

BIOCHEMICAL STUDIES ON IRON AND FOLIC ACID ADDITION TO BISCUIT

By

AZZA EMAD MOSTAFA EL-ANSARY

B.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2010

THESIS

**Submitted in Partial Fulfillment of the
Requirements for the Degree of**

MASTER OF SCIENCE

In

**Agricultural Sciences
(Agricultural Biochemistry)**

**Department of Agricultural Biochemistry
Faculty of Agriculture
Cairo University
EGYPT**

2018

APPROVAL SHEET

**BIOCHEMICAL STUDIES ON IRON AND FOLIC
ACID ADDITION TO BISCUIT**

**M.Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)**

By

AZZA EMAD MOSTAFA EL-ANSARY
B.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2010

APPROVAL COMMITTEE

Dr. AHMAD ELSAYED BASYONY.....
**Head Researcher, Food Technology Research Institute, Agricultural
Research Centre**

**Dr. ABD EL-KADER MOERSY EL-SAYED ABD EL-
SAMAD**.....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. MOHAMED SAAD ABDEL LATTIF
Associate Professor of Biochemistry, Fac. Agric., Cairo University

Dr. HODA GHARIB EL-AMRY.....
**Head Researcher, Food Technology Research Institute, Agricultural
Research Centre**

SUPERVISION SHEET

**BIOCHEMICAL STUDIES ON IRON AND FOLIC
ACID ADDITION TO BISCUIT**

**M.Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)**

BY

AZZA EMAD MOSTAFA EL-ANSARY
B.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2010

SUPERVISION COMMITTEE

DR. MOHAMED SAAD ABDEL LATTIF
Associate Professor of Biochemistry, Fac. Agric., Cairo University

DR. EMAN AHMED HANAFY AHMED
Lecturer of Biochemistry, Fac. Agric., Cairo University

DR. HODA GHARIB EL-AMRY
Head Researcher, Food Technology Research Institute, Agricultural Research Centre

Name of Candidate: Azza Emad Mostafa El-Ansary **Degree:** M.Sc.
Title of Thesis: Biochemical studies on iron and folic acid addition to biscuit
Supervisors: Dr. Mohamed Saad Abd-el latif
Dr. Eman Ahmed Hanafy
Dr. Hoda Gharib El-Amry.
Department: Agricultural Biochemistry **Approval:** 29/4/2018

ABSTRACT

This study was undertaken to create a healthy nutritive biscuit which can be considered as valuable source of non-heme iron in diet by fortifying the biscuit recipe with thyme to improve iron status in rats. The results showed that control (+ve) rat group showed a significant decrease in hemoglobin (12.69g/dl), hematocrit (39.07g/dl) & urea (25.07mg/dl). Moreover, it showed a significant increase in total cholesterol (97.04mg/dl) compared with control (-ve) group. Thyme 3% biscuits rat group showed a significant decrease in hemoglobin (10.67g/dl), hematocrit (32.03g/dl), urea (20.61mg/dl), total cholesterol (81.29mg/dl) & AST activity (69.95U/L) compared with control (-ve) group. Thyme 4.5% biscuits rat group showed a significant decrease in urea (20.86mg/dl) & total cholesterol (64.13mg/dl) and a significant increase in hemoglobin (13.87g/dl), hematocrit (41.63g/dl) & ALT activity (41.02U/L) compared with control (-ve) group. Ferrous sulfate 0.049% biscuits rat group showed a significant decrease in hemoglobin (10.81g/dl), hematocrit (32.54g/dl), urea (29.42mg/dl) & total cholesterol (66.94mg/dl) and a significant increase in ALT activity (41.27U/L) & AST activity (64.33U/L) compared with control (-ve) group. But ferrous sulfate 0.074% biscuits rat group showed a significant decrease in hemoglobin (11.33g/dl), hematocrit (33.99g/dl), urea (26.24mg/dl), total cholesterol (69.19mg/dl), ALT activity (26.33U/L) & AST activity (77.33U/L), compared with control (-ve) group. It could be concluded that thyme can be used as a safe source of iron in producing biscuits to overcome iron deficiency anemia.

Key words: anemia, Iron deficiency, Iron deficiency anemia, Folate deficiency, fortification, ferrous sulfate, thyme.

ACKNOWLEDGEMENT

In the name of ALLAH most merciful, all praise is to ALLAH, without whose bounty I would not have completed this work.

*I wish to express my sincere thanks, deepest gratitude and appreciation to **Dr. Mohamed Saad Abdel Lattif** Professor of Biochemistry, Faculty of Agriculture, Cairo University for supervision and his guidance and revision the manuscript of this thesis.*

*Grate thanks are also due to **Dr. Eman Ahmed Hanafy Ahmed** Lecturer of Biochemistry, Faculty of Agriculture, Cairo University, for her supervision and help during this work.*

*I would like to thank **Dr. Hoda Gharib EL-Amry** Head Researcher at Food Technology Research Institute, Agricultural Research Center, for her supervision, teaching, guidance, patience, constant support throughout this work and writing the thesis.*

Special deep appreciation is given to my father, my mother, my husband, my sisters and my lovely daughter.

LIST OF ABBREVIATIONS

No.	Abbreviation	
1.	ALT	Alanine transaminase
2.	AST	Aspartate transaminase
3.	B.W.	Body weight
4.	DWB	On dry weight basis
5.	Hb	Hemoglobin
6.	Hct	Hematocrit
7.	ID	Iron deficiency
8.	IDA	Iron deficiency anemia
9.	TP	Total protein

CONTENTS

	Page
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	7
1. Micronutrient malnutrition.....	7
2. Anemia.....	9
a. Iron deficiency anemia (IDA).....	13
b. Folate deficiency.....	19
c. Iron dietary sources.....	21
(1) Heme iron.....	21
(2) Non-heme iron.....	23
d. Iron chemical sources.....	24
1. Water soluble compounds.....	24
Ferrous sulphate.....	24
Ferrous gluconate.....	25
Ferric sodium ethylene diamine tetra acetate.....	26
Ferrous bisglycinate.....	27
2. Poorly water soluble /soluble in dilute acid.....	28
Ferrous fumarate.....	28
Ferrous succinate.....	29
3. Compounds water insoluble/poorly soluble in dilute acid.....	29
Elemental iron: Electrolytic – Carbonyl – Reduced	29
Ferric pyrophosphate - Ferric orthophosphate.....	30
e. Folic dietary sources.....	31
3. Effect of adding iron and folic acid.....	32
a. Effect of iron fortification and supplementation.....	32
b. Effect of folic acid fortification and supplementation.....	45
MATERIALS AND METHODS.....	
MATERIALS.....	47
1. Chemicals.....	47
2. Experimental animals.....	48
METHODS.....	48
1. Proximate analysis.....	48
a. Determination of moisture.....	48
b. Determination of ash content.....	48
c. Determination of crude protein.....	49

Continued.	
d. Determination of total lipid.....	49
e. Determination of crude fiber.....	49
f. Determination of total carbohydrate.....	50
g. Iron analysis.....	50
2. Rheological parameters	50
a. Farinograph test.....	50
b. Determination of gluten.....	51
3. Baking	51
a. Preparation of biscuit.....	51
b. Organoleptic evaluation of biscuits.....	51
4. Biological evaluation	52
a. Diet composition.....	52
b. Collection of blood samples.....	55
5. Biochemical analysis	56
a. Determination of hemoglobin.....	56
b. Determination of hematocrit.....	58
c. Determination of urea.....	58
d. Determination of cholesterol.....	59
e. Determination of alanine transaminase activity.....	61
f. Determination of aspartate transaminase activity.....	62
g. Determination of total protein in serum.....	64
6. Statistical analysis	65
RESULTS AND DISCUSSION	67
1. Chemical composition of wheat flour and thyme	67
2. Rheological parameters	71
a. Farinograph parameters of wheat flour and wheat flour blended with thyme.....	71
b. Gluten content.....	74
3. Organoleptic evaluation of biscuits	75
4. Chemical composition of biscuits	76
5. Biological evaluation of fortified biscuits	77
a. Effect of feed on fortified biscuits on body weight of rats	77
b. Organs weight of rats fed on fortified biscuits.....	81
c. Effect of feed on fortified biscuits on hemoglobin and hematocrit levels of rats.....	83
d. Effect of feed on fortified biscuits on Urea levels of rats	91

Continued.

e. Effect of feed on fortified biscuits on total cholesterol levels of rats.....	93
f. Effect of feed on fortified biscuits on alanine transaminases and aspartate transaminases activity of rats.....	97
g. Effect of feed on fortified biscuits on total protein levels of rats.....	103
SUMMARY.....	107
REFERENCES.....	113

LIST OF TABLES

No.	Title	Page
1.	Formulation of basal diet.....	52
2.	Composition of Salt mixture.....	53
3.	Composition of Vitamin mixture.....	54
4.	Composition of tested diets.....	56
5.	Chemical composition of raw materials.....	68
6.	Farinograph parameters of wheat flour blended with thyme.....	71
7.	Gluten contents and gluten index of wheat flour blended with thyme.....	74
8.	Organoleptic evaluation of biscuits fortified with ferrous sulfate or thyme as source of iron.....	75
9.	Chemical composition of biscuits fortified with ferrous sulfate or thyme as source of iron.....	77
10.	Effect of feed on fortified biscuits on body weight of rats	78
11.	Organs weight of rats fed on fortified biscuits	82
12.	Effect of feed on fortified biscuits on hemoglobin and hematocrit levels of rats.....	84
13.	Effect of feed on fortified biscuits on Urea levels of rats...	91
14.	Effect of feed on fortified biscuits on total cholesterol levels of rats.....	94
15.	Effect of feed on fortified biscuits on alanine transaminases and aspartate transaminases activity of rats	98
16.	Effect of feed on fortified biscuits on total protein levels of rats.....	104

LIST OF FIGURES

No.	Title	Page
1.	Farinograph of wheat flour 72%ext. and its blends with 3% and 4.5% thyme	72
2.	Mean value of body weight of different diets.....	79
3.	Mean value of hemoglobin level, of different diets.....	85
4.	Mean value of hematocrit level, of different diets.....	85
5.	Mean value of urea level of different diets.....	92
6.	Mean value of total cholesterol level of different diets.....	95
7.	Mean value of alanine transaminases ALT activity of different diets.....	99
8.	Mean value of aspartate transaminases AST activity of different diets.....	99
9.	Mean value of total protein level of different diets.....	105

INTRODUCTION

Nutritional anemia is an important health problem in many developing countries, and it leads to decreased capacity for physical work (Oppenheimer& Hendrickse, 1983). About 3.5 billion persons are affected by anemia in developing countries (ACC/SCN, 2000). Multiple micronutrient deficiencies have adverse effects on growth and development, especially in vulnerable groups like pregnant women and children (Arlappa *et al.*, 2011). In most cases anemia is caused by Iron deficiency (ID), although a smaller proportion is due to deficiencies of other micronutrients such as folate, and vitamins A and B12 (Iyengar&Nair, 2000) and even zinc (Hercberg *et al.*, 1987; Latham, 1997; Van den Broek& Letsky, 2000).Iron deficiency anemia (IDA) is frequently reported in chronic disorders (Weiss& Goodnough, 2005) including inflammatory bowel diseases (IBD) (Bager *et al.*, 2011) chronic heart failure (Enjuanes *et al.*, 2014) chronic kidney disease (Fishbane *et al.*, 2009) cancer (Gilreath *et al.*, 2014) rheumatoid arthritis (Masson, 2011) and obesity (Salgado *et al.*, 2014; Aigner *et al.*, 2014). Other studies have described an association between ID and obesity in children and adults (del Giudice *et al.*, 2009; Zekanowska *et al.*, 2011; Cheng *et al.*, 2012). IDA in children is a recognized public health problem that impacts adversely on child morbidity, mortality and impairs cognitive development (Habib *et al.*, 2016). Infants 6 months and older in developing countries are especially vulnerable because (1) the complementary foods offered to them are often not iron-dense and (2) although iron in human milk is highly bioavailable, it does not

provide sufficient quantities of iron to meet infants' requirements (Pachón *et al.*, 2008). Thus, the challenge in improving the iron status of infants from 6 months could be achieved by feeding infants iron-fortified foods coupled with an iron absorption enhancer (Lynch & Stoltzfus, 2003). After that period, the increased iron requirements for growth and a limited supply from breast milk (Abrams *et al.*, 1996) frequently result in ID with or without anemia, especially in many developing countries (Oski, 1993; Lonnerdal & Hernell, 1994). The prevalence of anemia is very high during pregnancy and was estimated to be 59% in 1980 in developing countries (DeMaeyer *et al.*, 1989). It is a major cause of maternal morbidity and mortality (Dallman, 1989), and it is one of the leading causes of anemia in infants and young children (Abu-Ouf & Jan, 2015).

ID is the single most common nutritional disorder worldwide. It is prevalent in most of the developing world and it is probably the only nutritional deficiency of consideration in industrialized countries (Rios *et al.*, 1983; DeMaeyer & Adiels-Tegman 1985; Viteri, 1994; De Benoist *et al.*, 2008; Domellof *et al.*, 2014; Quintaes *et al.*, 2015).

Folate deficiency is already an established risk factor for the development of certain types of cancer in the general population (Choi & Mason, 2000). A chronic deficiency of folate in the diet can cause anemia (Selhub & Rosenberg, 1996), but low concentrations of serum folate and vitamin B-12 and elevated concentrations of plasma total homocysteine have also been associated with psychiatric disorders (Sauberlich, 1991); the development of dementia, Alzheimer disease,

and cognitive dysfunction (Riggs *et al.*, 1996; Duthis *et al.*, 2002; Vermeer *et al.*, 2002; Seshadri *et al.*, 2002); a decline in physical function (Kado *et al.*, 2002); osteoporosis and hip fractures in the elderly (McLean *et al.*, 2004; Van Meurs *et al.*, 2004); and an increased risk of carotid artery stenosis (Selhub *et al.*, 1993). It has been associated with numerous diseases and birth defects including orofacial defects (Wahl *et al.*, 2015).

Iron fortification and supplementation are considered the major approaches to the control of ID and IDA. However, appropriate selection of iron fortificants remains an important technical issue (Ricardo *et al.*, 2002)

Supplementation is a short-term solution to acute nutritional deficiencies, (Van Stuijvenberg *et al.*, 1999). Some studies indicated that iron supplementation has positive effects on hemoglobin (Hb) levels and growth (Lawless *et al.*, 1994; Kanani and Poojara, 2000). There is concern that universal supplementation of children with iron and folic acid in areas of high malaria transmission might be harmful (Sazawal *et al.*, 2006).

Food fortification is often suggested as one of the most effective and sustainable strategies for increasing iron intake in the general population (Hurrell, 1997, 2002a, b). It offers a solution in both the medium and long term (Van Stuijvenberg *et al.*, 1999). Flour fortification with iron and other nutrients has been practiced in many countries (Sun *et al.*, 2007). For food fortification to be effective, it is important that a suitable vehicle be found (Van Stuijvenberg *et al.*,

1999). To increase the levels of folate and iron in the general population, compulsory fortification of foods with folic acid, iron and other nutrients has been implemented in many countries (Jacques *et al.*, 1999; Hertrampf *et al.*, 2003). Several international studies have reported improved folate status after fortification of foods, (Castilla *et al.*, 2003) since synthetic folic acid in fortified foods is more bioavailable than naturally occurring folate (Hertrampf & Cortés, 2004).

Cereals are a staple and healthy food, providing a good source of carbohydrates, fiber, and phytochemicals, and are low in fat. They are considered the major supplier of energy in the human diet with starch as the main component of the grain (Quintaes *et al.*, 2015). Since the importance of cereals in human nutrition has been recognized in past few decades, the production of all kinds of whole grain cereal based products has been growing constantly ever since (Vitali *et al.*, 2007). Those products include the new generation of enriched biscuits which are a basic and widely consumed food with different flavours, therefore, no longer considered just as high energy foods but also as sources of numerous nutritive and health protecting substances (Doner & Ege, 2004; Vitali *et al.*, 2007). The nutrition intervention of Bal *et al.*, (2015) clearly highlights biscuits as an ideal fortification vehicle for addressing anemia and to bring about a positive change in the anemia and health status in school aged children.

The aim of this study was to create a healthy nutritive biscuit which can be considered as valuable source of non-heme iron in diet by