ROLE OF ACTIVE COMPOUND OF SOME SPICES IN PROTECTION FROM IONIZING RADIATION EFFECT

WAFAA MOSTAFA ALI ELSAYED SHAHIN

B.Sc. Agric. Sc. (Food science and Technology), Cairo Univ. (2006) M.Sc. Agric. Sc. (Food science and Technology), Cairo Univ. (2013)

A Thesis Submitted in Partial Fulfillment
Of
The Requirements for the Degree of

in
Agricultural Sciences
(Food Science and Technology)

Department of Food Science and Technology
Faculty of Agriculture
Ain Shams University

Approval sheet

ROLE OF ACTIVE COMPOUND OF SOME SPICES IN PROTECTION FROM IONIZING RADIATION EFFECT

WAFAA MOSTAFA ALI ELSAYED SHAHIN

B.Sc. Agric. Sc. (Food science and Technology), Cairo Univ. (2006) M.Sc. Agric. Sc. (Food science and Technology), Cairo Univ. (2013)

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

Dr.	Ashraf Mahdy Sharoba
	Prof. of Food Science and Technology, Faculty of Agriculture, Benha
	University.
Dr.	Nessrien Mohamed Nabih Yasin
	Prof. of Food Science and Technology, Faculty of Agriculture, Ain
	Shams University.
Dr.	Ahmed Youssef Gibriel
	Prof. Emeritus of Food Science and Technology, Faculty of
	Agriculture, Ain Shams University.

Date of Examination: 12/10/2019

ROLE OF ACTIVE COMPOUND OF SOME SPICES IN PROTECTION FROM IONIZING RADIATION EFFECT

WAFAA MOSTAFA ALI ELSAYED SHAHIN

B.Sc. Agric. Sc. (Food science and Technology), Cairo Univ. (2006) M.Sc. Agric. Sc. (Food science and Technology), Cairo Univ. (2013)

Under the supervision of:

Dr. Ahmed Youssef Gibriel

Prof. Emeritus of Food Science and Technology, Food Science and Technology department, Faculty of Agriculture, Ain Shams University.

Dr. Hanan Mohamed Abdo

Prof. of Food Science and Technology, Food Science and Technology department, Faculty of Agriculture, Ain Shams University.

Dr. Mourad Aziz Hanna

Prof. Emeritus of Radiation Chemistry, Protection Dept., Nuclear Research Center, Atomic Energy Authority.

ABSTRACT

Wafaa Mostafa Ali Elsayed Shahin: Role of Active Compounds of Some Spices in Protection from Ionizing Radiation Effects. Unpublished PhD. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2019.

Rosemary leaves and clove buds were irradiated with γ -irradiation at different doses (0 to 30kGy) and stored for 12months at room temperature (22±3⁰C). The evaluation of irradiation depended on the chemical studies and antioxidant activity of extracted essential oils. Results showed significant difference for the essential oils yield at different doses during storage time. Compounds were fractionated and identified of extracted essential oils from each of non-irradiated and irradiated rosemary leaves and clove buds samples, the main components of essential oil extracted from either non-irradiated or irradiated rosemary were 1,8 cineole, camphor and γ-pinene 26.36, 12.63 and 16.98%, respectively, while, eugenol was the major component in clove essential oil (which extracted from non-irradiated and irradiated samples 81.69%). No differences were noticed in the % of essential oils constituents. Antioxidant activity as % of DPPH scavenging increased with the increasing of essential oils concentrations (250 up to 2500ppm). Furthermore, the effect of adding these extracted oils from non-irradiated and irradiated samples as well as their mixture (1:1) on the threshold odor scores of sunflower oil was observed. Rancimat test shown the high oxidative stability in sunflower oil which contained 0.3% clove essential oil which extracted from 15kGy irradiated sample was 11.99 hrs, its relative stability was 144.11% comparing with the oxidative stability of sunflower oil which contained 0.5% rosemary essential oil extracted from non-irradiated sample (9.76hrs) with 117.3% relative stability.

In the vivo study, 125 male rats exposed to 1 and 5Gy treated with clove and rosemary essential oils and their mixture (1:1) in three different intake times (21days before irradiation, 21days after exposing and 21

days before followed by 7days after irradiation) for evaluating the effect of both essential oils beside their mixture (1:1) on the tested physiological functions as a radioprotector. Assessment of essential oil treatments depending on measuring some physiological indicators, note the inducing change and recovery in these indicators. The best recovery was the sustained administration with clove essential oil for continuous 14days before gamma irradiation followed by 7days after 1Gy irradiation, while the lowest repair which administrated with the mixture of clove and rosemary essential oils (1:1) for 21consecutive days after rats exposing with 5Gy for all tested parameters. Also, during histopathological examination the liver more affected by irradiation than kidney. The treatment with clove essential oil induced best recovery compared with rosemary essential oil and their mixture.

Keywords: Rosemary leaves, Clove buds, Irradiation, Essential oil, GC/MS, Antioxidant activity, Rancimat, Biochemical tests and Histopathological examination.

ACKNOWLEDGEMENT

I feel myself inept as my words have lost their expressions, knowledge is lacking and diction is too short to express gratitude in the rightful manner to the blessings and support of **Allah**.

I wish to express my sincere thanks, deepest gratitude and appreciation to **Dr. Ahmed Youssef Gibriel** Prof. Emeritus of Food Technology, Food Science Department, Faculty of Agriculture, Ain Shams University, for his sincere help, continuous encouragement, keen supervision, generous support and giving every possible advice through the study and during writing of the manuscript.

I am very grateful to **Dr. Hanan Mohamed Abdo** Professor of Food Technology, Food Science Department, Faculty of Agriculture, Ain Shams University, for her sincere help, kind advice and supervision.

I would like to thank **Dr. Morad Aziz Hana** Professor of Radiation Chemistry, Bioassay unit, Radiation Protection Department, Nuclear Research Center, Atomic Energy Authority of Egypt for his sincere help, supervision and kind help through the work.

I would like to express my deep thanks **Dr. Abdullah Ahmed Gibriel** Biochemistry department, Faculty of Pharmacy, British
University for his kind and sincere help.

My deepest gratitude and appreciation to **Dr.Magda Amer** Professor of Physiology, Nuclear Research Center, Atomic Energy Authority of Egypt, for her encouragement, guidance, and unlimited help.

I dedicate this work to whom my heartfelt thanks; to my mother' soul, and would like to offer my deep thanks to my father, my brothers and sisters, my kind family who support me with all their effort, dear husband for his patience and help, my sons, and my cute daughters (Doaa and Ashrakat).

My deepest thanks to **Dr. Lamis A. Attia,** Bioassay unit, Radiation Protection Department, Nuclear Research Center, Atomic Energy Authority of Egypt for her help in laboratory work and preparation of this work.

My deepest thanks to **Dr. Karima S. Hammad,** Food Science Department, Faculty of Agriculture, Cairo University, for her sincere help, kind advice and supervision.

I want also to thank **all members**, of Clinical Chemistry unit, **Dr. Amal**, **Dr. Amaal**, **Dr. Doaa and Dr. Zeinab**, Nuclear Research center, Atomic Energy Authority of Egypt.

CONTENTS

	Page
LIST OF TABLES	
LIST OF FIGURES	
LIST OF ABBREVIATIONS	
INTRODUCTION	1
REVIEW OF LITERATURE	6
2.1. Essential oils	6
2.1.1. Using of essential oils in food industry	6
2.2. Antioxidant activity	7
2.3. Irradiation process	8
2.3.1. Irradiation of spices and herbs	9
2.3.1.1. Effect of γ -irradiation on the extracted essential oils	
content	10
2.3.1.2. The effect of γ - irradiation on the chemical constituents	
of extracted essential oils	11
2.3.1.3. Effect of γ -irradiation on the antioxidant activity of	
extracted essential oils	14
2.4. Biological effect of ionizing radiation	18
2.4.1. Direct effects of ionizing radiation	19
2.4.2. Indirect effects of ionizing radiation	19
2.4.3. Destructive changes caused by free radicals	20
2.4.4. Cell damage	21
2.4.5. Oxidative stress	23
2.4.5.1. Effect of exposure to ionizing radiation on the	
antioxidant defense status	24
2.4.5.1.1. Effect of exposure to ionizing radiation on the lipid	
peroxidation	24
2.4.5.1.2. Effect of exposure to ionizing radiation on the	
glutathione content (GSH)	25
2.4.5.1.3. Effect of exposure to ionizing radiation on the catalase	26
2.4.6. Effect of exposure to ionizing radiation on the liver	26
2.4.6.1. Effect of exposure to ionizing radiation on ALP activity	27

	Page
2.4.6.2. Effect of exposure to ionizing radiation on ALT and	
AST activities	28
2.4.7. Effects of exposure to ionizing radiation on the renal	
functions	29
2.4.7.1. Effect of exposure to ionizing radiation on the urea level	29
2.4.7.2. Effect of exposure to ionizing radiation on the creatinine	
level	30
2.4.8. Effect of exposure to ionizing radiation on the lipid	
metabolism	30
2.4.8.1. Effect of exposure to ionizing radiation on the	
cholesterol and triglycerides levels	31
2.4.9. Effect of exposure to ionizing radiation on the lipid profile	
(albumin content)	32
2.5. Role of spices in radiation recoveries	33
2.5.1. Mechanism of action	35
MATERIAL AND METHODS	37
3.1. Materials	37
3.1.1 Plant materials	37
3.1.2 Chemicals	37
3.1.3 Sun flower oil	37
3.1.4 Male albino rats "Swiss strain"	37
3.1.5 Animal diet	37
3.1.6. Reagent kits	38
3.2. Methods	38
3.2.1. Preparation of different samples	38
3.2.2. Technological methods	38
3.2.2.1. Irradiation of plant materials	38
3.2.2.2. Extraction of essential oils	38
3.2.3. Analytical methods	39
3.2.3.1. Identification of chemical components of essential oils	39
3.2.3.2. Percentages of essential oils	39
3.2.3.3. Physicochemical properties of essential oils	39
3.2.3.3.1. Specific gravity	39

	Page
3.2.3.3.2. Refractive index	40
3.2.3.3. Solubility in diluted alcohol	40
3.2.3.3.4. Acid value	40
3.2.3.3.5. Ester value	40
3.2.3.4. Evaluation of adding extracted essential oils on the odor	
of sunflower oil	40
3.2.3.5. Determination of the antioxidant activity of extracted	
essential oils	41
3.2.3.5.1. Measurement of scavenging activity	41
3.2.3.5.2. Measurement of sunflower oil stability as a food	
system by Rancimat apparatus	41
3.3. Biological evaluation of extracted essential oils and its	
protective effect against ionizing radiation	42
3.3.1. Animals housing and feeding	42
3.3.2. Animal irradiation	42
3.3.3. In vivo experiment design	42
3.3.4. Essential oil dose	43
3.3.5. Biochemical assay	43
3.3.5.1. Preparation of blood samples	44
3.3.5.2. Assay of various biochemical parameters in plasma	44
3.3.5.2.1. Determination of alanine amino transferase (ALT)	
activity	44
3.3.5.2.2. Determination of aspartate aminotransferase (AST)	
activity	44
3.3.5.2.3. Determination of alkaline phosphatase (ALP) activity	45
3.3.5.2.4. Determination of urea concentration	45
3.3.5.2.5. Determination of creatinine concentration	45
3.3.5.2.6. Determination of cholesterol concentration	45
3.3.5.2.7. Determination of triglyceride concentration	46
3.3.5.2.8. Determination of albumin concentration	46
3.3.5.3. Antioxidant defense status	47
3.3.5.3.1. Determination of plasma lipid peroxidation (MDA)	
level	47

	Page
3.3.5.3.2. Determination of plasma glutathione (GSH) content	47
3.3.5.3.3. Determination of plasma Catalase	47
3.3.6. Histopathological examination	48
3.4. Statistical analysis	50
RESULTS AND DISCUSSION	51
4.1. Extracted essential oils content (%)	51
4.2. Identification of extracted essential oil components	55
4.2.1. Identification of extracted essential oil components of	
rosemary leaves	55
4.2.2. Identification of extracted essential oil components of	
clove buds	58
4.3. Physico-chemical properties of extracted essential oils	60
4.4. Antioxidant activity of extracted essential oils	64
4.5. Acceptable odor levels of essential oils of rosemary leaves	
as well as clove buds added to sunflower oil	68
4.6. Oxidative stability of sunflower oil	71
4.7. Biochemical assay of irradiated rats	73
4.7.1. Liver function	74
4.7.1.1. Alkaline phosphatase (ALP) activities	75
4.7.1.2. Alanine aminotransferase (ALT) activities	77
4.7.1.3. Aspartate aminotransferase (AST) activities	80
4.7.2. Kidney function	84
4.7.2.1. Urea concentrations	84
4.7.2.2. Creatinine concentrations	87
4.7.3. Cholesterol levels	90
4.7.4. Triglyceride (TG) levels	92
4.7.5. Albumin levels	95
4.7.6. Antioxidant defense status	98
4.7.6.1. Lipid peroxidation levels (MDA)	100
4.7.6.2. Glutathione (GSH) contents	103
4.7.6.3. Catalase content	107
4.8. Histopathological examination	111
4.8.1. Liver tissue histopathology of irradiated rats	111

	Page
4.8.1.1. The liver tissue histopathology of normal rats	111
4.8.1.2. The liver tissue histopathology of treated rats with	
essential oils	112
4.8.1.3. The liver tissue histopathology of irradiated rats	112
4.8.1.4. The liver tissue histopathology of irradiated rats after	
treatment (timing A)	112
4.8.1.5. The liver tissue histopathology of irradiated rats before	
treatment (timing B)	119
4.8.1.6. The liver tissue histopathology of irradiated rats before	
and after treatment (timing C)	119
4.8.2. Kidney tissue histopathology of irradiated rats	125
4.8.2.1. The kidney tissue histopathology of normal rats	125
4.8.2.2. The kidney tissue histopathology of treated rats of	
essential oils	127
4.8.2.3. The kidney tissue histopathology of irradiated rats	129
4.8.2.4. The kidney tissue histopathology of irradiated rats in	
after treatment (timing A)	129
4.8.2.5. The kidney tissue histopathology of irradiated rats	
before treatment (timing B)	133
4.8.2.6. The kidney tissue histopathology of irradiated rats	
before and after treatment (timing C)	136
SUMMARY	139
REFERENCES	145
ARABIC SUMMARY	

LIST OF TABLES

No.		Page.
(1)	Essential oil content (%) of irradiated rosemary as	
	well as clove buds during storage at 0, 3, 6, 9 and	
	12months	53
(2)	Percentages of the identified components for	
	extracted essential oils of irradiated dry	
	rosemary leaves	57
(3)	Percentages of the identified components for	
	extracted essential oils of irradiated dry clove	
	buds	60
(4)	Physico-chemical properties of extracted essential	
	oils from dry rosemary leaves as well as dry clove	
	buds either non-irradiated or irradiated	62
(5)	Antioxidant activity of extracted essential oils	
	from either non-irradiated or irradiated rosemary	
	leaves and clove buds	66
(6)	Effect of irradiation on the threshold score of	
	rosemary, clove buds and their mixture (1:1)	
	essentials oil added to sun flower oil (Mean of	
	acceptable odor levels)	69
(7)	Effect of different concentrations of irradiated	
	rosemary leaves, clove buds essential oils and their	
	mixture on the oxidative stability of sunflower oil	
	assessed by Rancimat test	72
(8)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the alkaline phosphatase	
(0)	(ALP) activities of irradiated rats	76
(9)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the alanine amino	
(4.0)	transferase (ALT) activities of irradiated rats	79
(10)	Effect of extracted clove, rosemary essential oils	

	and their mixture (1:1) on the aspartate amino	
	transferase (AST) activities of irradiated rats	82
(11)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the Urea concentrations	
	of irradiated rats	86
(12)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the Creatinine	
	concentrations of irradiated rats	89
(13)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the cholesterol levels	
	of irradiated rats	91
(14)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the triglycerides levels	
	of irradiated rats	94
(15)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the albumin contents of	
	irradiated rats	97
(16)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the lipid peroxidation	
	(MDA) levels of irradiated rats	102
(17)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the glutathione (GSH)	
	contents of irradiated rats	104
(18)	Effect of extracted clove, rosemary essential oils	
	and their mixture (1:1) on the catalase levels of	
	irradiated rats	108

LIST OF FIGURES

No.		Page
(1)	Possible cellular changes following exposure to	
	ionizing radiation	23
(2)	The putative mechanisms of radioprotection by various	
	plants and herbs	36
(3)	Change in essential oil content (%) of irradiated	
	rosemary leaves during storage periods (months)	54
(4)	Change in essential oil content (%) of irradiated clove	
	buds during storage periods (months)	54
(5)	Physico-chemical properties of extracted essential oils	
	from dry rosemary leaves either non-irradiated or	
	irradiated	63
(6)	Physico-chemical properties of extracted essential oils	
	from clove buds either non-irradiated or irradiated	63
(7)	Antioxidant activity of extracted essential oils from	
	either non-irradiated or irradiated rosemary leaves	67
(8)	Antioxidant activity of extracted essential oils from	
	either non-irradiated or irradiated clove buds	67
(9)	Effect of irradiation on the threshold score of rosemary	
	essential oil added to sun flower oil (Mean of	
	acceptable odor levels)	70
(10)	Effect of irradiation on the threshold score of clove	
	buds essential oil added to sun flower oil (Mean of	
	acceptable odor levels)	70
(11)	Effect of irradiation on the threshold score of the	
	mixture of rosemary and clove (1:1) essentials oil	
	added to sun flower oil (Mean of acceptable odor	
	levels)	71
(12)	Effect of different concentrations of irradiated	
	rosemary leaves, clove buds essential oils and their	