BIOCHEMICAL STUDIES ON PHENOLIC COMPOUNDS OF BROCCOLI AND SWEET FENNEL VARIETIES

By

AIAA ABD ALLAH HASSAN GAAFAR

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Zagazig Univ., 1994 M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Zagazig Univ., 2002

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences (Biochemistry)

Department of Biochemistry
Faculty of Agriculture
Cairo University
EGYPT

2010

APPROVAL SHEET

BIOCHEMICAL STUDIES ON PHENOLIC COMPOUNDS OF BROCCOLI AND SWEET FENNEL VARIETIES

Ph.D. Thesis
In
Agric. Sci. (Biochemistry)

By

AIAA ABD ALLAH HASSAN GAAFAR

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Zagazig Univ., 1994 M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Zagazig Univ., 2002

Approval Committee

Researcher Professor of Microbiology, National Research Centre	•
Dr. Ahmed Mahmoud Mustafa Aboul-Enein Professor of Biochemistry, Fac. Agric., Cairo University	
Dr. Abdel Moneim Mohamed Naguib El-Nakkadi Professor of Biochemistry, Fac. Agric., Cairo University	

Date: 22/6/2010

SUPERVISION SHEET

BIOCHEMICAL STUDIES ON PHENOLIC COMPOUNDS OF BROCCOLI AND SWEET FENNEL VARIETIES

Ph.D. Thesis
In
Agric. Sci. (Biochemistry)

By

AIAA ABD ALLAH HASSAN GAAFAR

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Zagazig Univ., 1994 M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Zagazig Univ., 2002

SUPERVISION COMMITTEE

Dr. Abdel Moneim Mohamed Naguib El-Nakkadi Professor of Biochemistry, Fac. Agric., Cairo University

Dr. Hanaa Fawzy Mohamed Ali Assistant Professor of Biochemistry, Fac. Agric., Cairo University

Dr. Zeinab Hanem Abd El-Rahman Salama Researcher Professor of Plant Nutrition, National Research Centre Name of Candidate: Alaa Abd Allah Hassan Gaafar Degree: Ph.D.

Title of Thesis: Biochemical Studies on Phenolic Compounds of

Broccoli and Sweet Fennel Varieties

Supervisors: Dr. Abdel Moneim Mohamed Naguib El-Nakkadi

Dr. Hanaa Fawzy Mohamed Ali

Dr. Zeinab Hanem Abd El-Rahman Salama

Depeartment : Biochemistry **Approval:** 22/6/2010

ABSTRACT

A field experiment was carried out to study the effect of organic and bioorganic fertilizers on growth parameters yield and the quality of two broccoli cultivars (*Calabrese* and *Southern star*) and two sweet fennel cultivars (*Dolce* and *Zefa fino*). Bio-organic fertilizer gave better results for all vegetative growth parameters for *Southern Star* and *Zefa fino* cultivars. Total phenolics (TPC), total flavonoids (TFC), total glucosinolates (TGs) and vitamin C (Vit C) were almost higher in *Calabrese* and *Dolce* cultivars as a result of organic fertilizer application. The antioxidant activities of both plants were evaluated and *Calabrese* and *Dolce* cultivars showed the highest DPPH scavenging activity expressed as IC₅₀. In addition, *Calabrese* and *Dolce* cultivars showed the highest chelating power activities when organic fertilizer was applied.

The HPLC analysis of methanolic extracts of broccoli and sweet fennel showed the presence of many phenolics and flavonoids in variable levels. Gallic acid, chlorogenic acid, 3,5-dimethoxybenzyl, *P*-cumaric, quercetin, kampferol and eugenol were found as the most abundant consistuents in broccobi. While, the highest content of phenolic compounds content of sweet fennel were Pinostrobin, Pyrogallic acid, Chlorogenic acid, Protocatechuic acid, Salicylic acid and Rutin. Other compounds showed different concentrations depend on the cultivar and the type of fertilization treatment.

The hepatoprotective effect of methanolic extracts of broccoli and sweet fennel cultivars in a dose 100 and 200 mg/kg BW compared with silymarin as a reference agent at the same doses were evaluated against CCl₄-induced liver injury. Methanolic extracts of broccoli and sweet fennel were restorated and reversed the liver injury (CCl₄-induced hepatotoxicity) in rats.

The results indicated that there is a good margin for enhancing antioxidant compounds of broccoli and sweet fennel for economic production by using organic fertilization. The study indicated the potential application of broccoli and sweet fennel as potent natural sources of antioxidants and hepatoprotective agents.

Key words: Broccoli, sweet fennel, antioxidant activity, organic fertilizer, bioorganic fertilizer, phenolics, HPLC and hepatoprotective effect

DEDICATION

I dedicate this work to whom my heart felt thanks; to soul of my parents, my wife, my daughters, my brothers and my sister for their patience, help and for all the support they lovely offered along the period until now.

Also, I dedicate this work for my spiritual mother in my work and my life Dr. Zeinab Hanem Abd El-Rahman Salama.

ACKNOLEDGEMENT

Firstly, I am deeply thankful to merciful Allah who gave me everything I have and by the grace of whom the work was realized.

The author wishes to express thanks and gratitude to Dr.

Abd El-Moneim Mohamed Naguib El-Nakkadi Professor of

Biochemistry, Faculty of Agriculture, Cairo University for his

supervision, encouragement and constructive criticism during

the thesis.

Sincere thanks for **Dr. Hanaa Fawzy Mohamed Ali**Assistant Professor of Biochemistry Faculty of Agriculture,
Cairo University for her superversion.

I am heartily thankful to my supervisors, Dr. Zeinab Hanem Abd El-Rahman Salama, Professor of Plant Nutrition, National Research Centre, and Dr. Farouk Kamel El-Baz Professor of plant Biochemistry, National Research Centre, for suggestion the problem and their continuous encouragement, generous guidance, continued assistance, revision the manuscript and support from the initial to the final level enabled me to develop an understanding of the subject.

Sincere thanks to **Dr. Hanaa Abd El- Baky** Researcher Professor of Plant Biochemistry, National Research Centre for her help and encouragement.

Especially thanks to **Dr. Mohamad Fathy Zaki**Assistant Professor of Vegetable Crops, National Research
Centre, and **Dr. Eman Abd El-Motaleb** Senior Research of
pathology, Animal Health Research Institute for their sincere
help and assistance.

I would like to thank all colleges who have helped and inspired me during my doctoral study.

CONTENTS

INTRODUCTION	
INTRODUCTION	
REVIEW OF LIRTRATURE	
1. Broccoli	
a. Phytochemical constituents in broccoli	
2. Sweet fennel	
a. Phytochemical constituents in sweet fennel	
3. Influence of fertilizers on broccoli and sweet fennel	· · · · · ·
4. Phenolic compounds	
a. Health promoting effects of phenolic compounds	
b. Phenolic and flavonoid contents of broccoli	
c. Phenolic and flavonoids contents of sweet fennel	
5. Glucosinolates compounds	
a. Total glucosinolates content in broccoli	
6. Vitamin C (ascorbic acid)	
a. Vitamin C content of broccoli	
b. Vitamin C content of sweet fennel	
7. Antioxidant activity of broccoli and sweet fennel	
a. DPPH radical scavenging activities and iron chelating	
activity of broccoli	
b. DPPH. radical scavenging activities and iron chelating	
activity of sweet fennel	
8. Biological evaluation	
a. Hepatoprotective activity of some vegetables	
MATERIALS AND METHODS	
RESULTS AND DISCUSSION	
1. Broccoli study	
a. Influence of organic and bio-organic fertilizers	
b. Influence of organic and bio-organic fertilizers on total	
phenolic and total flavonoids contents	
c. Influence of organic and bio-organic fertilizers on total	
glucosinolates	
d. Influence of organic and bio-organic fertilizers on total	
vitamin C	
e. Effect of organic and bioorganic fertilization on yield of	 f
TPC TFC TGs and Vit C of broccoli florets	L

f. Antioxidant activities of broccoli
1. Scavenging activity on DPPH radicals of broccoli
cultivars
2. Ferrous ion chelating activities of broccoli cultivars 73
g. Identification of phenolic compounds of broccoli cultivars 77
2. Sweet fennel study 79
a. Influence of organic and bio-organic fertilizers
b. Influence of organic and bio-organic fertilizers on total
phenolic content and total flavonoids content
c. Influence of organic and bio-organic fertilizers on total
vitamin C content
d. Effect of organic and bioorganic fertilization on yield of
TPC, TFC and Vit C
e. Antioxidant activities of sweet fennel 85
1. Scavenging activity on DPPH radicals of sweet fennel
cultivars85
2. Ferrous ion-chelating activities of sweet fennel cultivar 88
f. Identification of phenolic compounds of sweet fennel
cultivars
3- Biological evaluation 94
a. Broccoli94
1. Effect of methanolic extracts of broccoli supplementation
on biochemical parameters
2. Effect of methanolic extracts of broccoli supplementation
on biochemical parameters of liver tissue
3. Effect of broccoli extracts on body and liver weight 10:
b. Sweet fennel 10.
1. Effect of methanolic extracts of sweet fennel
supplementation on biochemical parameters 103
2. Effect of methanolic extracts of sweet fennel
supplementation on biochemical parameters of liver
tissues
3. Effect of methnolic extracts of sweet fennel
supplementation on body and liver weight
4- Histopathological evaluation 115
SUMMARY 120
ARABIC SUMMARY

LIST OF TABLES

No.	Title	page
1.	Physical and chemical analysis of the soil	35
2.	The chemical composition of chicken manure	36
3.	Effect of organic and bioorganic fertilizer treatments on dry weight and yield of broccoli cultivars	61
4.	Effect of organic and bio-organic fertilizers on total phenolics (TPC), total flavonoids (TFC), total glucosinolates (TGsC) and Vitamin C (Vit C) contents of broccoli cultivars.	63
5.	Effect of organic and bioorganic fertilization on yield of total phenolics, total flavonoid total glucosinolates and vitamin C contents of florets in two cultivars of broccoli.	68
6.	Iron chelating activity by different concentrations of 80% methanolic extracts of broccoli cultivars in response to organic and bio-organic fertilizers	74
7.	Phenlic compounds of broccoli cultivars as affected by organic and bio-organic fertilization	78
8.	Effect of organic and bioorganic fertilizer treatments on dry weight and yield of sweet fennel cultivars	80
9.	Effect of organic and bio-organic fertilizer treatments on total phenolics (TPC), total flavonoids (TFC), and vitamin C (Vit C) of sweet fennel cultivars	82
10.	Effect of organic and bioorganic fertilization on yield of total phenolics, total flavonoids and vitamin C contents of bulbs in sweet fennel cultivars	84

11.	Iron chelating activity by different concentrations of 80% plant methanolic extracts of sweet fennel cultivars in response to organic and bio-organic fertilizers	89
12.	Phenlic compounds of sweet fennel cultivars as affected by organic and bio-organic fertilization	93
13.	Influence of broccoli extracts supplementation on blood serum marker enzymes and biochemical parameters against CCl ₄ -treated rats.	95
14.	Influence of broccoli extracts supplementation on total protein content and MDA, SOD and GST activities in the liver tissues of rats.	99
15.	Effect of broccoli extracts supplementation on body weight (BW) and liver weight (LW) of rats	102
16.	Influence of sweet fenne extracts supplementation on blood serum marker enzymes and biochemical parameters against CCl ₄ -treated rats	104
17.	Influence of sweet fennel extracts supplementation on total protein content and MDA, SOD and GST activities in the liver tissues of rats.	107
18.	Effect of sweet fennel extracts supplementation on body weight (BW) and liver weight (LW) of rats	109

LIST OF FIGURES

NO.	Title	page
1.	IC ₅₀ of DPPH free radical of 80% methanolic extract of broccoli cultivars in response to organic and bioorganic fertilizers.	70
2.	Correlation between IC ₅₀ and phenlics (a), flavonoids (b), glucosinolates (c) and vitamin C (d) of broccoli cultivars in response to organic and bio-organic fertilizers.	72
3.	Correlation between iron chelating activity at (30µg/m) phenolics (a), flavonoids (b), glucosinolates (c) and vitamin C (d) of broccoli cultivars in response to organic and bio-organic fertilizers	76
4.	IC ₅₀ of DPPH free radical of 80% methanolic extract of sweet fennel cultivars in response to organic and bioorganic fertilizers	86
5.	Correlation between IC ₅₀ and phenolics (a), flavonoids (b) and vitamin C (c) of sweet fennel cultivars in response to organic and bio-organic fertilizers	87
6.	Correlation between iron chelating activity at 30 μ g/ml and phenolics (a), flavonoids (b) and vitamin C (c) of sweet fennel cultivars in response to organic and bioorganic fertilizers.	91
7.	Normal liver with hepatic cord and control vein (H α E. X: 200)	120
8.	congestion of blood vessels of portal area, perivascular edema, the hepatocytes were suffering from vacuolar degenerative changes, and some hepatocytes were necrosis (H α E. X:400)	120

9.	Liver of rats treated with silymarin 100 mg/kg body weight after CCl_4 showing slight congestion of the hepatic vein with fewer cells suffered from vacuolar degenerative changes (H α E. X: 200)	121
10.	Liver of rats treated with silymarin 200 mg/kg body weight after CCl_4 showing few hepatocytes suffered from vacuolar degenerative changes (H α E. X: 200)	121
11.	Liver of rats treated with B1 100 mg/kg body weight after CCl_4 showing few hepatocytes suffered from vacular degenerative changes (H α E. X: 200)	122
12.	Liver of rats treated with B1 200 mg/kg body weight after CCl_4 showing slight edema dispersed hepatocytes (H α E. X 200)	122
13.	Liver of rats treated with B2 100 mg/kg body weight after CCl_4 showing congetion of the control vein with few focal area of heamorrhage , some hepatocytes suffered from vacular degenerative changes (H α E. X 200)	123
14.	Liver of rats treated with B2 200 mg/kg body weight after CCl_4 showing few numbers of mononuclear inflammatory cells, with slight edema dispersed the hepatocytes (H α E. X 200)	123
15.	Liver of rats treated with F1 100 mg/kg body weight after CCl_4 showing focal areas of hepatocytes suffered from vacculolar degenerative changes with infiltration of mononuclear inflammatory cells as well as activation of Van Kupfer cells (H α E. X: 200)	124
16.	Liver of rats treated with F1 200 mg/kg body weight after CCl ₄ showing large focal areas of hepatocytes suffered from vacculolar degenerative changes especially around the central vein (H α E. X:400)	124

17.	Liver of rats treated with F2 100 mg/kg body weight after CCl ₄ showing focal areas of hepatocytes suffered from vacculolar degenerative changes with activation of Van Kupfer cells (H α E. X:200)	125	
18.	Liver of rats treated with F2 200 mg/kg body weight after CCl_4 showing focal areas of hepatocytes suffered from vacculolar degenerative changes (H α E. X:200)	125	

INTRODUCTION

In recent years, increasing attention has been paid to the role of diet in human health. This is mainly attributed to the health promoting effect of fruit and vegetables. Several epidemiological studies have indicated that a high intake of fruits and vegetables is associated with a reduced risk of a number of chronic diseases, such as cancer and heart diseases (Law and Morris, 1998; Gosslau and Chen, 2004).

These protective effects are generally attributed to the presence of bioactive compounds such as phenolics, flavonoids, glucosinolates, vitamin C and minerals which possess antioxidants activity. Many of these compounds have bioactive mechanisms for scavenging reactive oxygen species (ROS) and reducing cell proliferation in cancer cell (Kris-Etherton *et al.*, 2002). Vegetables belonging to cruciferous family (broccoli, cauliflower, cabbage and turnip) have high antioxidant activity.

Broccoli has been a favorite test vegetable for several researchers due to its potential antioxidant properties (Zhang and Hamauzu, 2004; Piao *et al.*, 2005). It is considered a significant source for phenolics such as sinapic acid and caffiec acid derivatives and flavonoids (Vallejo *et al.*, 2003a). Broccoli also is considered a significant source of sulforaphane glucosinolates, a phytochemical that is believed to have strong anticarcinogenic properties (Beecher, 1994).

Sweet fennel (Foeniculum vulgare) belongs to family Apiaceae (Umbelliferae), which well known as annual vegetable,