



“Aspects in improvement of some functional foods using bio-synthesized nanoparticles”

A Thesis submitted for the degree of Doctor of Philosophy
in Science in Microbiology

By

Alyaa Talaat Gaber Ali

M.Sc. (Microbiology), Faculty of Science, Ain Shams University, 2014

To

Microbiology Department

Faculty of Science – Ain Shams University

Under Supervision of

Prof.

Yousseria Mohamed Hassan Shetaia

Professor of Microbiology (Mycology),
Faculty of Science, Ain Shams University

Prof.

Neimat Ali Hassan Elewa

Professor of Dairy Microbiology, Dairy
Science and Technology Department, Faculty
of Agriculture, Fayoum University

Prof.

El Sayed Abdel-Majid Al-Sherbini

Professor of Photochemistry,
National Institute of Laser Enhanced
Science, Cairo University

Prof.

Nahla Abd-Elfatah Mostafa Elbostany

Professor of Biochemistry,
Special food and Nutrition Department,
Food Technology Research Institute,
Agriculture Research Centre

2022

LIST OF CONTENTS

	<i>Page</i>
LIST OF TABLES	III
LIST OF FIGURES	IV
LIST OF ABBREVIATIONS	IX
ABSTRACT	XI
INTRODUCTION	1
AIM OF THE WORK	5
REVIEW OF LITERATURE	6
1. Nanotechnology	6
2. Methods for the synthesis of nanoparticles	7
3. Metal oxide nanoparticles (MONPs)	9
4. Surface modification of iron oxide nanoparticles	11
5. Antimicrobial activity of nanoparticles	13
6. Mechanism of antimicrobial action of iron oxide nanoparticles	14
7. Nanotechnology role in the improvement of food packaging	15
8. Safety concerns of nanoparticles in food sector	18
9. Toxicity of iron oxide nanoparticles	19
10. Functional food	22
11. Contaminated microorganisms in dairy Products	26
12. Biogenic amines in some functional foods	28
MATERIALS & METHODS	36
I. Materials	36
II. Methods	40

	<i>Page</i>
EXPERIMENTAL RESULTS	55
1. Isolation and identification of <i>Penicillium sp</i>	55
2. Molecular Identification of the fungal isolate	55
3. Biosynthesis of iron nanoparticles	56
4. Characterization of iron oxide nanoparticles (IONPs)	58
5. Silica-coated iron oxide nanoparticles	60
6. Characterization of silica coated iron oxide nanoparticles...	61
7. Assessment of antimicrobial activity of iron oxide nanoparticles and the modified iron oxide nanoparticles (Si-IONPs)	63
8. Cytotoxicity of applied nanoparticles	79
9. Results of fluorescence behavior of biogenic amines in the presence of IONPs or Si-IONPs in aqueous solutions in Vitro	80
10. Influence of coating Ras cheese with thin film containing 100 µg/ml of Si-IONPs	83
11. Assessment of biogenic amines content in untreated and treated Ras cheese during the ripening period using HPLC	95
DISCUSSION	99
SUMMARY	115
CONCLUSION & RECOMMENDATIONS	119
REFERENCES	120
ARABIC SUMMARY	-

LIST OF TABLES

Table No.	Title	Page
1	Principal preparation methods of iron oxide nanoparticles.....	11
2	Biogenic amines and their pharmacological effects.....	34
3	Statistically comparison between the inhibition zone diameter (mm) of IONPs and Si-IONPs against <i>Staphylococcus aureus</i> at different concentrations	64
4	Statistically comparison between the inhibition zone diameter (mm) of IONPs and Si-IONPs against <i>B. subtilis</i> at different concentrations	66
5	Statistically comparison between the inhibition zone diameter (mm) of IONPs and Si-IONPs against <i>S. typhimurium</i> at different concentrations.....	68
6	Statistically comparison between the inhibition zone diameter (mm) of IONPs and Si-IONPs against <i>E. coli</i> at different concentrations.....	70
7	The antibacterial activity of IONPs and Si-IONPs against G(+ve) and G(-ve) bacteria with different concentrations.....	71
8	Physico-Chemical analysis of untreated and treated chesses during the ripening period (6months).....	85
9	Microbiological population of treated and untreated Ras cheese during ripening	92
10	Biogenic amines levels (mg/100g) in treated and untreated Ras cheese during storage.	96

LIST OF FIGURES

Figure no.	Title	Page
1	Framework for integrating nano research areas and the food supply chain	7
2	Schematic illustration of the primary shells for the functionalization of iron oxide nanoparticles (IONPs)	12
3	The main mechanisms of action by which systems based on iron oxide nanoparticles (IONPs) generate cell toxicity.....	15
4	Formation of biogenic amines in food as a result of microbial metabolic activities	28
5	Denmark Roquefort cheese sample	40
6	Macroscopic and microscopic characteristics of the <i>Penicillium sp.</i> isolate.....	55
7	Neighbor-joining phylogenetic tree derived from 18S rRNA gene sequences	56
8	The confirmation sings of the biosynthesized IONPs	57
9	The purified powder of biosynthesized Iron oxide nanoparticle	57
10	UV-Vis spectra of the biosynthesized iron oxide nanopartcales using <i>penicillum roqueforti</i>	58
11	TEM micrograph of biosynthesized iron oxide nanoparticles.....	58
12	Scanning electron microscope image of mycelia of <i>p.roqueforti</i> and intracellular iron oxide nanoparticles embedded in fungal biomatrix.....	59
13	Energy Dispersive X-ray Spectroscopy by Scanning electron microscope EDX image of IO nanoparticles.....	59

Figure no.	Title	Page
14	The X-ray diffraction pattern of the biosynthesized iron oxide nanoparticles.....	60
15	The nanocomposite of silica coated the iron oxide nanoparticles	60
16	UV-Vis spectra of iron oxide nanopartcales coated by silica core shell	61
17	Transmission electron microscope image of Si coated IONPs	61
18	The SEM images of the Si coated IO nanoparticles.....	62
19	Energy dispersive X-ray analysis of Si-IO nanoparticles	62
20	The XRD pattern of silica coated iron oxide nanoparticles.....	63
21	The diameter of inhibition zones of both IONPs. and Si-IONPs against <i>Staphylococcus aureus</i>	64
22	The antibacterial effect of different concentrations of IONPs and Si-IONPs against <i>Staphylococcus aureus</i>	65
23	The diameter of inhibition zones of both IONPs. and Si-IONPs against <i>Bacillus subtilis</i>	66
24	The antibacterial effect of different concentrations of IONPs and Si-IONPs against <i>Bacillus subtilis</i>	67
25	The diameter of inhibition zones of both IONPs. and Si-IONPs against <i>Salmonella typhimurium</i>	68
26	The antibacterial effect of different concentrations of IONPs and Si-IONPs against <i>Salmonella typhimurium</i>	69
27	The diameter of inhibition zones of both IONPs. and Si-IONPs against <i>Escherichia coli</i>	70

Figure no.	Title	Page
28	The antibacterial effect of different concentrations of IONPs and Si-IONPs against <i>Escherichia coli</i>	71
29	Growth kinetic curves of <i>Staphylococcus aureus</i> with different concentrations of IONPs and SI-IONPs	73
30	Growth kinetic curves of <i>Bacillus subtilis</i> with different concentrations of IONPs and SI-IONPs	74
31	Growth kinetic curves of <i>Salmonella typhimurium</i> with different concentrations of IONPs and SI-IONPs	75
32	Growth kinetic curves of <i>Escherichia coli</i> with different concentrations of IONPs and SI-IONPs	76
33	Effect of antifungal activity of IONPs and Si-IONPs on the reduction of mycelium dry weight of <i>Aspergillus niger</i> ...	77
34	Effect of antifungal activity of IONPs and Si-IONPs on the reduction of mycelium dry weight of <i>Aspergillus fumigatus</i>	78
35	Effect of antifungal activity of IONPs and Si-IONPs on the reduction of mycelium dry weight of <i>Aspergillus flavus</i>	79
36	The cytotoxicity of Si-IONPs (200 µg/ml) on the normal Skin fibroblast (BJ1) <i>in vitro</i>	79
37	Absorption spectra of histamine in presence of different concentrations of IONPs and Si-IONPs.	80
38	Stern-volmer plot for histamine in presence of different concentrations of IONPs and Si-IONPs	81
39	Absorption spectra of tyramine in the presence of different concentrations of IONPs and Si-IONPs	82
40	Stern-volmer plot for tyramine with different concentrations of IONPs and Si-IONPs	83

Figure no.	Title	Page
41	The morphology of the treated cheese surface with 100 µg/ml of Si-IONPs during the ripening period (6months) ...	84
42	The morphology of the untreated Ras cheese surface, during the ripening period (6 months).....	84
43	The total protein (%) in treated and untreated samples during the ripening period	87
44	The moisture content (%) in treated and untreated samples during the ripening period	87
45	The Fat content (%) in treated and untreated samples during the ripening period.	88
46	The pH values in treated and untreated samples during the ripening period.....	88
47	The acidity (%) in treated and untreated samples during the ripening period.....	89
48	The total solids (%) in treated and untreated samples during the ripening period	89
49	The total nitrogen (%) in treated and untreated samples during the ripening period	90
50	The microbial count of total plate count bacteria (x10 ⁸ cfu/gm) in fresh, treated and untreated Ras cheese samples during the ripening period.	93
51	The microbial count of lactic acid bacteria (x10 ⁶ cfu/gm) in fresh, treated and untreated Ras cheese samples during the ripening period.....	93
52	The microbial count of Yeast and mold (x10 ² cfu/gm) in fresh, treated and untreated Ras cheese samples during the ripening period.....	94
53	The microbial count of coliform (x10 cfu/gm) in fresh,	94

Figure no.	Title	Page
	treated and untreated Ras cheese samples during the ripening period	
54	The microbial count of <i>Staphylococcus aureus</i> ($\times 10^2$ cfu/gm) in fresh, treated and untreated Ras cheese samples during the ripening period	95
55	Dansylated of six biogenic amines standard overviewed by high performance liquid Chromatography (HPLC)	95
56	Auto-Scaled Chromatogram of treated Ras cheese after 2 months.....	97
57	Auto-Scaled Chromatogram of treated Ras cheese after 4 months.....	97
58	Auto-Scaled Chromatogram of treated Ras cheese after 6 months.....	97
59	Auto-Scaled Chromatogram of untreated Ras cheese after 2 months.....	98
60	Auto-Scaled Chromatogram of untreated Ras cheese after 4 months.....	98
61	Auto-Scaled Chromatogram of untreated Ras cheese after 6 months.....	98

LIST OF ABBREVIATIONS

Abbreviation	Full term
°C	Celsius grade
AB assay	Alamar blue
ADA	American Dietetic Association
ATCC	American type culture collection
Bas	Biogenic amines
Cfu/gm	Colony-forming unit per gram
Cu	Copper
DAO	Diamine oxidase
Dm	Decimetre
Dm³	Cubic decimeter
DNA	Deoxyribonucleic Acid
Etc	Et cetera is a Latin phrase meaning and other similar things
et al	Comes from the Latin phrase meaning: and other
FAO	Food and Agriculture Organization of the United Nations
FeCl₂.4H₂O	Ferrous chloride Tetrahydrate
FeCl₃.6H₂O	Ferric chloride hexahydrate
FeSO₄.7H₂O	Ferrous sulfate heptahydrate
GI	Gastrointestinal
HCL	Hydrochloric Acid
HIV/AIDS	The human immunodeficiency virus is what ultimately causes AIDS (acquired immunodeficiency syndrome)
HPLC	High-performance liquid chromatography
Hrs	Hours
IONPs	Iron oxide nanoparticles
ITS	Internal transcribed spacer
K₂HPO₄	Dipotassium Phosphate
KCL	Potassium Chloride
Kg	Kilogram
LAB	Lactic acid bacteria
MAO	Mono-amino oxidase
Mg	Milligram
MgSO₄.7H₂O	Magnesium Sulfate (Sulphate) Heptaydrate
Min	Minute
ml	Millilitre

Abbreviation	Full term
Mm	Millimeter
MNPs	Magnetite nanoparticles
MSNs	Mesoporous silica nanoparticles
MTT assay	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaCl	Sodium chloride
NaHCO₃	Sodium bicarbonate
NaNO₃	Sodium Nitrate
NH₄OH	Ammonium hydroxide
NPS	Nanoparticles
NRU assay	Neutral red uptake
O.D	Optical density
PAO	Polyamine oxidases
PDA	Potato dextrose agar
r DNA	Ribosomal deoxyribonucleic acid
g- DNA	Genomic deoxyribonucleic acid
ROS	Reactive oxygen species
Rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscope
Si-IONPs	Nanocomposite of iron oxide nanoparticles modified by silica
SiO₂	Silica dioxide
TEM	Transmission electronic microscope
TEOS	Tetra-ethyl orthosilicate
UV	Ultra violet spectrophotometer
WHO	World Health Organization
XRD	X-ray diffraction
ZnO	Zinic oxide
A	Alpha
Γ	Gamma
Mg	Microgram
ml	Microliters

ABSTRACT

Nowdays, nanotechnology has received a lot of attention resulted in a wide application in medical environmental science, agriculture and food processing. Therefore, the aim of the present study was to investigate a novel antimicrobial agent such as iron oxide nanoparticles (IONPS) which biosynthesized by a simple, fast, eco-friendly efficient method using *Penicillium roqueforti* MK805460.1. The obtained iron oxide nanoparticles were modified by the shell formed by Stöber method. The biosynthesized nanoparticles were characterized by UV-Vis spectroscopy, Energy Dispersive X-ray, Scanning Electron Microscopy, Transmission Electron Microscopy and X-ray diffraction. Antibacterial activity of the nanoparticles with different concentrations of 50, 100, 150 and 200µg/ ml was examined against Gram-positive bacteria (*Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC6633), and Gram-negative bacteria (*Salmonella typhimurium* ATCC14028, *Escherichia coli* ATCC8739) by agar well-diffusion and kinetic bacterial growth method. While, the antifungal activity of the nanoparticles was tested against *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* using dry weight mycelia method. The cytotoxicity effect of nanocomposite (Si-IONPs) on the normal epithelial cells at Conc. 50, 100,150, and 200 µg/ml was investigated. Furthermore, the present study was evaluated the coating of Ras cheese with polymer contained Si-IONPs (100µg/ml) and its effect on microbiological, physical and chemical characteristics as compared with the untreated cheese samples. Also, the levels of the biogenic amines were determined in treated and untreated cheese samples by HPLC during ripening period (6 months). The results showed that upon UV-Vis spectroscopy analysis, the absorption band was observed at a wavelength ranged from 204 to 266 nm, which indicated the formation of iron oxide nanoparticles. Furthermore, transmission Electron

Microscopy was showed the spherical shape of iron oxide nanoparticles with a size of 5-16 nm. Also, the results indicated that modified silica form of nanoparticles have more significant antimicrobial activity than iron oxide nanoparticles. However, there is no cytotoxicity effect of nanocomposite (Si-IONPs) on the normal epithelial cells at Conc. 50,100, and 150 µg/ml. Coating of cheese decreased the moisture losses and affects the normal ripening changes in the microbiological and chemical of treated Ras cheese samples. Also, coating cheese with polymer film containing Si- IONPs eliminated mold growth on the cheese surface. According to the results of HPLC analysis, no detection of biogenic amines in Ras cheese treated samples, whereas the results illustrated the existence of putrescine, spermine, and tyramine in untreated cheese samples after 2, 4, and 6 months.