

**Inclusion Of *Lycium barbarum*, *Psidium guajava*, *Momordica grosvenori* And *Garcinia mangostana* In Yogurt And Their Effects  
On Fermentation And Exopolysaccharide-Production**

**ELHAM BAGHERI**

**DISSERTATION SUBMITED IN FULFILMENT OF  
THE REQUIREMENT FOR THE MASTER  
OF BIOTECHNOLOGY**

**INSTITUTE OF BIOLOGICAL SCIENCES  
FACULTY OF SCIENCE  
UNIVERSITY OF MALAYA  
50603 KUALA LUMPUR**

**2012**

## ABSTRACT

Yogurt is popularly consumed because of its high digestibility, refreshing organoleptic properties and the healthy bacteria it contains. Milk incubated with starter culture (SC) at 41°C in the presence of plants (*Momordica grosvenori*, *Psidium guajava*, *Lycium barbarum* or *Garcinia mangostana*) water extract were studied to examine the effects on acidification, microbial growth, gelation and exopolysaccharide (EPS) content. Initial pH values (5.8-6.2) of the mixture of milk, plant water extract and SC were not different ( $p>0.05$ ) from control (6.20; mixture of milk and SC). The rates of pH reduction for all herbal-yogurts (-0.40 pH unit/ hour), except *G. mangostana* and *M. grosvenori* (-0.33 and -0.32 respectively pH unit/hr) was similar to control (-0.43 pH unit/ hr) during the first 4 hours of incubation. Microbial mass increased for both *Lactobacillus* spp. and *Streptococcus thermophilus* in the presence of plant water extracts at 0.75%, 1.5% and 3% (w/v). *Lactobacillus* spp. counts in *L. barbarum*-, *P. guajava*- and *M. grosvenori*- yogurts (1.52, 1.32 and  $1.23 \times 10^6$  cfu/ ml, respectively,  $p>0.05$ ) with the exception of *G. mangostana*- yogurt ( $0.69 \times 10^6$  cfu/ml;  $p>0.05$ ) were higher compared to control ( $1.01 \times 10^6$  cfu/ ml). The *S. thermophilus* counts in *L. barbarum*-, *P. guajava*- and *M. grosvenori*- yogurts (785, 1045 and  $805 \times 10^6$  cfu/ ml respectively,  $p>0.05$ ) with the exception of *G. mangostana*- yogurt ( $610 \times 10^6$  cfu/ml;  $p>0.05$ ) were higher than that in control ( $615 \times 10^6$  cfu/ ml). The EPS content in *G. mangostana*-yogurt (113.1  $\mu\text{g}$ / ml,  $p>0.05$ ) was lower whereas *L. barbarum*-, *P. guajava*- and *M. grosvenori*- yogurts (181.4, 258.7 and 183.9  $\mu\text{g}$ / ml respectively,  $p<0.05$  for *P. guajava* -yogurt only) were higher than control (132  $\mu\text{g}$ / ml). Syneresis (S) was increased in *P. guajava*- but decreased in *G. mangostana*-yogurts (3.61 and 2.90% respectively;  $p>0.05$ ) whereas water holding capacity (WHC) was reduced in *P. guajava* (22.8%;  $p>0.05$ ) but increased in *G.*

*mangostana* (28.1%;  $p<0.05$ ) compared to control ( $S= 3.23\%$ , WHC= 24.1%). In summary, the presence of plant water extracts affected yogurt fermentation by modifying of yogurt bacteria growth and metabolism which resulted in the enhancement of acidification of yogurt.

## ABSTRAK

Yogurt popular dimakan kerana penghadaman yang tinggi, sifat-sifat organoleptik yang menyegarkan dan bakteria sihat yang dikandunginya. Susu dieram dengan kultur pemula (SC) pada  $41^{\circ}\text{C}$  di dalam kehadiran ekstrak air tumbuh-tumbuhan (*Momordica grosvenori*, *Psidium guajava*, *Lycium barbarum* atau *Garcinia mangostana*) telah dikaji bagi meneliti kesan pada pengasidan, pertumbuhan mikrob, pengentalan dan kandungan eksopolysakarida (EPS). Nilai-nilai awal pH (5.8-6.2) campuran susu, ekstrak tumbuhan air dan SC tidak berbeza ( $p > 0.05$ ) daripada kontrol (6.20; campuran susu dan SC). Kadar pengurangan pH untuk semua yogurt herba (-0,40 pH unit/ jam), kecuali *G. mangostana* dan *M. grosvenori* (-0,33 -0,32 masing-masing pH unit/ jam) adalah sama untuk kontrol (-0,43 pH unit/ jam) dalam tempoh 4 jam pertama pengeraman. Jisim mikrob meningkat bagi kedua-dua *Lactobacillus spp.* dan *Streptococcus thermophilus* dalam kehadiran ekstrak tumbuhan air 0.75%, 1.5% dan 3% (w / v). Kiraan *Lactobacilus spp.* dalam yogurt *L. barbarum*, *P. guajava* dan *M. grosvenori* (1.52, 1.32 dan  $1.23 \times 10^6$  CFU / ml masing-masing,  $p > 0.05$ ) kecuali yogurt *G. mangostana* ( $0.69 \times 10^6$  CFU / ml;  $p > 0.05$ ) adalah lebih tinggi berbanding kontrol ( $1.01 \times 10^6$  CFU / ml). Kiraan *S. thermophilus* dalam yogurt *L. barbarum*, *P. guajava* dan *M. grosvenori* (785, 1045 dan  $805 \times 10^6$  CFU / ml masing-masing,  $p > 0.05$ ) kecuali yogurt *G. mangostana* ( $610 \times 10^6$  CFU / ml;  $p > 0.05$ ) adalah lebih tinggi berbanding kontrol ( $615 \times 10^6$  CFU / ml). Kandungan EPS dalam *G. mangostana*-yogurt (113.1  $\mu\text{g}$  / ml,  $p > 0.05$ ) adalah lebih rendah manakala *L. barbarum*, *P. guajava* dan *M. grosvenori*-yogurt (181.4, 258.7 dan 183.9  $\mu\text{g}$  / ml masing-masing,  $p < 0.05$  untuk *L. barbarum*-yogurt sahaja) adalah lebih tinggi daripada kawalan (132  $\mu\text{g}$  / ml). Syneresis *P. guajava* meningkat tetapi menurun bagi *G. mangostana*-yogurt (3.61 dan 2.90% masing-masing;  $p > 0.05$ ) manakala kapasiti menahan air (WHC) telah berkurangan bagi *P. guajava* (22.8%;  $p > 0.05$ ) tetapi meningkat bagi *G. mangostana* (28.1%;  $p < 0.05$ )

berbanding kontrol ( $S = 3.23\%$ ,  $WHC = 24.1\%$ ). Kesimpulannya, kehadiran ekstrak air mempengaruhi fermentasi yogurt melalui pengubahsuaian pertumbuhan dan metabolisma yogurt bakteria yang menyebabkan peningkatan pengasidan yogurt.

## **ACKNOWLEDGEMENT**

I would like to express my gratitude to all those who gave me the possibility to complete this project. First of all I want to thank Allah for giving me this opportunity to do my masters and for blessing me with the courage and strength. My special thanks are also dedicated to my supervisor, Associate Professor Dr. Ahmad Salihin Baba, who has been very helpful providing me with the guidance to completing this project.

Besides, I also would like to thank my research partner, Ms. Chin, who had been through a rough and enduring process with me until the end of this project.

I would like to thank Cik Hazwani, Cik Zanariah and other lab assistants for their advice to use the equipments in the laboratories.

Finally, special thanks to my parents and friends for their continuous encouragement, moral support and understanding towards me, which did help me overcome difficulties that I encountered while accomplishing this project.

ELHAM BAGHERI

SEPTEMBER, 2011

## **CONTENTS**

<b>ABSTRACT.....</b>	II
<b>ABSTRAK.....</b>	IV
<b>AKNOWLEDGEMENT.....</b>	VI
<b>LIST OF ABBREVIATIONS.....</b>	XIII
<b>LIST OF FIGURES.....</b>	XV
<b>LIST OF TABLES.....</b>	XVII
<b>LIST OF PICTURES.....</b>	XVIII
<b>CHAPTER1: INTRODUCTION.....</b>	1
<b>CHAPTER 2: LITERATURE REVIEW.....</b>	4
2.1 <i>Momordica grosvenori</i> .....	5
2.2 <i>Psidium guajava</i> .....	7
2.2.1 Traditional medicine usage of <i>P. guajava</i> .....	7
2.2.2 Phytochemistry of <i>P. guajava</i> leaves.....	7
2.2.3 Biological activity of <i>P. guajava</i> .....	8
2.2.3.1 Anti-diarrheal.....	8
2.2.3.2 Anti-bacterial effects.....	9
2.2.3.3 Anti-tussive effects.....	9
2.2.3.4 Anti-oxidant effects.....	9
2.2.3.5 Anticancer/antitumour effects.....	10
2.2.3.6 Anti-inflammatory/analgesic.....	10

2.2.3.7 Cardiovascular, hypotensive effects.....	11
2.2.3.8 Anti-diabetic effect.....	11
2.3 <i>Lycium barbarum</i> .....	12
2.3.1 Cardiovascular benefits of <i>L. barbarum</i> .....	12
2.3.2 Effect of <i>L. barbarum</i> on diabetes.....	13
2.3.3 Eye health benefits.....	13
2.3.4 Anti-oxidant activity.....	14
2.3.5 Anti-inflammatory.....	14
2.3.6 Anti-cancer.....	14
2.3.7 Lycium barbarum polysaccharide.....	14
2.4 <i>Garcinia mangostana</i> .....	15
2.4.1 Health Benefits of <i>G. mangostana</i> .....	16
2.4.2 Xanthones activity.....	16
2.4.3 Anticancer activity.....	18
2.4.4 Anti-inflammatory activity.....	19
2.4.5 Antibacterial activity.....	21
2.5 Exo-polysaccharide (EPS).....	21
2.5.1 EPS structure.....	22
2.5.2 Effect of carbohydrate and protein on EPS.....	23
2.5.3 Ropy and capsular EPS.....	24
2.5.4 EPS effects and health benefits.....	24

2.6 <i>Streptococcus thermophilus</i> .....	25
2.6.1 Role of <i>S. thermophilus</i> in dairy products.....	25
2.6.2 EPS production by <i>S. thermophilus</i> .....	26
2.6.3 Probiotic strains of <i>S. thermophilus</i> .....	26
2.7 <i>Lactobacillus delbrueckii</i> .....	27
2.8 Colony forming unit (CFU).....	28
2.9 Syneresis.....	29
2.9.1 Methods to determination of whey syneresis.....	31
2.9.1.1 Drainage method.....	31
2.9.1.2 Centrifugation method.....	31
2.10 Total solid.....	32
2.11 Yogurt.....	34
2.12 Bio-yogurt.....	35
<b>CHAPTER 3: MATERIALS AND METHODS.....</b>	<b>36</b>
3.1 MATERIALS	
3.1.1 Starter culture.....	36
3.1.2 Milk.....	36

## 3.2 METHODS

3.2.1 Plant materials .....	36
3.2.2 Preparation of plant water extract.....	37
3.2.3 Yogurt.....	37
3.2.3.1 Starter culture preparation.....	37
3.2.3.2 Yogurt preparation.....	38
3.2.4 pH measurement and Total Titratable Acid (TTA %).....	38
3.2.5 OPTICAL DENSITY (OD).....	39
3.2.5.1 Preparation of yogurt bacteria suspension.....	39
3.2.5.2 Assessment of yogurt bacteria growth in suspension by measuring OD <sub>600</sub> .....	40
3.2.6 Enumeration of probiotic bacteria (CFU).....	41
3.2.6.1 MRS agar preparation.....	41
3.2.6.2 M17 agar preparation.....	41
3.2.6.3 Preparation of Peptone Water Buffer.....	42
3.2.6.4 Pour plate method using MRS media.....	42
3.2.6.5 Spread plate method using M17 media.....	43
3.2.7 Exopolysaccharide (EPS) isolation.....	43
3.2.7.1 Phenol-sulfuric method.....	44
3.2.8 Syneresis.....	45
3.2.9 WATER HOLDING CAPACITY (WHC).....	45
3.2.10 TOTAL SOLID.....	45
3.2.11 STATISTICAL ANALYSIS.....	46

<b>CHAPTER 4: RESULTS.....</b>	47
<b>4.0 RESULTS.....</b>	47
<b>4.1 Effects of <i>M. grosvenori-</i>, <i>P. guajava-</i>, <i>L. barbarum-</i> and <i>G.mangostana</i>-yogurts on changes of pH and TTA in yogurt.....</b>	47
<b>4.2 Growth of bacteria in medium suspension.....</b>	49
<b>4.2.1 Effects of plant water extract on <i>Lactobacillus spp.</i> in MRS growth medium.....</b>	49
<b>4.2.2 Effects of plant water extract on <i>S. thermophilus</i> growth in peptone buffer.....</b>	52
<b>4.3 Comparison of <i>Lactobacillus spp.</i> and <i>S. thermophilus</i> density at 3% plant water extract.....</b>	55
<b>4.4 Enumeration of yogurt bacteria in yogurts.....</b>	57
<b>4.5 Exopolysaccharide content in yogurts.....</b>	58
<b>4.6 Physicochemical properties of plain- and herbal-yogurts.....</b>	59
<b>4.7 Correlation between acidification changes and EPS content with Syneresis and WHC.....</b>	59
<b>CHAPTER 5: DISCUSSION.....</b>	60
<b>5.0 DISCUSSION.....</b>	60
<b>5.1 Effects of <i>M. grosvenori</i>, <i>P. guajava</i>, <i>L. barbarum</i> and <i>G. mangostana</i> on the acidification of yogurt.....</b>	60
<b>5.2 Effects of plants water extract on the growth of <i>Lactobacillus ssp.</i> and <i>S. thermophilus</i>.....</b>	61

5.2.1 Measurement of yogurt bacteria cell mass by turbidity measurement...	61
5.2.2 Measurement of yogurt bacteria cell numbers by indirect viable cell count.....	62
5.3 Effects of plant water extracts on EPS production by yogurt bacteria.....	64
5.4 Effects of plant extracts on physicochemical properties of yogurt.....	66
<b>CONCLUSION.....</b>	<b>70</b>
<b>REFERENCES.....</b>	<b>71</b>
<b>APPENDICES.....</b>	<b>109</b>

## **LIST OF ABBREVIATIONS**

% = percentage

°C = Degree Celcius

& = and

etc = et cetera

i.e = for example

vs = versus

min = minute(s)

mg = milligram(s)

L = liter (s)

ml = milliliter(s)

µl = microliter(s)

g = gram (s)

µg = microgram(s)

m = meter

nm = nanometer

Da = Dalton

Wt = weight

V = volume (s)

N = normality (s)

Min = minute (s)

Hr = hour (s)

EPS = exopolysaccharides

LAB = lactic acid bacteria

dH<sub>2</sub>O = distilled water

TCA = trichloroacetic acid

HCl = hydrochloric acid

NaOH = sodium hydroxide

TTA = Titratable Acidity

WHC = water holding capacity

CFU = Colony forming unit

OD = optical density

Tr cell = T regulatory cell

Th cell = T helper cell

TNF = Tumor necrosis factor

IL-6 = Interleukin-6

SD rats = Sprague Dawley rats

SSUV = solar-simulated Ultraviolet

GML = Garcinia mangostana Linn

DMH = 1,2-dimethylhydrazine

*S. thermophilus* = *Streptococcus thermophilus*

*G. mangostana* = *Garcinia mangostana*

*L. barbarum* = *Lycium barbarum*

*M. grosvenori* = *Momordica grosvenori*

*P. guajava* = *Psidium guajava*

## LIST OF FIGURES

Figure 2.1: Structures of sweet glycosides isolated from the fruits of <i>M. grosvenori</i> .....	5
Figure 2.2: Xanthone nucleus with IUPAC numbers of carbons and chemical structure of the most studied xanthones.....	17
Figure 2.3: Exopolysaccharide of <i>S. thermophilus</i> .....	22
Figure 2.4: Exopolysaccharide of <i>L. delberueckii bulgaricus</i> .....	23
Figure 2.5: Structure of exopolysaccharide was produced by <i>Lactobacillus acidophilus</i> .....	23
Figure 4.1: pH changes in yogurts during the first 4 hours of yogurt fermentation at 41°C.....	47
Figure 4.2: TA% changes in yogurts during the first 4 hours of fermentation at 41°C.....	48
Figure 4.3: Effects of different concentration of <i>L. barbarum</i> on the changes in optical density (OD) of <i>Lactobacillus spp.</i> growing in MRS broth.....	50
Figure 4.4: Effects of different concentration of <i>P. guajava</i> on the changes in optical density (OD) of <i>Lactobacillus spp.</i> growing in MRS broth.....	51
Figure 4.5: Effects of different concentration of <i>M. grosvenori</i> on the changes in optical density (OD) of <i>Lactobacillus spp.</i> growing in MRS broth.....	51
Figure 4.6: Effects of different concentration of <i>G. mangostana</i> on the changes in optical density (OD) of <i>Lactobacillus spp.</i> growing in MRS broth.....	52
Figure 4.7: Effects of different concentration of <i>L. barbarum</i> on the changes in optical density (OD) of <i>S. thermophilus</i> growing in peptone buffer.....	53

Figure 4.8: Effects of different concentration of <i>P. guajava</i> water extract on the changes in optical density (OD) of <i>S. thermophilus</i> growing in peptone buffer.....	53
Figure 4.9: Effects of different concentration of <i>M. grosvenori</i> on the changes in optical density (OD) of <i>S. thermophilus</i> growing in peptone buffer.....	54
Figure 4.10: Effects of different concentration of <i>G. mangostana</i> on the changes in optical density (OD) of <i>S. thermophilus</i> growing in peptone buffer.....	55
Figure 4.11: Changes in the optical density (OD) ratio with time for <i>Lactobacillus spp.</i> grown in 3% plant water extracts in relation to their respective control.....	56
Figure 4.12: Changes in the density (OD) ratio with time for <i>S. thermophilus</i> grown in 3% plant water extracts in relation to their respective control.....	57

## LIST OF TABLES

Table 2.1: Antitumoral properties of xanthones isolated from <i>Garcinia mangostana</i> .....	18
Table 2.2: Anti-inflammatory effects of GML.....	19 & 20
Table 4.1: Colony forming unit (CFU) of <i>Lactobacillus spp.</i> and <i>S. thermophilus</i> from plain- and herbal- yogurts.....	58
Table 4.2: Purified exopolysaccharide from plain- and herbal-yogurts.....	58
Table 4.3: Syneresis, water holding capacity (WHC) and total solids of plain- and herbal-yogurts.....	59
Table 4.4: Effects of plant water extracts on the physicochemical properties and EPS production .....	59
Table 5.1. The acidification of yogurt and the yogurt bacteria counts in herbal- and plain-yogurts at pH=4.5.....	63
Table 5.2. Relationship between acidification and EPS content on syneresis and water holding capacity of yogurt.....	68

## **LIST OF PICTURES**

Picture 2.1: <i>M. grosvenori</i> fruits.....	6
Picture 2.2: Leaves and fruit of <i>P. guajava</i> .....	11
Picture 2.3: <i>L. barbarum</i> fruits.....	15
Picture 2.4: <i>G. mangostana</i> fruit.....	21
Picture 2.5: Form of <i>S. thermophilus</i> in yogurt.....	27
Picture 2.6: <i>Lactobacillus spp.</i> forms in yogurt.....	28