

بسم الله الرحمن الرحيم



HOSSAM MAGHRABY



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



HOSSAM MAGHRABY

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
على هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

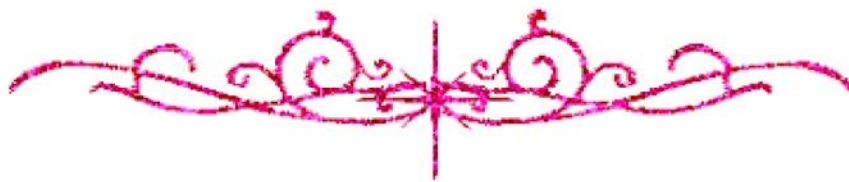
تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HOSSAM MAGHRABY



بعض الوثائق الأصلية تالفة



HOSSAM MAGHRABY



بالرسالة صفحات

لم ترد بالأصل

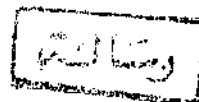


HOSSAM MAGHRABY

ACCELERATING CHEESE RIPENING WITH BACTERIAL PROTEINASES

By

ALI ABDEL- AZIZ ALI

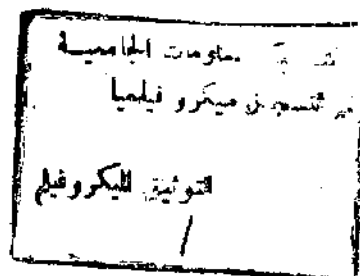


A thesis submitted in partial fulfillment
of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

637.3
A.A



**Agricultural Science
(Dairy Science and Technology)**

Department of Food Science
Faculty of Agriculture
Ain Shams University

٧٩٥٤

1994



Approval Sheet

ACCELERATING CHEESE RIPENING WITH BACTERIAL PROTEINASES

By

ALI ABDEL- AZIZ ALI

B. Sc. Agric. (Dairying), 1981, Ain Shams University

M. Sc. Agric. (Dairying), 1987, Ain Shams University

This thesis for Ph.D degree in Dairy Science has been approved by :

Prof. Dr. Abdel- Wahab M. Abdel- Hafez *A. Abdel Hafez*

Professor of Agricultural Microbiology, and
President of Ain Shams University, Cairo, Egypt.

Prof. Dr. M. M. El- Abd *M. M. EL ABD*

Professor of Dairy Science, Faculty of Agric.,
Cairo University, Giza, Egypt.

Prof. Dr. Abdou E. Shehata *A. Shehata*

Professor of Dairy Science and Technology, and Dean of
Faculty of Agric., Ain Shams University, Cairo, Egypt. (Supervisor)

Date of Examination : 2 / 2 / 1994

ACCELERATING CHEESE RIPENING WITH BACTERIAL PROTEINASES

By

ALI ABDEL- AZIZ ALI

**B. Sc. Agric. (Dairying), 1981, Ain Shams University
M. Sc. Agric. (Dairying), 1987, Ain Shams University**

Under the supervision of :

Prof. Dr. Abdou E. Shehata

**Professor of Dairy Science and Technology, and Dean,
Faculty of Agric., Ain Shams University**

Prof. Dr. Abdel- Moneim E. Hagra

**Professor of Dairy Science and Technology,
Faculty of Agric., Ain Shams University**

Prof. Dr. T. E. Shehata

**Professor of Food Microbiology, and Director of the
office of International programs, University of Maryland**

Abstract

Two different proteases of *B. subtilis* ATCC 35854 and *P. fluorescens* strain P 27 were recovered, purified and characterized. Results showed that the two proteases were heat-stable, neutral and metalloenzymes. The two purified proteases were involved in Ras cheese slurry using different concentrations. The best concentration (2 units/10 g curd) of

ii

the two proteases were incorporated into Ras cheese either free or in DRV liposomes. Using DRV in Ras cheese had a better quality and saved about 50 % of the ripening period.

Key words

Accelerating - Cheese - Ripening - Proteases - Heat-stable -
Bacterial - Slurry - Liposomes - DRV liposomes - Ras cheese

ACKNOWLEDGMENTS

I would like to express sincere appreciation to my Major Professor Dr. A. E. Shehata, Professor of Dairy Science, and Dean of faculty of Agriculture, Ain Shams University, for his guidance. It has been a pleasure to be his student. Gratitude is also expressed to Dr. A. E. A. Hagrass, Professor of Dairy Science, Ain Shams University, and Dr. T. E. Shehata, Professor of Food Science, and Director of the Office of International Programs, University of Maryland, USA, members of my committee, for their suggestions and helpful criticism.

I would like also, to thank Dr. E. M. Sybert, Manager of Biotechnology Program, University of Maryland and Mr. Tom M. Davis, Laboratory Director of Environmental Systems Service, Maryland, USA, for the use of their laboratory facilities.

A special thanks is extended to all the staff members of Food Science Department, Ain Shams University, for their encouragement.

CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	7
I- Characterization of bacterial proteases	7
II- Accelerated ripening of cheese	21
A- Use of bacterial proteases for accelerated cheese ripening	24
1- Utilization of bacterial proteases for accelerated ripening of cheese slurry	24
2- Utilization of microbial proteases for accelerated ripening of cheese	29
B- Acceleration by enzymes entrapped in liposomes	36
MATERIALS AND METHODS	41
Materials	41
Methods	42
I- Production, purification and characterization of proteases	42
A- Selection and maintenance of cultures	42
B- Enzyme production	43
C- Measurement of enzyme activity	44
D- Determination of protein	46
E- Enzyme purification	46
F- Characterization of enzyme	50

	Page
II- Application of the ample proteases in Ras cheese slurry	52
A- cheesemaking	52
1- Ras cheese manufacture	52
2- Preparation of Ras cheese slurry	53
B- Analysis of slurries	54
1- Moisture	54
1- Fat	54
1- Salt	54
1- pH	54
2- Soluble nitrogen/Total nitrogen (SN/TN)	54
3- Protein breakdown	54
4- Sensory evaluation	55
III - Use of liposomes for bacterial proteases addition to accelerate ripening of Ras cheese	55
A- Cheesemaking	55
B- Preparation of liposomes	56
C- Estimation of enzyme entrapment in liposomes	57
D- Cheese sampling	58
E- Analysis of Ras cheese	58
1- Chemical analysis	58
a- Moisture, fat, salt, pH, acidity and SN/TN	58
b- Determination of free amino acids	58
c- Free fatty acids determination	61

	Page
d- Determination of cheese protein degradation	61
2- Scoring the cheese	62
3- Statistical analysis	62
RESULTS AND DISCUSSIONS	63
I- Production, purification and characterization of proteases	63
II - Utilization of bacterial proteases for accelerated ripening of Ras cheese slurry	91
III- Use of liposomes for proteases addition to accelerate ripening of cheese	126
SUMMARY AND CONCLUSION	177
REFERENCES	184

LIST OF TABLES

<u>Table Nr.</u>		<u>Page</u>
1	Alternative modes of proteinase addition to cheese	4
2	Percent of retention of different types of liposomes in cheese	5
3	Protein standards for gel electrophoresis	49
4	Recovery and purification of proteases of <i>B. subtilis</i> (ATCC 35854)	65
5	Recovery and purification of proteases of <i>P. fluorescens</i> (P 27)	66
6	Heat-stability of purified proteases of <i>B. subtilis</i> (ATCC35854) and <i>P. fluorescens</i> (P27)	76
7	Effect of the temperature on the activity of purified proteases of <i>B. subtilis</i> (ATCC 35854) and <i>P. fluorescens</i> (P 27)	79
8	Activation energy of purified proteases of <i>B. subtilis</i> (ATCC 35854) and <i>P. fluorescens</i> (P27)	79
9	Effect of pH on the activity of purified proteases of <i>B. subtilis</i> (ATCC35854) and <i>P. fluorescens</i> (P27)	84
10	Effect of cations and chelating agent on the activity of purified proteases of <i>B. subtilis</i> (ATCC 35854) and <i>P. fluorescens</i> (P 27)	89
11	Moisture content of Ras cheese slurries manufactured with different concentrations of purified bacterial proteases during incubation at 30°C for 7 days	94
12	Analysis of variance for the effect of type of enzymes (E), incubation period (D) and concentrations of enzyme added (U) on the moisture content of Ras cheese slurries	95
13	Fat / dry matter (FDM) content of Ras cheese slurries manufactured with different concentrations of purified bacterial proteases during incubation at 30°C for 7 days	98

	page
14 Analysis of variance for the effect of type of enzymes (E), incubation period (D) and concentrations of enzyme added (U) on the FDM content of Ras cheese slurries	99
15 Salt in water phase of Ras cheese slurries manufactured with different concentrations of purified bacterial proteases during incubation at 30°C for 7 days	103
16 Analysis of variance for the effect of type of enzymes (E), incubation period (D) and concentrations of enzyme added (U) on the salt in water phase of Ras cheese slurries	104
17 pH values of Ras cheese slurries manufactured with different concentrations of purified bacterial proteases during incubation at 30°C for 7 days	108
18 Analysis of variance for the effect of type of enzymes (E), incubation period (D) and concentrations of enzyme added (U) on the pH values of Ras cheese slurries	109
19 Soluble nitrogen as percentage of total nitrogen (SN/TN) of Ras cheese slurries manufactured with different concentrations of purified bacterial proteases during incubation at 30°C for 7 days	113
20 Analysis of variance for the effect of type of enzymes (E), incubation period (D) and concentrations of enzyme added (U) on the SN/TN of Ras cheese slurries	114
21 Trichloroacetic acid soluble products (TCA-SN) of Ras cheese slurries manufactured with different concentrations of purified bacterial proteases during incubation at 30°C for 7 days	118
22 Analysis of variance for the effect of type of enzymes (E), incubation period (D) and concentrations of enzyme added (U) on the TCA-SN of Ras cheese slurries	119
23 Sensory evaluation of Ras cheese slurries manufactured with different concentrations of purified bacterial proteases during incubation at 30°C for 7 days	123