ABSTRACT

Yogurt is generally recognized as a healthy and multifunctional food. It is a coagulated milk product obtained from the lactic acid fermentation by the action of Lactobacillus bulgaricus and Streptococcus thermophilus. In this present studies, dragon fruit Hylocereus polyrhizus and Hylocereus undatus extract was added in milk and their effects during yogurt fermentation were investigated. The changes in pH and Titratable Acidity (TA) were measured during fermentation and after 14 days of storage at 4°C. The yogurt extracts were subsequently analysed for their syneresis, proteolysis, peptide content, total phenolic content, antioxidant activities, inhibitory activities on α -amylase and α -glucosidase, presence of exopolysaccharides (EPS) and organoleptic properties. pH values for both dragon fruits yogurt showed significant reduction while TA showed higher percentages compared to plain-yogurts during fermentation and after 14 days of storage. Syneresis in yogurt (52.93%) also has been increased (p>0.05) with the addition of *H. polyrhizus* (57. 19-63.16%) and *H. undatus* (57.76-70.32%) compared to control (52.93%). The total phenolic content (TPC) in both dragon fruits yogurt (36.44-64.43ugGAE/ml) showed a greater increase (p<0.05) than plain yogurt (20.25ugGAE/ml). The addition of dragon fruits also increased (24.97-45.74%; p<0.05) antioxidant capacity compared to plain yogurt (19.16%). The peptides content in both dragon fruit yogurts were not different (p>0.05) from plain yogurt. Higher inhibitory activities (p<0.05) on α -amylase (17.30-52.20%), α -glucosidase (8.70-34.02%) and EPS production (214-738mg/L) in dragon fruit yogurts than in plain-yogurts were recorded (19.70%, 9.21%, and 181mg/L respectively). H. polyrhizus showed the highest score for visual appearance (7.77), aroma (5.9) and sweetness (4.22) while plain yogurt showed highest scores for body texture (6.81), sourness (7.13) and overall taste (5.45). In conclusion, the addition of *H. polyrhizus* and *H.* undatus in yogurt gave enhanced effects on physicochemical, therapeutic properties, production of EPS and organoleptic properties in yogurt.

ABSTRAK

Yogurt dikenali umum sebagai makanan yang berkhasiat yang mana ianya merupakan makanan yang terhasil daripada proses fermentasi susu oleh Lactobacillus bulgaricus dan Streptococcus thermophilus. Dalam kajian ini, ekstrak buah naga Hylocereus polyrhizus dan Hylocereus undatus telah dicampur semasa proses fermentasi susu dan kesan-kesanya turut dikaji. Perubahan daripada segi bacaan pH dan TA (titratble acid) telah diperhati dan diukur semasa proses fermentasi susu dan juga selepas 14 hari penyimpanan dibawah suhu 4°C. Selepas itu analisa berkaitan syneresis, proteolysis, kandungan peptida, jumlah kandungan sebatian berfenol, aktiviti antioksidan, penyekatan aktiviti α -amylase and α -glucosidase, penghasilan eksopolisakarida (EPS) dan ciri-ciri organoleptic dijalankan. Yogurt kedua-dua jenis buah naga mencatatkan peningkatan nilai pH dan penurunan nilai TA berbanding kawalan (plain yogurt). Syneresis yogurt (52.93%) bertambah (p<0.05) dengan penambahan kedua-dua buah naga (57.76-70.32%) berbanding kawalan (52.93%). Jumlah kandungan sebatian berfenol (36.44-64.43ugGAE/ml) juga menunjukkan peningkatan (p<0.05) berbanding kawalan (20.25ugGAE/ml). Peningkatan yang signifikan (p<0.05) juga dicatatkan untuk aktiviti antioksida dalam yogurt buah naga (24.97-45.74%) berbanding kawalan (19.16%). Walaubagaimanapun, kandungan peptida yogurt tidak berubah secara signifikan (p>0.05). Penambahan buah naga ke dalam yogurt memberikan peningkatan dalam peratus penyekatan (p<0.05) terhadap aktiviti enzim α -amylase (17.30-52.20%) dan α-glucosidase (8.70-34.02%) serta penghasilan EPS (214-738mg/L) berbanding kawalan (19.70%, 9.21%, and 181mg/L). yogurt buah naga merah (H. polyrhizus) mencatatkan nilai yang tinggi untuk ciri penampilan visual (7.77), aroma (5.9) dan tahap kemanisan (4.22) manakala control mencatatkan nilai tertinggi untuk ciri tekstur yogurt (6.81), tahap kemasaman (7.13) dan keseluruhan rasa (5.45). Kesimpulannnya, penambahan kedua-dua buah naga memberikan kesan yang positif dari segi fisikokemikal, ciri-ciri therapeutik, penghasilan eksopolisakarida dan juga ciri-ciri organoleptic dalam yogurt.

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim, I would like to express my gratitude to all those who have assisted me to complete this project. First of all, Alhamdulillah, Allah has given me this big opportunity to complete my studies and blesses me with the courage and strength. My special thanks are also dedicated to my beloved parents and siblings who always support me with full encouragement. Furthermore, I would like to thank my supervisor, Associate Professor Dr. Ahmad Salihin Baba who is very helpful in providing me with a good supervision and guidance towards in the execution of experiments and writing of this thesis.

I am indebted to my labmates Shamrul, Sara, Shabboo and Amal, for their excellent assistance, help, advice and guidance throughout the period. I would like to thank Mr. Asokan, Cik Hazwani, Miss Ng, Encik Izuan, Mr. Shanmugam, Pn. Maziah, and Pn. Zanariah for their guidance and assistance regarding the use of lab equipments.

Last but not least, I would like to express my sincere and heartiest thanks to all persons who helped me during the completion of my project and thesis but are not mentioned here. I am very grateful for all your help. Thank you. May Allah blesses you all.

CONTENTS

ACKNOWLEDGEMENT	i
ABSTRACT	ii
ABSTRAK	iii
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	3
2.1 Milk	3
2.2 Fermentation of milk	3
2.3 Fermented milk	3
2.4 Yogurt	4
2.4.1 Yogurt as a functional food	5
2.4.1(i) Control of intestinal infections	5
2.4.1(ii) Reducing lactose intolerance	6
2.4.1(iii) Discouraging vaginal infections	6
2.4.1(iv) Reducing serum cholesterol level	7
2.4.1(v) Anticarcinogenic activity	7
2.5 Probiotics	8
2.6 Extracellular polysaccharides (Exopolysaccharides)	11
2.6.1 Exopolysaccharides in yogurt	12
2.7 Syneresis	14
2.8 Proteolysis	15
2.8.1 O-phthaldehyde in the evaluation of proteolysis	16
2.9 α -Amylase and α -glucosidase enzyme	18
2.9.1 Inhibition of pancreatic α -amylase and intestinal α -glucosidase	18
2.10 Antioxidant activity	20
2.10.1 Oxidative stress and antioxidants	20
2.10.2 Determination of Antioxidant Capacity	21
2.11 Phenolic phytochemicals	22
2.14 Dragon fruit (Pitaya)	24

iv

3.0 MATERIALS AND METHODS

3.1 Materials	27
3.1.1 Fruits	27
3.1.2 Yogurt bacteria	27
3.2 Methods	27
3.2.1 Apparatus	27
3.2.2 Preparation of starter culture.	28
3.2.3 Preparation of yogurt	28
3.2.4 Preparation of yogurt water extract	28
3.2.5 pH and titratable acid measurement	29
3.2.6 Syneresis measurement	30
3.2.7 The total phenolic content assay	30
3.2.8 Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhib	oition
assay	30
3.2.9 Proteolysis assessment of yogurt	31
3.2.9.1 Preparation of OPA reagent	31
3.2.9.2 OPA assay	31
3.2.10 Inhibition of α -glucosidase activity	32
3.2.10.1 Preparation of reagents	
(i) α-Glucosidase enzyme solution	32
(ii) 0.1 M potassium phosphate buffer (pH6.90)	32
$3.2.10.2 \alpha$ -Glucosidase inhibition assay	32
3.2.10.3 Preliminary studies on the inhibition of enzymes by yogurt water e	xtract 33
(i) The optimum enzyme concentration and incubation time	33
(ii) The optimum dilution factor of yogurt extracts	34
3.2.11 α-amylase inhibition assay	34

27

- (iii) Starch solution
- (iv) Dinitrosalicyclic acid (DNSA) reagent
- 3.2.11.2 α-Amylase inhibition assay35
- 3.2.11.3 Preliminary experiments on optimisation of α -amylase assay conditions 36
 - (i) The optimal composition of DNSA reagent
 - (ii) The optimum dilution factor of yogurt extracts
 - (iii) The optimum incubation time
 - (iv) The optimum boiling time
- 3.2.12 Production of exopolysaccharides in dragon fruits yogurts383.2.12.1 Extraction, purification and quantification of exopolysaccharides38
- 3.2.12.2 Phenol-sulphuric acid assay3.2.13 Sensory evaluation of yogurts
- 3.2.14 Statistical analysis40

4.0 RESULTS

4.1 DPPH inhibition: Preliminary experiment	41
4.2 OPA assay: Preliminary experiment	43
4.3 α-Amylase Inhibition assay	44
4.3.1 The optimum composition of DNSA reagent	44
4.3.2 The optimum dilution factor of yogurt water extracts	46
4.3.3 The optimum incubation time after the addition of substrate	47
4.3.4 The optimum boiling time after the addition of DNSA reagent	48
4.4 α -Glucosidase inhibition assay: Preliminary experiments	49
4.4.1 The optimum enzyme concentration and incubation time	49

38

39

41

4.4.2 The optimum dilution factor yogurt water extracts	50
4.5 Experiments on plain and dragon fruit-yogurt	51
4.5.1 Reduction of yogurt pH during fermentation	51
4.5.2 Titratable acid	54
4.5.3 Syneresis measurement	56
4.5.4 Total phenolic content (TPC) in dragon fruit yogurts	57
4.5.5 Antioxidant activity of dragon fruit yogurts	58
4.5.6 Effects of dragon fruit extract on proteolysis of milk protein	59
4.5.7 α -Glucosidase inhibition assay	60
4.5.8 α-Amylase inhibition assay	62
4.5.9 Sensory evaluation	64
4.5.10 Exopolysaccharides productions	65
5.0 DISCUSSION	66
5.1 Effects of dragon fruits on the changes of physicochemical properties of yogurt	66
5.1.1 pH of yogurt	66
5.1.2 Titratable acid of yogurt	67
5.1.3 Syneresis of dragon fruits yogurts	68
5.2 Effects of dragon fruits on therapeutical properties of yogurt	69
5.2.1 Total phenolic content (TPC)	69
5.2.2 Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical	
inhibition assay	70
5.2.3 α-Glucosidase inhibitory potentials of yogurt	72

vii

5.2.4 α-Amylase inhibitory potentials of yogurt	73
5.3 Effects of dragon fruits on proteolysis of yogurt	74
5.4. Effects of dragon fruits on exopolysaccharides production of yogurt	75
5.5. Effects of dragon fruits on organoleptic properties of yogurt	76
CONCLUSIONS	77
REFERENCES	78
APPENDICES	95

LIST OF FIGURES

Figure 2.0 (a) Hylocereus undatus	25
Figure 2.0 (b) Hylocereus polyrhizus	25
Figure 2.1 Sugar composition of <i>Hylocereus undatus</i> and <i>Hylocereus polyrhizus</i> extracts analysed by Wichienchot <i>et al.</i> , (2010)	26
Figure 4.1 The changes in absorbance readings at 517nm after the addition of yogurt water extracts in 30µM DPPH reagent	41
Figure 4.2 The changes in absorbance readings at 517nm after the addition of yogurt water extracts in 60µM DPPH reagent	41
Figure 4.3 The changes in absorbance readings at 517nm after the addition of yogurt water extracts in 120µM DPPH reagent	42
Figure 4.4 The changes of absorbance readings with time after the mixing of yogurt water extracts with OPA reagent	43
Figure 4.5 The α -amylase inhibitory activity of yogurt water extracts at different concentration of DNSA	45
Figure 4.6 Absorbance readings of yogurt water extracts at 540 nm with and without phenol and bisulfite in α -amylase inhibition assay	45
Figure 4.7 The α -amylase inhibitory activity of yogurt water extracts at different dilution factors	46
Figure 4.8 The α -amylase inhibitory activity of yogurt extracts at different incubation time	47
Figure 4.9 The α -amylase inhibitory activity of yogurt water extracts at different boiling time	e 48
Figure 4.10 The α -glucosidase activity of control sample (without inhibitor) with different dilution factors of enzyme solution	49
Figure 4.11 The α -glucosidase activities of plain yogurt water extract at different dilution factors	50
Figure 4.12 Changes in pH during yogurt fermentation in the absence or presence of dragon fruit (10, 20 or 30% w/w)	52
Figure 4.13 Changes in titratable acid (lactic acid percentage) during fermentation of yogurt	55
Figure 4.14 Changes in syneresis in yogurt in the presence of dragon fruit.	56

Figure 4.15 Total phenolic content in red and white dragon fruit yogurt	57
Figure 4.16 DPPH antioxidant activities in yogurts. Values represent mean of 3 independent experiments (n=3) and bar represent standard error on the means (SEM)	58
Figure 4.17 Proteolysis in dragon fruit yogurts as determined using O-phthaldehyde assay	59
Figure 4.18 Inhibition on α -glucosidase activity by dragon fruit yogurts extracts	60
Figure 4.19 IC ₅₀ values for α -glucosidase inhibition by dragon fruit yogurts	61
Figure 4.20 Inhibition on α -amylase activity by dragon fruit yogurts extracts	63
Figure 4.21 IC ₅₀ values for α -glucosidase inhibition by dragon fruit yogurts	63
Figure 4.22 Sensory evaluation score of plain and dragon fruit yogurts	64
Figure 4.23 Exopolysaccharides concentration in plain and dragon fruits yogurts	65

LIST OF TABLES

Table 2.1: Benefits and potential therapeutical applications	10
Table 3.1: Criteria evaluated in sensory evaluation	40
Table 4.1 pH values of plain and dragon fruit-yogurt on the 14 th day of storage	53
Table 4.2: Titratable acid (TA) of dragon fruit yogurts on the 14 th day of storage	54

LIST OF ABBREVIATIONS

LAB	Lactic acid bacteria
°C	Degree Celcius
L. acidophilus	Lactobacillus acidophilus
S. thermophilus	Streptococcus thermophiles
L .delbrueckii ssp. Bulgaricus	Lactobacillus delbrueckii ssp Bulgaricus
L. casei	Lactobacillus casei
L. plantarum	Lactobacillus plantarum
B. bifidum	Bifidobacteria bifidum
B. infantis	Bifidobacteria infantis
cfu	Colony forming unit
ml	Millilitre
μΙ	Microliter
μg	Microgram
mg	Milligram
nm	Nanometer
mm	Millimeter
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
ТА	Titratable acid
dH ₂ O	Distilled water
EPS	Exopolysaccharides

GRAS	Generally recognized as safe
TNBS	Trinitrobenzenesuphonic
OPA	O-phthaldehyde
NADH	Nicotinamide adenine dinucleotide
ATP	Adenosine triphosphate
GSH	Glutathione
FCR	Folin-Ciocalteau's reagent
DNSA	Dinitrosalicyclic acid
Da	Dalton unit