

TABLE OF CONTENTS

Declaration	1
Certificate	2
Acknowledgements	3-4
Abstract	5-8
Table of Contents	9-15
List of Tables	16-17
List of Figures	18-20
List of Abbreviations	21-22
Chapter – 1	23-27
1.0 Introduction	23
Introduction.....	24-27
Chapter – 2	28-75
2.0 Literature Review	28
2.1. Trace elements – Functions and deficiencies.....	29-31
2.2. Iron.....	31-39
2.2.1. Importance and Deficiency.....	31
2.2.2. Prevalence of iron deficiency.....	31-35
2.2.3. Solution for iron deficiency.....	35-39
2.3. Types of Fortification.....	39-40
2.3.1. Mass fortification.....	39
2.3.2. Targeted fortification.....	39
2.3.3. Market driven fortification.....	39-40
2.4. Strategies of fortification.....	40-53

2.4.1. Microencapsulation.....	41-46
2.4.2. Double fortified salt (DFS).....	47-48
2.4.3. Ultra rice.....	48
2.4.4. Fe EDTA.....	48-49
2.4.5. Iron-protein complexes.....	50
2.4.6. Micronization (Sun active Fe).....	50-51
2.4.7. Biofortification.....	51-53
2.5. Microorganisms as biosorbents.....	53-55
2.5.1. Bacteria.....	53-54
2.5.2. Algae and Fungi.....	54-55
2.6. Factors affecting biosorption.....	55-57
2.7. Mechanism of biosorption.....	57-58
2.7.1. Physical adsorption.....	57
2.7.2. Ion exchange.....	58
2.7.3. Precipitation.....	58
2.7.4. Complexation.....	58
2.8. Isotherm modeling of biosorption.....	59-60
2.8.1. Langmuir isotherm model.....	59
2.8.2. Freundlich isotherm model.....	59-60
2.8.3. Temkin isotherm model.....	60
2.9. Kinetic modeling of biosorption.....	60-61
2.10. Techniques for characterization of biosorbent.....	61-65
2.10.1. Fourier Transform Infrared Spectroscopy (FTIR).....	61-62
2.10.2. Scanning Electron Microscope – Energy Dispersive X- ray Spectroscopy (SEM –EDX).....	62-64

2.10.3. X- ray Diffraction (XRD).....	64-65
2.11. Uptake/Bioaccumulation.....	65-66
2.12. Bioavailability of metal from fortified food.....	66-73
2.12.1. Mechanism of iron absorption.....	66-67
2.12.2. Enhancers and inhibitors of iron absorption.....	67-68
2.12.3. <i>in vitro</i> methods.....	68-70
2.12.3.1. Solubility.....	69
2.12.3.2. Dialyzability.....	69
2.12.3.3. Simulated gastrointestinal digestion.....	69
2.12.3.4. Caco2 model.....	70
2.12.4. <i>in vivo</i> studies.....	70-73
2.12.4.1. Chemical balance methods.....	72
2.12.4.2. Isotopic methods.....	72-73
2.12.4.3. Area under the curve for serum iron.....	73
2.13. Determination of iron by acid digestion.....	74
2.14. Exopolysaccharides.....	74-75
Chapter – 3.....	76-107
3.0 Objective – 1.....	76
3.1. Materials and Methods	
3.1.1. Food grade Microorganisms.....	77
3.1.2. Preparation of yeast enriched with iron.....	77-78
3.1.3. Preparation of Biosorbent.....	78
3.1.4. Preparation of Fe (II) solution.....	78
3.1.5. Batch biosorption experiments.....	78
3.1.6. Determination of iron bound to biomass.....	79

3.1.7. Evaluation of Fe (II).....	79
3.1.8. Optimization of biosorption parameters.....	80
3.1.8.1. Influence of initial metal concentration.....	79-80
3.1.8.2. Influence of contact time.....	80
3.1.8.3. Influence of biosorbent dose.....	80
3.1.8.4. Influence of pH of the solution.....	80
3.1.9. Modeling of biosorption isotherms.....	80-81
3.1.10. Biosorption kinetics.....	81-82
3.1.11. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectrometry (EDX) analysis.....	82
3.1.12. Fourier Transform Infrared spectroscopy (FTIR) analysis.....	82
3.1.13. X-ray diffraction (XRD) analysis.....	82
3.1.14. Evaluation of point zero charge (pH_{pzc}) of the biosorbent.....	83
3.1.15. Characterization of metal binding potential of exopolysaccharide (EPS).....	83-85
3.1.15.1. Isolation of EPS producing lactic acid bacteria (LAB).....	83
3.1.15.2. Identification of EPS strain.....	83
3.1.15.3. Production and isolation of EPS.....	83-84
3.1.15.4. Determination of molecular weight of the purified EPS.....	84
3.1.15.5. Determination of monomer composition of EPS.....	84-85
3.1.15.6. Biosorption of iron by EPS.....	85
3.2. Results and Discussion	
3.2.1. Influence of initial metal concentration.....	85-86
3.2.2. Influence of Biosorbent dose.....	86-87
3.2.3. Influence of pH.....	87-88

3.2.4. Influence of contact time.....	88-89
3.2.5. Biosorption experiments.....	89-90
3.2.6. Determination of iron from iron bound biomass.....	90-91
3.2.7. Iron enriched yeast.....	91-92
3.2.8. Isotherm modeling.....	92-94
3.2.9. Kinetic modeling.....	94-95
3.2.10. SEM – EDX analysis.....	96-99
3.2.11. FTIR analysis.....	99-102
3.2.12. XRD analysis.....	102-103
3.2.13. Point zero charge of biosorbent (pH _{pzc}).....	103-104
3.2.14. Characterization of metal binding potential of EPS.....	104-107
3.2.14.1. Isolation of EPS producing lactic acid bacteria.....	104
3.2.14.2. Production and isolation of EPS.....	105
3.2.14.3. Identification of micro-organism.....	105
3.2.14.4. Molecular weight estimation of EPS.....	105-106
3.2.14.5. Monosaccharide composition.....	106
3.2.14.6. Biosorption experiments.....	107
Chapter – 4.....	108-125
4.0 Objective – 2.....	108
4.1. Materials and Methods	
4.1.1. Simulated gastrointestinal (<i>in vitro</i>) digestion studies.....	109
4.1.2. Animal (<i>in vivo</i>) study.....	110-114
4.1.2.1. Diets.....	110-111
4.1.2.2. Iron analysis.....	111
4.1.2.3. Animals.....	111

4.1.2.4. Experimental design.....	111-112
4.1.2.5. Collection of blood and tissues.....	112-113
4.1.2.6. Iron bioavailability and hematological parameters.....	113
4.1.2.7. Histological analysis.....	114
4.1.3. Statistical analysis.....	114
4.2. Results and Discussion	
4.2.1. Determination of iron from fortified chocolate by simulated Gastro-intestinal (<i>in vitro</i>) digestion.....	114-116
4.2.2. Animal study.....	116-125
4.2.2.1. Diet analysis.....	116-117
4.2.2.2. Body weight and food consumption.....	117-119
4.2.2.3. Iron bioavailability and hematological parameters.....	119-123
4.2.2.4. Histological analysis.....	123-125
Chapter – 5.....	126-144
5.0 Objective – 3.....	126
5.1. Materials and Methods	
5.1.1. Preparation of various treatments of chocolate.....	127-128
5.1.2. Preparation of various treatments of curd/dahi.....	128-129
5.1.3. Analysis of fortified curd/dahi by thiobarbituric acid (TBA) method.....	129
5.1.4. Analysis of fortified chocolate by thiobarbituric acid (TBA) Method.....	130
5.1.5. Enumeration of microbes in curd/dahi and chocolate (microbiological analysis) treatments.....	130
5.1.6. Determination of iron in fortified chocolate by acid digestion.....	130
5.1.7. Availability of iron from fortified chocolate by simulated	

Gastro-intestinal (<i>in vitro</i>) digestion.....	130-131
5.2. Results and Discussion	
5.2.1. Appearance of fortified chocolate.....	131-135
5.2.2. Oxidative changes in fortified chocolate and curd/dahi.....	135-139
5.2.3. Microbiological analysis of curd/dahi and chocolate.....	139-140
5.2.4. Acid digestion of fortified chocolate.....	140-142
5.2.5. Availability of iron from fortified chocolate by simulated	
Gastro-intestinal (<i>in vitro</i>) digestion.....	142-144
6.0 Conclusion.....	145-147
7.0 References.....	148-179
8.0 List of Publications.....	180