ethanol), ketones (i.e. ethyl acetate and ethyl butyrate), esters (i.e. 2-butanone, acetone, 3-hydroxy-2-butanone or acetoin and 2,3-butanedione or diacetyl) and aldehydes (i.e. acetaldehyde) were considered for further analysis. The release of the corresponding volatile flavor compounds into the headspace was monitored during fermentation and 4 weeks of refrigerate storage, using GC coupled to flame ionization detector (FID). In fermented products devoid of *K. marxianus* subsp. *marxianus* LAF4, acetoin was seen to be the most prominent volatile flavor substance at the end of fermentation, ranging from 59.5 to 104.2 mg L<sup>-1</sup>. While, in those co-inoculated with the yeast strain ethanol was observed to be the most abundant volatile flavor compound ranged between 964 and 6522 mg L<sup>-1</sup>.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia Sebagai memenuhi keperluan untuk ijazah

**PERTUMBUHAN MIKROORGANISMA, PENGASIDAN DAN  
PEMBENTUKAN METABOLISMA SEJUK PRODUK SUSU  
TERFERMENTASI MENGGUNAKAN KOMBINASI PELBAGAI KULTUR  
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Penyelidikan ini mengkaji tentang pertumbuhan mikrob, sifat pengasidan, dan perubahan dalam penentuan hasil sebatian metabolisma semasa fermentasi susu skim terbentuk semula disterilkan. Fermentasi dijalankan menggunakan kultur tunggal dan kultur campuran *Lactobacillus acidophilus* LA5 (strain probiotik; A), *L. delbrueckii* subsp. *bulgaricus* LB12 (B), *Streptococcus thermophilus* TH4 (T) dan *Kluyveromyces marxianus* subsp. *marxianus* LAF4 (M). Masa fermentasi, masa adaptasi, dan julat keasidan terbolehtitrat akhir, masing-masing adalah 225-721 min, 2-31 min dan 0.76-1.25 % (w/v) asid laktik. Di akhir fermentasi, pengiraan tertinggi bagi *L. acidophilus* LA5, *L. delbrueckii* subsp. *bulgaricus* LB12, *S. thermophilus* TH4 dan *K. marxianus* subsp. *marxianus* LAF4, masing-masing adalah 8.42, 8.56, 8.91, dan 8.69 log<sub>10</sub> cfu mL<sup>-1</sup>. Dalam semua kultur tunggal dan kultur campuran yang mengandungi *L. acidophilus* LA5, pengiraan sel probiotik hidup telah mencapai tahap unjuran keberkesanan minimum yang diperlukan bagi memperlihatkan kesan positif keatas kesihatan.



Dalam kajian ini juga, produk terfermentasi dengan kultur yogurt tradisional (kultur campuran *L. delbrueckii* subsp. *bulgaricus* LB12 and *S. thermophilus* TH4) telah menunjukkan keasidan terboleh titrat yang paling tinggi dan pH yang paling rendah semasa keseluruhan tempoh penyimpanannya. Produk yang paling stabil dalam terma perubahan keasidan terboleh titrat semasa tempoh penyimpanan telah dicampurkan bersama kultur tunggal *S. thermophilus* TH4. Walaubagaimana pun, strain ini telah didapati berkemungkinan penyebab kepada post-pengasidan bila mana digabungkan dalam kultur campuran. Pengiraan sel hidup bagi LAB dan yis sentiasa kekal lebih tinggi dari  $7 \log_{10} \text{cfu mL}^{-1}$ , walaubagaimana pun, dalam beberapa produk yang lain, setiap perubahan dalam pengiraannya diperhatikan semasa 4 minggu dalam penyimpanan berhawa dingin.

Perubahan pada laktosa dan jumlah glukosa dan galaktosa dipantau semasa fermentasi dan penyimpanan berhawa dingin dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC) yang digabungkan bersama pengesanan indeks biasan (RI). Penggunaan laktosa diperhatikan dalam semua produk dan *K. marxianus* subsp. *marxianus* LAF4 menunjukkan ia lebih tinggi secara signifikannya ( $P < 0.05$ ) terhadap penggunaan laktosa dalam penyimpanan berhawa dingin. Ianya diperhatikan bahawa perubahan dalam jumlah kandungan glukosa dan galaktosa tidak berkait dengan asimilasi laktosa semasa fermentasi.

Penyaringan kepekatan asid sitrik, orotik, piruvik, suksinik, laktik, formik, asetik, urik, propionik, butirik dan hippurik, dijalankan menggunakan HPLC digabungkan bersama



pengesan ultraviolet (UV). Secara amnya, jumlah setiap asid organik yang terhasil adalah berbeza-beza mengikut kultur pemula semasa proses fermentasi dan penyimpanan. Walaubagaimana pun, didapati bahawa asid laktik adalah asid organik yang paling banyak didapati dalam majoriti produk-produk di akhir proses fermentasi dan keseluruhan masa penyimpanan. Kandungan akhir laktik asid adalah di antara 1167 dan 8895 mgL<sup>-1</sup>. Asid organik yang paling menonjol dalam produk yang defermentasikan oleh kultur tunggal *L. delbrueckii* subsp. *bulgaricus* LB12 dan kultur campuran *L. delbrueckii* subsp. *bulgaricus* LB12 dan *K. marxianus* subsp. *marxianus* LAF4, masing-masing adalah asid asetik dan asid sitrik, diikuti oleh asid laktik.

Empat puluh sebatian mudah meruap telah dikesan pada mulanya menggunakan kromatografi gas dan digabungkan bersama spektrometer jisim 'time-of-flight' (GC-TOFMS). Beberapa kumpulan sebatian mudah meruap yang berbeza iaitu, alkohol (etanol), keton (etil asetat dan etil butirir), ester (2-butanol, aseton, 3-hidroksil-2butanol atau asetoin dan 2,3-butanedion atau diasetil) dan aldehid (asetaldehid) dipartimbangkan untuk analisis selanjutnya. Pembebasan sebatian perasa mudah meruap yang setara ke kawasan "headspace" dipantau semasa fermentasi dan penyimpanan sejuk selama 4 minggu, menggunakan kromatografi gas (GC) yang digandingkan bersama pengesan pengionan nyalaan (FID). Dalam produk fermentasi yang tidak menggunakan *K. marxianus* subsp. *marxianus* LAF4, asetoin dilihat sebagai bahan perasa mudah meruap yang paling menonjol sekali, di antara 59.5 dan 104.2 mg L<sup>-1</sup>. Walaubagaimana pun, bagi produk fermentasi yang menggunakan strain yis sebagai

inokulat utama, etanol dilihat sebagai sebatian perasa mudah meruap yang paling banyak sekali, di antara 964 dan 6522 mg L<sup>-1</sup>.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. 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 UPM or at any other institution.

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**AREZOU AGHLARA**

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