



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF SINGLE- AND MIXED-CULTURE FERMENTATION OF  
RECONSTITUTED SKIM MILK AND SUBSEQUENT COLD STORAGE  
ON MICROBIAL SURVIVAL, POST-ACIDIFICATION AND SELECTED  
METABOLITE CONCENTRATIONS**

**MARYAM MOLAVI SADR  
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**By**

**MARYAM MOLAVI SADR**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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**October 2009**



Dedicated to  
my beloved mother,  
for being a constant role model of  
commitment and unconditional love.

She has provided me with good  
example of faith in God, strength and determination.

Without her endless support, I am not sure any of  
this would have ever been possible.

I love you.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**EFFECTS OF SINGLE- AND MIXED-CULTURE FERMENTATION OF RECONSTITUTED SKIM MILK AND SUBSEQUENT COLD STORAGE ON MICROBIAL SURVIVAL, POST-ACIDIFICATION AND SELECTED METABOLITE CONCENTRATIONS**

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**MARYAM MOLAVI SADR**

**October 2009**

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

**Faculty : Food Science and Technology**

The use of *Bifidobacterium* spp. in fermented milks in order to improve the microbial balance in the human gut has become very popular in recent years. The present study was carried out to investigate the effect of culture composition and storage time on microbial growth, acidification properties and formation of metabolites in fermented milks containing *Bifidobacterium pseudocatenulatum* G4, *Streptococcus thermophilus* TH4 and *L. delbrueckii* subsp. *bulgaricus* LB12, after fermentation and during 4 weeks of storage at 4 °C. All strains used for inoculation were in pure and mixed cultures, including all the possible combinations between them. Fermentation and lag times



ranged from 266 to 743 min and 5 to 35 min in single and mixed starter cultures, respectively. The titratable acidity and pH showed similar increasing or decreasing pattern after preparation and storage of fermented milks. The least pH and highest titratable acidity during the storage period was observed with single strain of *L. delbrueckii* subsp. *bulgaricus* LB12. The highest counts of *L. delbrueckii* subsp. *bulgaricus* LB12, *S. thermophilus* TH4 and *B. pseudocatenulatum* G4 was 8.54, 8.89, and 8.52 log<sub>10</sub> cfu/mL, respectively. In single and all mixed cultures containing *B. pseudocatenulatum* G4, the viable probiotic cell count remained above 7 log<sub>10</sub> cfu/mL over 4 weeks of storage.

Carbohydrates, organic acids and amino acids were measured by high performance liquid chromatography method using refractive index, ultra violet and photodiode array detectors, respectively. Accumulation of glucose and galactose due to lactose degradation was found in the prepared fermented milks. The highest lactose breakdown during fermentation was observed in products fermented with mixed culture of *B. pseudocatenulatum* G4 and *L. delbrueckii* subsp. *bulgaricus* LB12 with decomposition of 41.71 % of the initial lactose content. The levels of certain organic compounds (citric, acetic, lactic, pyruvic and formic) were significantly ( $P < 0.05$ ) affected by the type of starter culture. The concentration of lactic acid increased after fermentation and during storage, reaching to a maximum content of 8335 mg/L in traditional yoghurt culture (LS) by Week 4. A reduction in citric acid content was observed in all the prepared fermented milks throughout the fermentation and storage. The considerable ( $P < 0.05$ ) increase in the content of essential free amino acids was observed in phenylalanine,

methionine, valine, and histidine levels. A higher concentration of free amino acids observed were in mixed culture of *B. pseudocatenulatum* G4, *L. delbrueckii* subsp. *bulgaricus* LB12 and *S. thermophilus* TH4 compared with the other cultures. The quantities of free amino acids were found to be much lower in single culture than mixed culture fermentations.

Thirty seven volatile compounds were detected using gas chromatography coupled to time-of-flight mass spectrometer (GC-TOFMS). However, only eight of them were further monitored as they represented more than 98% of headspace volatile flavor compounds of the fermented milk. Equilibrium volatile headspace concentration of acetaldehyde, acetone, ethyl acetate, 2-butanone, ethanol, 2,3-butanedione (diacetyl), ethyl butyrate and 3-hydroxy-2-butanone (acetoin) concentrations were above the taste threshold level of 0.06 mg/L in almost all the prepared fermented milks. The acetaldehyde levels in the fermented milk samples ranged from 5.29 to 14.08 mg/L, with single strain of *L. delbrueckii* subsp. *bulgaricus* LB12 producing the highest level of acetaldehyde. Equilibrium volatile headspace concentration of ethanol decreased when bifidobacteria was used in the mixed cultures. Single culture of *L. delbrueckii* subsp. *bulgaricus* LB12 was observed to be the most active strain in synthesizing acetoin.



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

**KESAN FERMENTASI KULTUR TUNGGAL DAN CAMPURAN DALAM PEMBENTUKAN SEMULA SUSU TANPA LEMAK SERTA PENYIMPANAN SEJUK BEKU SECARA BERKALA TERHADAP KETAHANAN MIKROBIAL, POS-PENGASIDAN DAN KEPEKATAN METABOLIT TERTENTU**

Oleh

**MARYAM MOLAVI SADR**

**October 2009**

**Pengerusi : Profesor Mohd Yazid Abdul Manap, PhD**

**Fakulti : Sains dan Teknologi Makanan**

Penggunaan *Bifidobacterium spp.* di dalam susu fermentasi untuk meningkatkan keseimbangan mikrob di dalam perut manusia telah menjadi sangat popular pada masa ini. Kajian telah dijalankan untuk mengenalpasti kesan komposisi kultur dan masa storan pada pertumbuhan mikro-organisma, pengasidan dan pembentukan metabolisma yang terhasil di dalam fermentasi susu yang mengandungi *Bifidobacterium pseudocatenulatum* G4, *Streptococcus thermophilus* TH4 dan *L. delbrueckii* subsp. *bulgaricus* LB12, selepas penapaian selama 4 minggu pada suhu penyimpanan 4 °C. Semua sarin telah digunakan untuk penginokulatan ke dalam kultur tulen dan campuran, termasuk keseluruhan penggabungan yang mungkin berlaku antara mikrob. Penapaian dan masa-masa susulan berkadar dari 266 hingga 743 min dalam kultur pemula dan 5 hingga 35 min dalam kultur campuran. Keasidan tertitrat dan pH menunjukkan pola pertambahan atau penurunan yang serupa selepas penyediaan dan penyimpanan



fermentasi susu. Strain tunggal *L. delbrueckii* subsp. *bulgaricus* LB12 telah menunjukkan bacaan pH terendah dan keasidan tertitrat yang tertinggi semasa tempoh penyimpanan. Bacaan tertinggi bagi *L. delbrueckii* subsp. *bulgaricus* LB12, *S. thermophilus* TH4 dan *B. pseudocatenulatum* G4 adalah 8.54, 8.89, dan 8.52 log<sub>10</sub> cfu/mL. Dalam kultur tunggal dan kesemua kultur campuran mengandungi *B. pseudocatenulatum* G4, bilangan sel probiotik kekal atas baccan 7 log<sub>10</sub> cfu/mL setelah lebih 4 minggu penyimpanan.

Karbohidrat, asid organik dan asid amino telah diukur dengan menggunakan kaedah kromatografi cecair prestasi tinggi menggunakan pengesanan indeks biasan, ultra ungu dan tatasusun fotodiod. Pengumpulan glukosa dan galaktosa akibat daripada degradasi laktosa telah ditemui dalam fermentasi susu. degradasi laktosa tertinggi semasa fermentasi telah diperhatikan dalam produk penapaian dengan kultur campuran *B. pseudocatenulatum* G4 dan *L. delbrueckii* subsp. *bulgaricus* LB12 dengan penguraian sebanyak 41.71 % pada permulaan kandungan laktosa. Tahap amaun bagi sebatian-sebatian organik tertentu (sitrik, asetik, laktik, piruvik dan formik) adalah ternyata ( $P < 0.05$ ) terjejas oleh kultur jenis pemula. Tahap kepekatan asid laktik bertambah selepas penapaian dan semasa penyimpanan, sehingga mencapai kandungan maksimum 8335 mg/L dalam kultur yogurt tradisional (LS) dalam minggu ke-4. Penurunan dalam kandungan asid sitrik telah diperhatikan di dalam semua susu fermentasi sepanjang penapaian dan penyimpanan. Peningkatan ( $P < 0.05$ ) yang bererti pada kandungan asid amino bebas penting telah diperhatikan dalam fenilalanina, metionina, valina, dan histidina. Kepekatan asid amino bebas yang tinggi telah didapati dalam strain tunggal *B. pseudocatenulatum* G4, *L. delbrueckii* subsp. *bulgaricus* LB12 dan *S. thermophilus* TH4



bebanding kultur-kultur lain. Kuantiti asid amino bebas dalam kultur asli didapati kurang berbanding kultur bercampur.

Tiga puluh tujuh sebatian meruap telah dikesan menggunakan gas kromatografi yang dipasang dengan spektrometer jisim 'time-of-flight' (GC-TOFMS). Namun begitu, hanya lapan sebatian yang terus diawasi kerana menunjukkan lebih dari 98% ruang tutup pemeruapan sebatian dalam fermentasi susu. Keseimbangan kepekatan ruang tutup pemeruapan bagi asetaldehid, aseton, etil asetat, 2-butanon, etanol, 2,3- butanadion (diasetil), etil butirate dan 3-hidroksi-2butanon (asetoin) adalah berada atas tahap ambang rasa 0.06 mg/L dalam semua susu fermentasi. Tahap asetaldehid di dalam sampel fermentasi susu adalah berkadar dari 5.29 hingga 14.08 mg/L, dengan menunjukkan strain tunggal *L. delbrueckii* subsp. *bulgaricus* LB12 telah menghasilkan tahap asetaldehid tertinggi. Keseimbangan kepekatan ruang tutup pemeruapan etanol berkurang apabila bifidobacteria telah digunakan dalam kultur campuran. Kultur tunggal *L. delbrueckii* subsp. *bulgaricus* LB12 telah didapati sebagai strain paling aktif dalam pensintesisan asetoin.



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I certify that an Examination Committee met on October 01, 2009 to conduct the final examination of Maryam Molavi Sadr on his Master of Science thesis entitled “Effect of Single and Mixed Culture Fermentation of Reconstituted Skim Milk and Subsequent Cold Storage on Microbial Survival, Post-acidification and Selected Metabolite Concentrations” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Master of Science degree.

Members of the Examination Committee are as Follows:

**Jamilah Bakar, PhD**

Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Chairman)

**Hasanah Mohd Ghazali, PhD**

Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Internal Examiner)

**Lasekan Olusegun, PhD**

Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Internal Examiner)

**Zaiton Binti Hasan, PhD**

Associate Professor  
Faculty of Science and Technology  
Universiti Sains Islam Malaysia  
(External Examiner)

---

**Bujang Kim Huat, PhD**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:



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

**Mohd Yazid Abdul Manap, PhD**

Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Chairman)

**Nazamid Saari, PhD**

Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Member)

**Sharifah Kharidah Syed Muhamad, PhD**

Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Member)

**Syed Hamed Mirhosseini, PhD**

Lecturer  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Member)

---

**HASANAH MOHD GHAZALI, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 11 February 2010



## **DECLARATION**

I declare that the thesis is my original work except for quotations and citation 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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**MARYAM MOLAVI SADR**

Date: 22 February 2010



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## LIST OF ABBREVIATIONS

$\mu\text{L}$	Micro liter
$^{\circ}\text{C}$	degree Celsius
AOAC	Association of Official Analytical Chemists
<i>B.</i>	<i>Bifidobacterium</i>
CAR	carboxen
cfu	Colony forming unit
$\text{CO}_2$	Carbon dioxide
d	Day
e.g.	<i>Example gratia</i> ( for example)
<i>et al.</i>	Etcetera (and company)
FID	Flame ionization detector
GC	Gas chromatography
GIT	Gastrointestinal tract
h	Hour
$\text{H}^+$	Hydrogen ion
$\text{H}_2\text{SO}_4$	Sulphuric acid
HCL	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HS	Head space
IDF	International Dairy Federation
<i>L.</i>	<i>Lactobacillus</i>
LAB	Lactic Acid Bacteria
LOD	Limit of detection
Log	Logarithm





LOQ	Limit of quantitation
min	minute
mL	Mililiter
MRS	de Man Rogosa Sharpe Medium
NaOH	Sodium Hydroxide
NNLP	nalidixic acid, neomycine sulfate, lithium chloride and paromomycine sulfate
PDMS	Polydimethylsiloxane
RI	Refractive index
rpm	Revolutions per minute
<i>S.</i>	<i>Streptococcus</i>
S.D.	Standard Deviation
SPME	Solid Phase Microextraction
ssp.	Species
Subsp.	Subspecies
TOFMS	Time-of-flight mass spectrometry
UV	Ultra Violet
WHO	World Health Organization

