USE OF EXOPOLYSACHARIDE PRODUCING STARTER CULTURES IN THE PRODUCTION OF FUNCTIONAL DAIRY PRODUCTS

By

DOAA MAAMOUN ABD ALLAH

B.Sc. Agric. Sc. (Dairy Science and Technology), Fac. Agric., Ain Shams Univ., 2004M.Sc. Agric. Sc. (Dairy Science and Technology), Fac. Agric., Ain Shams Univ., 2014

A Thesis Submitted in Partial Fulfillment Of the Requirements for the Degree of

DOCTOR OF PHYLOSOFY in Agricultural Sciences (Dairy Science and Technology)

Department of Food Science Faculty of Agriculture Ain Shams University

2020

CONTENTS

	Page
LIST OF TABLES	VI
LIST OF FIGURES	IX
LIST OF ABBREVIATIONS	XII
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	4
1. Importance of dairy functional foods	4
2. Importance of milk fat in dairy product	5
3. Low- fat dairy products: an overview	7
4. Production and utilization of exopolysaccharides (EPS)	8
using (LAB)	
4.1. EPS in fermented milks manufacture	11
4.2. EPS in cheese manufacture	21
III. MATERIALS AND METHODS	31
1. MATERIALS	31
1.1. Bacterial strains	31
1.2. Raw milk	31
1.3. Rennet	31
1.4. Sodium Chloride (NaCl) and Calcium chloride (CaCl ₂)	32
1.5. Microbiological media	32
2. Methods of manufacture	32
A. yoghurt manufacture	32
B. Ras cheese manufacture	33
3. Methods of analysis	34
3.1. Experimental procedure	34
3.1.1. Screening tests for mucoidy and ropiness	35
3.1.2. Isolation of exopolysaccharides	35
3.2. Chemical analysis	35
3.2.1. Determination of total solids content	35
3.2.2. Determination of fat content	36

	Page
3.2.3. Determination of protein content	36
3.2.4. Determination of titratable acidity (TA%)	36
3.2.5. Determination of Ash content	36
3.2.6. Calculation of carbohydrate content	36
3.2.7. Determination of Acetaldehyde and diacetyl contents	36
3.2.8. pH value measurment	37
3.2.9. Determination of Total volatile fatty acids (TVFA)	37
contents	
3.2.10. Determination of Soluble tyrosine and Tryptophan	37
content	
3.2.11. Determination of Salt content	37
3.3. Microbiological examinations	37
3.3.1. Total bacterial counts	37
3.3.2. Lb. delbrueckii subsp. bulgaricus counts	37
3.3.3. Streptococcus thermophilus counts	37
3.4. Rheological analysis	38
3.4.1. Determination of Syneresis and water holding capacity	38
3.4.2. Measurement of viscosity	38
3.5. Texture profile analysis (TPA)	38
3.6. Microstructure examinations (SEM)	40
3.7. Sensory evaluation	40
3.8. Statistical Analysis.	41
IV. RESULTS AND DISCUSSION	42
Part I: Using of Eexopolysaccharides producing LAB starter	42
in making functional low-fat yoghurt	
1. Gross Chemical composition	42
1.1.Total solids	42
1.2. Acetaldehyde and diacetyl contents	47
1.2.1. Acetaldehyde content	47
1.2.2 diacetyl content	50
2. Acidity and pH values	52

	Page
2.1. Acidity percent	52
2.2. pH values	54
3. physical properties of functional low-fat yoghurt made with	55
exopolysaccharides producing cultures when zero and during	
storage at 5 ± 1 °C.	
3.1. Apparent Viscosity	55
3.2. Syneresis	58
3.3. Water- holding capacity (WHC)	59
4. Bacteriological properties (log cfu/g) of the produced	61
functional yoghurt	
4.1. Total bacterial count (log cfu/g) of the produced functional	62
yoghurt	
4.2. Str. theromphilus count (log cfu/g) of the produced	63
functional yoghurt	
4.3. Lb. delbrueckii subsp. bulgaricus count (log cfu/g) of the	64
produced functional yoghurt	
5. Texture profile analysis (TPA) of functional low- fat yoghurt	66
made with exopolysaccharides producing cultures when zero	
and during storage	
6. Scanning Electron Microscopy (SEM) of functional low-fat	74
yoghurt made with exopolysaccharides producing cultures	
7. Sensory evaluation	76
Part II: Making functional low-fat Ras cheese using of	82
Exopolysaccharides producing LAB starter culture	
1. Yield and chemical composition of functional low-fat Ras	82
cheese made with exopolysaccharides producing cultures either	
commercial or laboratory when zero and during ripening	
period	
1.1. Yield percent	82
1.2. Moisture content	83
1.3. Total Nitrogen content	85

	Page
1.4. Fat content	87
1.5. Ash content	88
1.6. Salt precent	89
2. Titratable acidity (%) and pH values	91
2.1. Acidity percent	91
2.2. pH values	93
3. Ripening indices	94
3.1. Soluble Tyrosine content	94
3.2. Soluble Tryptophan content	97
3.3. Total Volatile Fatty Acids (TVFA) content	99
4. Bacteriological properties	100
4.1. Total bacterial count (log cfu/gm)	101
4.2. Str. theromphilus count	103
4.3. Lb. delbrukii subsp bulgaricus count	104
5. Texture profile analysis (TPA)	105
6. Scanning Electron Microscopy (SEM) of fresh functional	114
low-fat Ras cheese made with exopolysaccharides producing	
cultures	
7. Sensory evaluation scores of functional low-fat Ras cheese	117
made with exopolysaccharides producing cultures when zero	
and during ripening period at 15±2 °C	
V. SUMMARY	122
VI. REFERENCES	129
ARABIC SUMMARY	1

LIST OF TABLES

NO.		Page
1	Used bacterial strains	34
2	Gross chemical composition of functional low-fat yoghurt	44
	made with exopolysaccharides producing cultures when	
	zero and at the end of storage at $5 \pm 1 \ ^{\circ}C$	
3	Acetaldehyde and Diacetyl (ppm) contents of functional	48
	low-fat yoghurt made with exopolysaccharides producing	
	cultures when zero and during storage at 5 ± 1 °C	
4	Titratable acidity (%) and pH values of functional low- fat	52
	yoghurt made with exopolysaccharides producing cultures	
	when zero and during storage at $5 \pm 1 \ ^{\circ}C$	
5	physical properties of functional low-fat yoghurt made	56
	with exopolysaccharides producing cultures when zero	
	and during storage at $5 \pm 1 \ ^{\circ}\text{C}$	
6	Bacteriological properties (log cfu/ml) of functional low-	61
	fat yoghurt made with exopolysaccharides producing	
	cultures when zero and during storage at 5 ± 1 °C	
7	Texture profile analysis (TPA) of functional low-fat	67
	yoghurt made with exopolysaccharides producing cultures	
0	when zero and during storage at $5 \pm 1 ^{\circ}\text{C}$	-
8	Sensory evaluation of functional low-fat yoghurt made	78
	with exopolysaccharides producing cultures when zero	
0	and during storage at $5 \pm 1 ^{\circ}\text{C}$	0.4
9	Yield and gross chemical composition of functional low- fat Bag chaose made with exemply applying and using	84
	fat Ras cheese made with exopolysaccharides producing	
	cultures when zero and during ripening period 90 days at 15±2 °C	
10		92
10	Titratable acidity (%) and pH values of functional low- fat Ras cheese made with exopolysaccharides producing	72
	cultures when zero and during ripening period at 15 ± 2	
	cultures when zero and during ripering period at 13 ± 2	

NO.

°C

- Soluble tyrosine, tryptophan (mg/100g) and TVFA(ml 96
 0.1N NaOH/100g) of functional low-fat Ras cheese made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 12 Bacteriological properties (log cfu/g) of functional low-fat 101 Ras cheese made with exopolysaccharides producing cultures when zero and during ripening period at 15 ± 2 °C
- 13 Texture profile analysis of functional low-fat Ras cheese 107 made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 14 Sensory evaluation scores of functional low-fat Ras 118 cheese made with exopolysaccharides producing cultures when zero and during ripening period at $15 \pm 2 \,^{\circ}C$

LIST OF FIGURES

1	Standard of texture profile analysis curve (TMS-Pro) 20
1	Standard of lexine brothe analysis curve (TMS-F10	1 37

No.

- 2 Total solids (%) of functional low-fat yoghurt made with 45 exopolysaccharides producing cultures when zero and at the end of storage at $5 \pm 1^{\circ}$ C
- 3 Protein (%) of functional low-fat yoghurt made with 45 exopolysaccharides producing cultures when zero and at the end of storage at 5 ± 1 °C
- 4 Fat (%) of functional low-fat yoghurt made with 46 exopolysaccharides producing cultures when zero and at the end of storage at 5 ± 1 °C
- 5 Ash (%) of functional low-fat yoghurt made with 46 exopolysaccharides producing cultures when zero and at the end of storage at 5 ± 1 °C
- 6 Carbohydrate (%) of functional low-fat yoghurt made 47 with exopolysaccharides producing cultures when zero and at the end of storage at 5 ± 1 °C
- 7 Acetaldehyde (ppm) of functional low-fat yoghurt made 49 with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 8 diacetyl (ppm) of functional low-fat yoghurt made with 51 exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 9 Acidity (%) of functional low-fat yoghurt made with 53 exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 10 pH values of functional low-fat yoghurt made with 54 exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 11 Viscosity (cp) of functional low-fat yoghurt made with 57

VII

Page

exopolysaccharides producing cultures when zero and during storage at 5 \pm 1 $^{o}\mathrm{C}$

- 12 Syneresis (%) of functional low-fat yoghurt made with 59 exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 13 Water- holding capacity (WHC) of functional low-fat 60 yoghurt made with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 14 Total bacterial count (log cfu/g) of functional low-fat 63 yoghurt made with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 15 Str. thermophilus count (log cfu/g) of functional low-fat 64 yoghurt made with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 16 *Lb. delbrueckii* subsp. *bulgaricus* count (log cfu/g) of 66 functional low-fat yoghurt made with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 17 Hardness values (N) of functional low-fat yoghurt made 68 with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 18 Adhesiveness values (mJ) of functional low-fat yoghurt 70 made with exopolysaccharides producing cultures when zero and during storage at $5 \pm 1 \,^{\circ}\text{C}$
- 19 Cohesiveness values of functional low-fat yoghurt made 70 with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 20 Springiness values (mm) of functional low-fat yoghurt 71 made with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 21 Gumminess values (N) of functional low- fat yoghurt 72

Page

made with exopoly saccharides producing cultures when zero and during storage at 5 \pm 1 $^{o}\mathrm{C}$

- 22 Chewiness values (mJ) of functional low-fat yoghurt made 73 with exopolysaccharides producing cultures when zero and during storage at $5 \pm 1^{\circ}$ C
- 23 Scanning electron microscopy (SEM) of fresh functional 75 low- fat yoghurt made with exopolysaccharides producing cultures
- 24 Flavour score of functional low-fat yoghurt made with 79 exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 25 Body & texture score of functional low-fat yoghurt made 79 with exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 26 Appearance score of functional low-fat yoghurt made with 80 exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 27 Total Score of functional low-fat yoghurt made with 80 exopolysaccharides producing cultures when zero and during storage at 5 ± 1 °C
- 28 Moisture (%) of functional low-fat Ras cheese made with 85 exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 29 Total Nitrogen (%) of low fat Ras cheese with 86 exopolysaccharides producing cultures when fresh and during ripening period at 15±2 °C
- 30 Fat (%) of functional low-fat Ras cheese made with 88 exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 31 Ash (%) of functional low-fat Ras cheese made with 89 exopolysaccharides producing cultures when zero and

Page

during ripening period at 15±2 °C

- 32 Salt percent of functional low-fat Ras cheese functional 90 made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 33 Acidity (%) of functional low-fat Ras cheese made with 93 exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 34 pH values of functional low-fat Ras cheese made with 94 exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 35 Soluble tyrosine (mg/100g) of functional low- fat Ras 97 cheese made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 36 Soluble tryptophan (mg/100g) of functional low-fat Ras 98 cheese made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 37 TVFA(ml 0.1NNaOH/100g) of low fat Ras cheese with 99 exopolysaccharides producing cultures when fresh and during ripening period at 15±2 °C
- 38 Total bacterial count (log cfu/g) of functional low- fat Ras 102 cheese made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 39 *Str. theromphilus* (log cfu/g) of functional low- fat Ras 104 cheese made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 40 *Lb. delbrukii* subsp *bulgaricus* count (log cfu/g) of 105 functional low fat Ras cheese made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 41 Hardness (N) values of functional low- fat Ras cheese 108 made with exopolysaccharides producing cultures when

zero and during ripening period at 15±2 °C
Adhesiveness (mJ) values of functional low- fat Ras
cheese made with exopolysaccharides producing cultures
when zero and during ripening period at 15±2 °C
Cohesiveness values of functional low-fat Ras cheese
made with exopolysaccharides producing cultures when
zero and during ripening period at 15±2 °C
Springiness (mm) values of functional low-fat Ras cheese

42

43

- 44 Ras cheese 111 Spring made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 45 Gumminess (N) values of functional low-fat Ras cheese 112 made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 46 Chewiness (mJ) values of functional low-fat Ras cheese 113 with made exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 47 Scanning electron microscopy (SEM) of fresh functional 116 low- fat Ras cheese made with exopolysaccharides producing cultures
- 48 Flavour scores of functional low-fat Ras cheese made with 119 exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 49 Body & texture scores of functional low-fat Ras cheese 120 made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 50 Appearance scores of functional low-fat Ras cheese made 121 with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C
- 51 Total sensory scores of functional low-fat Ras cheese 121 made with exopolysaccharides producing cultures when zero and during ripening period at 15±2 °C

Page

109

111

ABSTRACT

Doaa M. Abd Allah, Use of Exopolysacharide Producing Starter Cultures in the Production of Functional Dairy Products. Unpublished Doctor of Science Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2020.

The objective of the present study was to improve the functional and sensory characteristics of low fat yoghurt and Ras cheese by using EPS-producing cultures. The study was conducted in two parts, after selection of bacterial cultures. Seven different bacterial cultures were screened for their ability to produce exopolysaccharides. Out of the Seven tested bacterial cultures of *Lactobacillus dulbruekii subsp. bulgaricus* DSM 20080, *Streptococcus thermophilus* CH-1, thermophilic culture Yoflex Express 1.0 and thermophilic culture Yoflex CH-1 were the best. Therefore, these bacterial cultures were selected for further investigations.

In first part of this study, exopolysaccharides producing culture in low-fat yoghurt making. The results showed that addition of exopolysaccharides producing cultures into low-fat milk had no effect on T.S and chemical composition of the resultant yoghurts. Use of exopolysaccharides into low- fat yoghurt improved significant the yoghurt viscosity. Values of the viscosity were increased with extending the storage period in all treatments including the two controls. Low-fat yoghurt with laboratory exopolysaccharides producing culture was the lowest significant syneresis among all treatments. Low-fat yoghurt with exopolysaccharides producing cultures either commercial or laboratory achieved the significantly greatest acetaldehyde and diacetyl content in both fresh and cold stored yoghurt samples compared to low- fat yoghurt control. Also, fresh and stored yoghurts made from low- fat milk with laboratory exopolysaccharides producing culture showed highest counts of *Lb.bulgaricus* and *Str. theromphillus* among all treatments including two the controls (full- and low-fat). The data shown that use of exopolysaccharides into low-fat yoghurt led to significant decrease of hardness compared to low-fat yoghurt control. Also, the hardness decreased by advancing the storage in all treatments including two controls. Microstructure of fresh yoghurt showed that addition of EPSproducing cultures either commercial or laboratory into low- fat buffaloes milk improved the texture of the resultant yoghurt. All yoghurt treatments were sensory acceptable but the best yoghurt was low- fat yoghurt with laboratory EPS- producing culture.

In the second part, exopolysaccharides producing culture in low-fat Ras cheese making. Fresh low- fat Ras cheese with laboratory EPSproducing culture had the highest yield percent while low- fat Ras cheese control showed the lowest among all treatments. The salt/moisture percent and ash content of all Ras cheese samples tended to increase gradually along the ripening period. Use of exopolysaccharides into lowfat Ras cheese led to significant increase in the soluble tryptophan, soluble tyrosine and TVFA contents compared to low- fat yoghurt control. Total bacterial count, Str. theromphillus and Lb. delbrueckii ssp. *bulgaricus* counts in Ras cheese made from low- fat milk with laboratory exopolysaccharides producing culture showed highest counts among all treatments including the two controls (full- and low- fat). Use of exopolysaccharides into low- fat Ras cheese led to significant decrease of hardness compared to low- fat Ras cheese control. Microstructure showed that fresh cheese containing exopolysaccharides exhibit protein network with more and large opened cavities than low- fat Ras cheese control. The body and texture scores in full- fat Ras cheese control and low- fat Ras cheeses including EPS-producing cultures had significantly higher points than those of the cheese 10w- fat Ras cheese control. All Ras cheese treatments were sensory acceptable but the best cheese was low- fat Ras cheese with laboratory EPS- producing culture as well as the full- fat control.

Key words: Exopolysaccharides starter cultures, Low- fat yoghurt, Ras cheese, Textural properties, Scanning Electron Microscopy, Sensory evaluation.