



AIN SHAMS UNIVERSITY  
FACULTY OF SCIENCE  
BIOCHEMISTRY DEPARTMENT

# **Biochemical Studies on Antioxidant Activity of Certain Irradiated Agro Industrial Wastes**

**SUBMITTED BY**  
**ASHRAF MOHAMED MONIR SHAABAN**

M. Sc. Biochemistry Ain Shams University (2008)

For Fulfillment of PhD Degree in Biochemistry

## **Supervising Committee**

**Dr. Dina Mohamed Seoudi    Prof. Dr. M. Diah El-Din H. Farag**

Assistant Prof. of Biochemistry  
Biochemistry Department  
Faculty of Science  
Ain Shams University

Prof. of Biological Sciences  
Chairman of Industrial Irradiation  
Division, Former  
National Center for Radiation  
Research and Technology  
Atomic Energy Authority

**Dr. Nahla Samir Hassan**

Assistant Prof. of Biochemistry  
Biochemistry Department  
Faculty of Science  
Ain Shams University

**Dr. Manal Asem Emam**

Assistant Prof. of Biochemistry  
Biochemistry Department  
Faculty of Science  
Ain Shams University

*Department of Biochemistry  
Faculty of Science  
Ain Shams University*

**2016**

# **Biochemical Studies on Antioxidant Activity of Certain Irradiated Agro Industrial Wastes**

## ***Board of Scientific Supervision***

### ***Dr. Dina Mohamed Seoudi***

Assistant Prof. of Biochemistry  
Biochemistry Department  
Faculty of Science  
Ain Shams University

### ***Prof. Dr. M. Diao El-Din H. Farag***

Prof. of Biological Sciences  
Chairman of Industrial Irradiation Division, Former  
National Center for Radiation Research and Technology  
Atomic Energy Authority

### ***Dr. Nahla Samir Hassan***

Assistant Prof. of Biochemistry  
Biochemistry Department  
Faculty of Science  
Ain Shams University

### ***Dr. Manal Asem Emam***

Assistant Prof. of Biochemistry  
Biochemistry Department  
Faculty of Science  
Ain Shams University

## **Approval Sheet**

# **Biochemical Studies on Antioxidant Activity of Certain Irradiated Agro Industrial Wastes**

By

**Ashraf Mohamed Monir Shaaban Mabrouk**

B. Sc. of Science, Bio Chemistry (1995)

M. Sc. of Science in Biochemistry (2008)

**Biochemistry Department, Faculty of Science,  
Ain Shams University**

**This thesis for PhD degree has been approved by:**

**Prof. Dr. Samir Mostafa Abdel Aziz** .....

Prof. of Biochemistry, Director of Middle Eastern Regional  
Radioisotope Centre for the Arab Countries, Former.

**Prof. Dr. Amal Saad Eldin Hussein** .....

Prof. of Environmental & Preventive Medicine, Head of Environmental  
& Occupational Medicine Department, Environmental Research  
Division, National Research Center.

**Prof. Dr. Mohamed Diao El-Din H. Farag** .....

Prof. of Biological Science, Chairman of Industrial Irradiation Division,  
Former, National Center for Radiation Research and Technology,  
Atomic Energy Authority.

**Dr. Dina Mohamed Seoudi** .....

Assistant Prof. of Biochemistry, Biochemistry Department, Faculty of  
Science, Ain Shams University.

## **ACKNOWLEDGMENTS**

*First and foremost all praise to **Allah** the most gracious and most merciful, who guides the straight path, for all **His** gifts throughout life, for helping to complete this work. And the peace and greeting are upon the prophet **Mohamed** as well as his family and his companions.*

*I wish to express my sincere appreciation, infinite gratitude to **Dr. Dina Mohamed Seoudi, Dr. Nahla Samir Hassan, and Dr. Manal Asem Emam**, Assistant Professors of Biochemistry, Faculty of Science, Ain Shams University for their great support, their meticulous supervision, patience, encouragement and finally I wish to thank them for everything I had been taught from them.*

*My thanks also due to **Prof. Dr. M. Diao El-Din H. Farag**, Prof. of Biological Sciences, Chairman of Industrial Irradiation Division, Former, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority for his supervision, suggesting the point of research, guiding, inspiring discussion, criticism, building up this thesis and finally I wish to thank him for everything I had been taught.*

*Finally, special debt of gratitude to my father's soul **Prof. Dr. Mohamed Monir Shaaban** whom I miss his presence and support but I will not ignore that he was the mentor that guided and inspired me, all my thanks also goes to my mother, brother, wife, all my colleges at the Food Irradiation Research Department, NCRRT and to my other friends, too numerous to mention who helped me in one way or the others.*

**Ashraf Mohamed Monir Shaaban**

## Contents

<i>Subject</i>	<i>Page no.</i>
<b>Abstract</b> .....	I-III
List of tables .....	
List of figures .....	
List of abbreviations .....	
<b>Introduction</b> .....	1-5
<b>Aim of the work</b>	6
<b>Review</b>	7-51
1-Antioxidants and Their Physiological and Biochemical Effects.	7
1.1 Antioxidants and Rheumatoid Arthritis.....	9
1.2 Antioxidant and Cancer.....	12
1.3 Antioxidants and diabetes .....	17
1.4 Antioxidant and atherosclerotic risk factors.....	22
2-Dietary antioxidants.....	25
2.1 Grapes and its by-products.....	25
2.2 Tomato and its pomace.....	30
2.3 Artichoke and its leaves.....	37
3-Irradiation processing for food, feed and agro-industrial by-products.....	44
<b>Materials and methods</b>	52-73
<b>1. Materials</b>	52
1.1. Plant material.....	52
1.2. Radiation Processing.....	52
1.3. Animals .....	53
<b>2. Methods</b>	55
2.1. Chemical composition .....	55
2.1.1. Moisture content.....	55
2.1.2. Crude fat .....	55
2.1.3. Crude protein (CP).....	56
2.1.4. Ash content.....	58
2.1.5. Crude fiber content (CF).....	58
2.1.6. Total carbohydrate (Nitrogen free extract, NFE).....	59
2.1.7. Lipid extraction and determination of fatty acids ....	59
2.1.8. Amino acids determination.....	60
2.1.9. Tannins analysis.....	61

<i><b>Subject</b></i>	<i><b>Page no.</b></i>
2.1.10. Antioxidant activities (DPPH assay).....	62
2.1.11. Total Phenolic compounds.....	63
2.2. Biochemical parameters.....	64
2.2.1. Serum ALT (Alanine Aminotransferase) .....	65
2.2.2. Serum AST (Aspartate aminotransferase) .....	66
2.2.3. Serum Cholesterol.....	67
2.2.4. Serum HDL- C.....	68
2.2.5. Serum Triglycerides.....	69
2.2.6. Serum LDL-C.....	71
2.2.7. Serum VLDL-C .....	71
2.2.8. Atherogenic Index (AI) .....	71
2.2.9. Plasma glucose.....	71
2.3. Organs weights.....	72
2.4. Statistical analysis.....	73
<b>Results</b> .....	74-134
<b>Discussion</b> .....	135-156
<b>Summary</b> .....	157-164
<b>References</b> .....	165-209
<b>Arabic Summary</b> .....	I-II
<b>Arabic Abstract</b> .....	III-IX

# Abstract

### Abstract

***Ashraf Mohamed Monir Shaaban "Biochemical studies on antioxidant activity of certain irradiated agro industrial wastes".  
PhD. Thesis, Ain-Shams University, Faculty of Science,  
Biochemistry Department.***

Food irradiation is a method of preservation; it is used to extend the shelf life of food products fresh and/or dried, destroy the contaminating harmful pathogens and modify the activity of bioactive compounds present in food materials.

The present study was conducted to test the possible biochemical impacts of radiation processing on three agro industrial wastes (grape seed-skin, tomato pomace and artichoke leaves), as well as to elucidate the physiological and biochemical effects of feeding growing male Wister rats on diets supplemented with the processed agro industrial wastes at dose levels of 10, 20 and 30 kGy.

The chemical composition of processed agro industrial wastes at the above mentioned doses showed no significant differences in corresponding to the non-irradiated one. The orthogonal statistical analysis of the data revealed that the linear, quadratic and/or cubic effects were not significantly different; however, an exception for these general observations was observed for fiber content where it was significantly reduced accompanied with increase in nitrogen free extract.

## Abstract

---

Statistical analysis of polyphenol, tannin content and free radical scavenging activity results showed that irradiation processing caused an increase in polyphenols as a function of radiation dose, meanwhile grape seed-skin processed by 20 kGy gamma rays showed a decrease by 15.38%, tannin content exhibited an increase in both grape seed-skin and artichoke leaves at all radiation doses used. In contrast, grape seed-skin irradiated at dose level 10 kGy has lost 25 % corresponding to their control one, same observation of reduction in tannins content of tomato pomace, was recorded. Regarding to the impact of irradiation treatment up to 30 kGy on free radical scavenging activity of grape seeds-skin, tomato pomace and artichoke leaves, the results indicated that there was a significant reduction in their ability to scavenge free radicals, while an exception was observed for artichoke leaves processed by 10 kGy.

The amino acid pattern was significantly affected by gamma irradiation, where some amino acids increased; others decreased, or did not change. It was observed that the changes in amino acids pattern as affected by irradiation up to and including 30 kGy was occurred without a specific trend in the three agro industrial wastes under investigation.

The fatty acid profile of the three agro industrial wastes under investigation was significantly affected by radiation processing which caused an increase in some fatty acids, decrease

## Abstract

---

in others and some did not change, compared to those corresponding fatty acids in non-irradiated three agro industrial wastes, with no specific trend.

The physiological and biochemical performance of growing male Wister rats as affected by feeding high fat diets supplemented with non-irradiated or irradiated up to and including 30 kGy agro industrial wastes under investigation for 8 weeks, showed better results when compared with those rats fed on reference diet. It has been manifested in body weight gain and internal organ weight alongside biochemical aspects such as serum total cholesterol, triglycerides, LDL-C, HDL-C, VLDL-C, AI, ALT, AST and serum glucose.

## *List of Tables*

<b>Table No.</b>	<b>Legend</b>	<b>Page</b>
<b>Table (1)</b>	Effects and applications of $\gamma$ -rays doses in food irradiation	48
<b>Table (2)</b>	Composition of reference diet	54
<b>Table (3)</b>	Chemical composition of raw and gamma irradiated grape seeds-skin	75
<b>Table (4)</b>	Chemical composition of raw and gamma irradiated tomato pomace	78
<b>Table (5)</b>	Chemical composition of raw and gamma irradiated artichoke leaves	81
<b>Table (6)</b>	Amino acids composition of irradiated and non-irradiated grape seed - skin	84
<b>Table (7)</b>	Amino acids composition of irradiated and non-irradiated Tomato pomace	87
<b>Table (8)</b>	Amino acids composition of irradiated and non-irradiated Artichoke leafs	90
<b>Table (9)</b>	Fatty acids (%) of raw and gamma irradiated wastes	94
<b>Table (10)</b>	Antioxidant content and activity of raw and gamma irradiated grape seeds-skin	97
<b>Table (11)</b>	Antioxidant content and activity of raw and gamma irradiated tomato pomace	100
<b>Table (12)</b>	Antioxidant content and activity of raw and gamma irradiated artichoke leaves	103
<b>Table (13)</b>	Body mass of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets	106
<b>Table (14)</b>	Body mass of rats fed on non-irradiated and irradiated Tomato Pomace incorporated in high fat diets	109
<b>Table (15)</b>	Body mass of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets	112
<b>Table (16)</b>	Relative weight of organs of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets	115
<b>Table (17)</b>	Relative weight of organs of rats fed on non-irradiated and irradiated Tomato pomace incorporated in high fat diets	118

<b><i>Table No.</i></b>	<b><i>Legend</i></b>	<b><i>Page</i></b>
<b>Table (18)</b>	Relative weight of organs of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets	121
<b>Table (19)</b>	Some biochemical parameters of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diet	125
<b>Table (20)</b>	Some biochemical parameters of rats fed on non-irradiated and irradiated Tomato pomace incorporated in high fat diets	129
<b>Table (21)</b>	Some biochemical parameters of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets	133

## *List of Figures*

<b>Figure No.</b>	<b>Legend</b>	<b>Page</b>
<b>Fig. ( 1 )</b>	Chemical composition of raw and gamma irradiated grape seeds–skin	76
<b>Fig. ( 2 )</b>	Chemical composition of raw and gamma irradiated tomato pomace	79
<b>Fig. ( 3 )</b>	Chemical composition of raw and gamma irradiated artichoke leaves	82
<b>Fig. ( 4 )</b>	Percentage change of calculated parameters of amino acids of raw and $\gamma$ -irradiated grape seeds-skin	85
<b>Fig. ( 5 )</b>	Percentage change of calculated parameters of amino acids of raw and $\gamma$ -irradiated tomato pomace	88
<b>Fig. ( 6 )</b>	Percentage change of calculated parameters of amino acids of raw and $\gamma$ -irradiated artichoke leaves	91
<b>Fig. ( 7 )</b>	Changes of fatty acids content of raw and $\gamma$ -irradiated grape seeds-skin as being affected by irradiation treatment	95
<b>Fig. ( 8 )</b>	Changes of fatty acids content of raw and $\gamma$ -irradiated tomato pomace as being affected by irradiation treatment	95
<b>Fig. ( 9 )</b>	Changes of fatty acids content of raw and $\gamma$ -irradiated artichoke leaves as being affected by irradiation treatment	95
<b>Fig. (10)</b>	percentage change of antioxidant parameters of $\gamma$ -irradiated grape seeds-skin compared to non-irradiated	104
<b>Fig. (11)</b>	Percentage change of antioxidant parameters of $\gamma$ -irradiated tomato pomace compared to non-irradiated	104
<b>Fig. (12)</b>	Percentage change of antioxidant parameters of $\gamma$ -irradiated artichoke leaves	104
<b>Fig. (13)</b>	Body mass of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets	107
<b>Fig. (14)</b>	Body mass of rats fed on non-irradiated and irradiated Tomato pomace incorporated in high fat diets	110

<b><i>Figure No.</i></b>	<b><i>Legend</i></b>	<b><i>Page</i></b>
<b>Fig. (15)</b>	Body mass of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets	113
<b>Fig. (16)</b>	Relative weight of organs of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets	116
<b>Fig. (17)</b>	Relative weight of organs of rats fed on non-irradiated and irradiated Tomato pomace incorporated in high fat diets	119
<b>Fig. (18)</b>	Relative weight of organs of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets	122
<b>Fig. (19-a)</b>	Some biochemical parameters of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets	126
<b>Fig. (19-b)</b>	Atherogenic index and AST/ALT ratio of rats fed on non-irradiated and irradiated Grape seed-skin incorporated in high fat diets	126
<b>Fig. (20-a)</b>	Some biochemical parameters of rats fed on non-irradiated and irradiated Tomato pomace incorporated in high fat diets	130
<b>Fig. (20-b)</b>	Atherogenic index and AST/ALT ratio of rats fed on non-irradiated and irradiated Tomato pomace incorporated in high fat diets	130
<b>Fig. (21-a)</b>	Some biochemical parameters of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets	134
<b>Fig. (21-b)</b>	Atherogenic index and AST/ALT ratio of rats fed on non-irradiated and irradiated Artichoke leaves incorporated in high fat diets	134

### *List of Abbreviations*

<b><i>Abb.</i></b>	<b><i>Full Name</i></b>
<b>A.O. A. C.</b>	Association of Official Analytical Chemists
<b>AI</b>	Atherogenic index
<b>ALT</b>	Alanine Aminotransferase
<b>AST</b>	Aspartate aminotransferase
<b>CF</b>	Crude fiber
<b>CP</b>	Crude protein
<b>DPPH</b>	2, 2-diphenyl-1-picrylhydrazyl
<b>DWG</b>	Daily weight gain
<b>FAO</b>	Food and Agriculture Organization of the United Nations
<b>FW</b>	Final weight
<b>HDL-C</b>	High density lipoproteins cholesterol
<b>IAEA</b>	International Atomic Energy Agency
<b>IW</b>	Initial weight
<b>LDL-C</b>	Low density lipoproteins cholesterol
<b>Mo</b>	Moisture
<b>NFE</b>	Nitrogen free extract
<b>RNS</b>	Reactive nitrogen species
<b>ROS</b>	Reactive oxygen species
<b>TAA</b>	Total amino acids
<b>TEAA</b>	Total essential amino acids
<b>TNEAA</b>	Total non-essential amino acids
<b>TWG</b>	Total weight gain
<b>VLDL-C</b>	Very low density lipoproteins cholestero;
<b>WHO</b>	World Health Organization
<b><math>\gamma</math></b>	Gamma irradiation