

**EFFECT OF ENHANCED ATMOSPHERIC CARBON
DIOXIDE ON THE NUTRITIONAL QUALITY OF
SELECTED DIETARY VEGETABLES**



Ph.D Thesis

By

ANDALEEB AZAM

**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
PAKISTAN**

March 2012

**EFFECT OF ENHANCED ATMOSPHERIC CARBON
DIOXIDE ON THE NUTRITIONAL QUALITY OF
SELECTED DIETARY VEGETABLES**

By

ANDALEEB AZAM

DISSERTATION

**SUBMITTED TO THE UNIVERSITY OF PESHAWAR IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN CHEMISTRY**

**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
PAKISTAN**

March 2012

INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
PAKISTAN

Certified that **Ms Andaleeb Azam** D/O Mir Azam Khan has carried out her research and experimental work on the topic entitled as “**Effect of Enhanced Atmospheric Carbon Dioxide on the Nutritional Quality of Selected Dietary Vegetables**” under our guidance and supervision. Her research work is original and her dissertation is worthy of presentation to the University of Peshawar for the award of degree of **Doctor of Philosophy in Chemistry**.

SUPERVISOR

Prof. Dr. Ikhtiar Khan
Institute of Chemical Sciences,
University of Peshawar, Pakistan

CO-SUPERVISOR

Dr. Abid Mahmood
Ayub Agriculture Research
Institute, (AARI)
Faisalabad, Pakistan.

EXTERNAL EXAMINER

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Dedicated
To
To my Loving Parents

Acknowledgements

All praise be to Allah the Most Gracious and the Most Merciful, for giving me the strength and ability to complete this thesis. Peace and blessing be upon Prophet Muhammad SAW., his families, his relatives and all his followers.

First and foremost, I would like to express my sincere gratitude to my teacher and supervisor Prof. Dr. Ikhtiar Khan for his patience, motivation, enthusiasm, and immense knowledge. It has been an honor to be his student. I appreciate all his contributions of time and ideas to make my Ph.D. experience productive and stimulating. The joy and enthusiasm he has for research work was contagious and motivational for me. He has contributed immensely to my personal and professional grooming at this Institute.

I owe my deepest gratitude to my co-supervisor Dr. Abid Mahmood for devoting his precious time, providing numerous ideas, valuable suggestions and research facilities at Barani Agriculture Research Institution, Chakwal, which indeed helped to improve quality of this research work. Without his help and guidance, this work would not have been possible.

I am truly indebted and thankful to my foreign supervisor Dr. Barry Lomax, University of Nottingham, for his support and guidance during my six month research at University of Nottingham, United Kingdom.

*I gratefully acknowledge funding provided by the **Higher Education Commission, (HEC) of Pakistan** for my Ph.D studies at the Institute of Chemical Sciences, University of Peshawar through Indigenous Ph.D Fellowship Program and at University of Nottingham, United Kingdom through International Research Support Initiative Program. I am also thankful to HEC for providing funds to Pakistan Council of Scientific and Industrial Research (PCSIIR), Peshawar for sample analysis.*

*I would like to thank **Dr. Imdadullah**, Professor and Director Institute of Chemical Sciences and **Prof. Dr. Imtiaz Ahmad** Director QEC University of Peshawar, for all their help and support.*

*My time at the institute and at hostel was made enjoyable and unforgettable in large part due to my friends that became a part of my life. I am grateful to **Maria Sadia, Beshisht Ara** and **Humaira Inayat**. Their moral support is a precious asset of my life.*

*Very special thanks to my friend **Ms. Nazia Gul** who supported me morally throughout my Ph.D work.*

*I am grateful to scholar colleagues for providing a stimulating and decent environment to learn and grow. I am especially thankful to **Ms. Syeda Sohaila Naz, Ms. Asma Ijaz, Nasirullah, Rizwan Alam** and **Abdul Hameed**.*

*Finally, I would like to express cordial and special thanks to my parents, **Mr. Mir Azam Khan** and **Mrs. Nasima Azam**, for their prayers and love. They helped me to concentrate on completing*

this dissertation and supported me mentally during the course of this work. Without their help and encouragement, this study would not have been completed. I wish to thank my entire extended family for providing a loving and supportive environment for me. My brothers Muhammad Asad, Muhammad Asghar and Muhammad Nauman and my sister Mrs. Sabeena Amjid were particularly supportive.

*Andaleeb Azam
University of Peshawar
March 2012*

CONTENTS

S. No.	TITLE	Page No.
	LIST OF TABLES	i-iii
	ABSTRACT	iv-vi
	CHAPTER-1: INTRODUCTION	
1.0	Introduction	1-13
1.1	Climate Change	1
1.2	Increases in Atmospheric CO ₂ Concentration	2
1.3	Sources and Sinks of Atmospheric CO ₂	4
1.3.1	Natural sources and sinks	4
1.3.2	Anthropogenic sources and sinks	5
1.4	Environmental Effects of Increase in Atmospheric CO ₂	6
1.4.1	Increase in temperature	6
1.4.2	Changes in cryosphere	7
1.4.3	Sea level rise	8
1.4.4	Changes in precipitation, drought and monsoon	8
1.4.5	Changes in the extreme events	9
1.5	Increase in Atmospheric CO ₂ and Agriculture	9
1.5.1	Enhanced atmospheric CO ₂ and photosynthesis	10
1.5.2	Enhanced atmospheric CO ₂ and crop yield	11
1.5.3	Enhanced atmospheric CO ₂ and respiration	12
1.5.4	Enhanced atmospheric CO ₂ and transpiration	12
1.5.5	Enhanced atmospheric CO ₂ and food quality	13
1.6	Objectives of the Present Study	13
	CHAPTER-2: LITERATURE REVIEW	
2.0	Literature Review	14-24
	CHAPTER-3: MATERIALS AND METHODS	
3.0	Materials and methods	25-42
3.1	Experimental Design	25
3.1.1	Plant material and growth	25

3.1.2	Greenhouses	27
3.1.3	CO ₂ Monitoring	27
3.1.4	Temperature measurement	28
3.1.5	Soil analysis	30
3.1.6	Harvest and sampling	30
3.2	Chemical Analysis	31
3.2.1	Fruit number and fresh weight	31
3.2.2	Protein determination	31
3.2.3	Sugars determination	32
3.2.4	Ascorbic acid/Vitamin C determination	35
3.2.5	Total titratable acidity determination	37
3.2.6	Crude fat determination	37
3.2.7	Crude fiber determination	38
3.2.8	Ash determination	39
3.2.9	Elemental analysis	39
3.2.10	Fatty acids analysis	40
3.3	Statistical Analysis	42

CHAPTER-4: RESULTS AND DISCUSSION

4.0	Results and Discussion	43-141
4.1	Effect of Enhanced CO ₂ on Tomato (<i>Lycopersicon esculentum</i>) Fruit	43
4.1.1	Effect on yield	43
4.1.2	Effect on proximate composition	44
4.1.3	Effect on elemental composition	49
4.1.4	Effect on fatty acid composition	52
4.2	Effect of Enhanced CO ₂ on Chili (<i>Capsicum annuum</i>) Fruit	54
4.2.1	Effect on yield	55
4.2.2	Effect on proximate composition	56
4.2.3	Effect on elemental composition	64
4.2.4	Effect on fatty acid composition	67
4.3	Effect of Enhanced CO ₂ on Onion (<i>Allium cepa</i>) Bulb	69

4.3.1	Effect on yield	69
4.3.2	Effect on proximate composition	70
4.3.3	Effect on elemental composition	74
4.3.4	Effect on fatty acid composition	78
4.4	Effect of Enhanced CO ₂ on Okra (<i>Abelmoschus esculentus</i>) Fruit	79
4.4.1	Effect on yield	80
4.4.2	Effect on proximate composition	81
4.4.3	Effect on elemental composition	85
4.4.4	Effect on fatty acid composition	87
4.5	Effect of Enhanced CO ₂ on Cucumber (<i>Cucumis sativus</i>) Fruit	88
4.5.1	Effect on yield	89
4.5.2	Effect on proximate composition	89
4.5.3	Effect on elemental composition	92
4.5.4	Effect on fatty acid composition	94
4.6	Effect of Enhanced CO ₂ on Spinach (<i>Spinacia oleracea</i>) Leaves	96
4.6.1	Effect on yield	96
4.6.2	Effect on proximate composition	96
4.6.3	Effect on elemental composition	98
4.6.4	Effect on fatty acid composition	100
4.7	Effect of Enhanced CO ₂ on Carrot (<i>Daucus carota</i>) Root Tuber	101
4.7.1	Effect on yield	102
4.7.2	Effect on proximate composition	102
4.7.3	Effect on elemental composition	104
4.7.4	Effect on fatty acid composition	106
4.8	Effect of Enhanced CO ₂ on Pea (<i>Pisum sativum</i>) Seeds	108
4.8.1	Effect on yield	108
4.8.2	Effect on proximate composition	109
4.8.3	Effect on elemental composition	111
4.8.4	Effect on fatty acid composition	113
4.9	Effect of Enhanced CO ₂ on Cauliflower (<i>Brassica oleracea</i>) Flower	115

4.9.1	Effect on yield	115
4.9.2	Effect on proximate composition	115
4.9.3	Effect on elemental composition	118
4.9.4	Effect on fatty acid composition	119
4.10	Effect of Enhanced CO ₂ on Radish (<i>Raphanus sativus</i>) Root Tuber	121
4.10.1	Effect on yield	122
4.10.2	Effect on proximate composition	122
4.10.3	Effect on elemental composition	125
4.10.4	Effect on fatty acid composition	127
4.11	Effect of Enhanced CO ₂ on Turnip (<i>Brassica rapa</i>) Root Tuber	128
4.11.1	Effect on yield	129
4.11.2	Effect on proximate composition	129
4.11.3	Effect on elemental composition	132
4.11.4	Effect on fatty acid composition	134
4.12	Effect of Enhanced CO ₂ on Eggplant (<i>Solanum melongena</i>) Fruit	136
4.12.1	Effect on proximate composition	136
4.12.2	Effect on elemental composition	138
4.12.3	Effect on fatty acid composition	140
	Conclusions	142-145
	References	146-166
	Appendix	167-184

LIST OF TABLES

S. No.	TITLE	Page. No.
3.1	List of dietary vegetables and their varieties used in the study	26
3.2	Date of growing and final harvesting of vegetables	27
3.3	Temperature record (°C) of the greenhouse	29
3.4	Physical and chemical characteristics of soil used in experiment	30
3.5	Factors for 10mL of Fehling solution used against sugar solution	35
3.6	Column oven programming of GC-MS	42
4.1	Yield of tomato fruits grown at two levels of atmospheric CO ₂	44
4.2	Proximate composition of tomato fruits grown at two levels of atmospheric CO ₂	48
4.3	Elemental composition of tomato fruits grown at two levels of atmospheric CO ₂	51
4.4	Fatty acid composition of tomato fruits grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	54
4.5	Yield of capsicum fruits grown at two levels of atmospheric CO ₂	56
4.6	Proximate composition of capsicum fruits grown at two levels of atmospheric CO ₂	61
4.7	Elemental composition of capsicum fruits grown at two levels of atmospheric CO ₂	65
4.8	Fatty acid composition of capsicum fruits grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	68
4.9	Yield in terms of fresh weight of onion bulbs grown at two levels of atmospheric CO ₂	70
4.10	Proximate composition of onion bulbs grown at two levels of atmospheric CO ₂	61
4.11	Elemental composition of onion bulbs grown at two levels of atmospheric CO ₂	65
4.12	Fatty acid composition of onion bulbs grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	79

4.13	Yield of okra fruits grown at two levels of atmospheric CO ₂	80
4.14	Proximate composition of okra fruits grown at two levels of atmospheric CO ₂	84
4.15	Elemental composition of okra fruits grown at two levels of atmospheric CO ₂	86
4.16	Fatty acid composition of okra fruits grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	88
4.17	Yield and proximate composition of cucumber fruits grown at two levels of atmospheric CO ₂	92
4.18	Elemental composition of cucumber fruits grown at two levels of atmospheric CO ₂	94
4.19	Fatty acid composition of cucumber fruits grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	95
4.20	Yield and proximate composition of spinach leaves grown at two levels of atmospheric CO ₂	98
4.21	Elemental composition of spinach leaves grown at two levels of atmospheric CO ₂	100
4.22	Fatty acid composition of spinach leaves grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	101
4.23	Yield and proximate composition of carrot root grown at two levels of atmospheric CO ₂	104
4.24	Elemental composition of carrot root grown at two levels of atmospheric CO ₂	106
4.25	Fatty acid composition of carrot root grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	107
4.26	Yield and proximate composition of pea seeds grown at two levels of atmospheric CO ₂	111
4.27	Elemental composition of pea seeds grown at two levels of atmospheric CO ₂	113
4.28	Fatty acid composition of pea seeds grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	114

4.29	Yield and proximate composition of cauliflower grown at two levels of atmospheric CO ₂	117
4.30	Elemental composition of cauliflower grown at two levels of atmospheric CO ₂	119
4.31	Fatty acid composition of cauliflower grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	121
4.32	Yield and proximate composition of radish root grown at two levels of atmospheric CO ₂	125
4.33	Elemental composition of radish root grown at two levels of atmospheric CO ₂	127
4.34	Fatty acid composition of radish root grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	128
4.35	Yield and proximate composition of turnip root grown at two levels of atmospheric CO ₂	132
4.36	Elemental composition of turnip root grown at two levels of atmospheric CO ₂	134
4.37	Fatty acid composition of turnip root grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	135
4.38	Proximate composition of eggplant fruits grown at two levels of atmospheric CO ₂	138
4.39	Elemental composition of eggplant fruits grown at two levels of atmospheric CO ₂	140
4.40	Fatty acid composition of eggplant fruits grown at two levels of atmospheric CO ₂ (determined as corresponding methyl ester)	141

ABSTRACT

Besides increasing global temperature, enhanced atmospheric carbon dioxide is affecting physico-chemical and nutritional characteristics of crops and vegetables. In order to evaluate the hypothesis that climate change is threatening food quality, the effect of enhanced atmospheric CO₂ on nutritional, elemental and fatty acid composition of dietary vegetables has been investigated. Dietary vegetables including tomato (*Lycopersicon esculentum*), chili (*Capsicum annum*), onion (*Allium cepa*), okra (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*), spinach (*Spinacia oleracea*), carrot (*Daucus carota*), pea (*Pisum sativum*), cauliflower (*Brassica oleracea*), radish (*Raphanus sativus*), turnip (*Brassica rapa*) and eggplant (*Solanum melongena*) were grown in ambient (400 $\mu\text{mol mol}^{-1}$) and elevated (1000 $\mu\text{mol mol}^{-1}$) concentration of CO₂ in green houses. Edible parts of the vegetables (fruit/flower/tuber/seeds or leaves) were collected on maturity and analyzed. Enhanced CO₂ has largely disturbed the nutritional balance of vegetables. A significant increase in carbohydrates and crude fiber at the cost of protein, vitamin C and fat contents was observed. Elements behaved inconsistently with a general decreasing trend. The results revealed that with a substantial increase in yield, nutritional quality of dietary vegetables unfavorably altered under CO₂ enrichment with accumulated sugars and diminished proteins and vitamin C.

Plants were examined for their physical characteristics and chemical composition. Previously known standard procedures were applied for chemical analysis. Samples were analyzed in triplicate and standard deviation was calculated, Student's *t* test was applied on data using SPSS 16. Results were quoted as significant at ($P \leq 0.05$), non-significant (ns) at $P > 0.1$ and trend at $0.05 < P \leq 0.1$. Nutritional balance of majority of the vegetables studied was disturbed by CO₂ enriched atmosphere.

Two varieties of tomato were analysed and it was observed that protein content of tomato varieties were reduced by 13.64% and 18.27% and vitamin C by 20.02% and 24.72% for mature stages and 9.59% for premature stage. Increase in sugar content with elevated CO₂ was 16.12% and 20.85% for mature and 14.16% for premature tomato. Elemental composition of tomato was disturbed by enhanced CO₂ with increased C, H, Ca, Fe and

Cu and reduced N, Mg, Zn, Mn, Pb, Ni, Cr and Cd. Most of the fatty acids including essential fatty acids i.e. linoleic acid and linolenic acid, were reduced by elevated CO₂.

Enhanced CO₂ disturbed nutritional, elemental and fatty acid composition of capsicum varieties. Five varieties of capsicum were analysed. Observed reduction in protein was from 25.10% to 31.62% and in vitamin C it was from 11.84% to 15.66% for mature red stages and 8.98% to 12.12% for premature green stages of capsicum. Sugar contents were increased in the range 11.83% to 13.86% in red stages and 9.66% for the green stage of on variety. Elemental composition of capsicum varieties was disturbed by elevated level of CO₂. Elements like C, H, Fe and Mn were increased while Ca, Mg, N, Zn and Pb were decreased. Response of capsicum fatty acids to enhanced CO₂ was not same, however a general decreasing trend was observed.

Enhanced CO₂ altered nutritional quality of onion with reduced protein and vitamin C and increased sugars. For four varieties of onion the observed decrease in protein with elevated CO₂ ranged from 12.01% to 19.53% and that of vitamin C ranged from 17.14% to 21.64%. Total sugar content was increased by 11.24%. Among the elements, C and Zn were increased while N, Mn, Fe, Pb and Cr were decreased. Different fatty acids of onion bulbs responded differently to enhanced CO₂, with a general decreasing trend.

Elevated CO₂ reduced the nutrient composition of okra. Protein content of okra was reduced by 23.95% and 18.24% and vitamin C content was reduced by 17.72% and 13.66% for two varieties. Total sugar content of okra increased by 18.73% and 19.34%. Elemental composition of okra was disturbed by elevated CO₂ with increased C, Ca and Fe and decreased N, Mg, Zn, Mn and Pb. Fatty acids of okra were mostly decreased by enhanced CO₂.

Enhanced CO₂ decreased the protein content of cucumber by 11.15%, vitamin C by 18.57% and increased total sugars by 15.20%. Elements like C, H, Ca and Mg were increased while N, Zn, Mn and Fe were decreased by elevated CO₂. Elevated CO₂ mostly decreased the fatty acid content of cucumber with reduced linolenic acid, and linoleic acid.

Atmospheric CO₂ enrichment disturbed the nutritional balance of spinach with 15.88% reduction in protein and 15.72% reduction in vitamin C. Among elements, C, H and Ca were increased while N, Zn, Mn, Fe, Pb, Ni, Cu and Cr were decreased. Elevated CO₂ decreased almost all of the fatty acids in spinach leaf. The decrease was more pronounced for major fatty acids as compared to minor fatty acids.

Elevated CO₂ affected the nutritional composition of root vegetables. Protein content of carrot, radish and turnip root tubers was decreased by 24.30%, 18.83% and 18.17% respectively by enhanced CO₂. Vitamin C was reduced by 9.09% for carrot, 12.93% for radish and 21.87% for turnip. Sugar content was increased by 12.99% and 19.64% by CO₂ enrichment for radish and turnip respectively. Elemental and fatty acid composition of root vegetables were also disturbed by enhanced CO₂.

Nutritional composition of pea was disturbed by enhanced CO₂ with 13.42% reduction in protein, 13.95% reduction in vitamin C and 13.14% increase in total sugars. Elements like C, H and Mn were increased while N, Ca, Mg, K and Fe were reduced. Elevated CO₂ decreased linoleic, linolenic and oleic acids in pea lipids.

Elevated CO₂ decreased the protein content of cauliflower by 15.55% and Vitamin C by 18.59%. Concentration of C and H were increased and that of N, S, Ca, Zn, Mn, Fe, Cu and Cr were decreased. Enhanced CO₂ decreased the fatty acid content of cauliflower oil including linolenic, palmitic and linoleic acid.

Protein and vitamin C content of eggplant were decreased with enhanced CO₂. The decrease was 11.36% for protein and 15.96% for vitamin C. Elements like C, H and Ca were increased while N, Mg, K, Zn, Mn and Fe were decreased. Most of the fatty acids were reduced by elevated CO₂.

Vegetables responded differently to enhanced CO₂ and more interestingly, even different varieties of the same vegetable showed different changes. Although the data is on a limited scale, the message is loud and clear - enhanced atmospheric CO₂ has adversely affected the nutritional balance of dietary vegetables.

INTRODUCTION

Chapter 1

INTRODUCTION

1.1 Climate Change

Global climate is constantly changing due to natural processes as well as through human activities. Climate change can be defined as any change in the statistical distribution of weather conditions over periods of time that range from decades to millions of years. It can either be a change in the average weather or a change in the distribution of weather events around an average (for example, greater or fewer extreme weather events). Such change may be limited to a specific region, or it may occur across the globe. Natural processes that are responsible for climate change involve changes in the earth's orbit around the sun, the sun's intensity and changes in ocean circulation, while human activities involve burning of fossil fuels, deforestation, urbanization, desertification etc. Human activities mostly lead to changes in atmospheric composition by releasing different pollutants to the atmosphere which increase the temperature of the planet earth.

According to the Fourth Assessment report of Intergovernmental Panel on Climate Change (IPCC), atmospheric carbon dioxide (CO₂) concentration has increased from a pre-industrial value of 280 parts per million (ppm) to about 380 ppm in 2005. Atmospheric methane (CH₄) concentration has increased by 148% of its pre-industrial value, which varied between 580 and 730 parts per billion (ppb) over the past 10,000 years and increased to 1774 ppb in 2005. Nitrous oxide (N₂O) was 319 ppb in 2005 which is 18% higher than its pre-industrial value. Similarly ozone (O₃) concentration has increased by 36% compared to its pre-industrial value [1].

Chlorofluorocarbons (CFCs) and hydrochlorofluorocarbons (HCFCs) were used widely since their introduction in 1828 and their concentrations increased steadily but have declined due to natural removal process after their use was banned by the 1987 Montreal Protocol. Flourinated gases like hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulphur hexafluoride (SF₆) are nowadays used as substituent for CFCs and their concentrations are lower in atmosphere but these are increasing rapidly.

All these gases are greenhouse gases and they lead to an increase in temperature of the earth. Although the exact rate and extent of increase in temperature is difficult to calculate with certainty but it is estimated that in the past 100 years, temperature has increased by an average of $0.76^{\circ}\text{C} \pm 0.19^{\circ}\text{C}$. Most of this temperature increase is due to global warming by greenhouse gases. The effect due to urbanization and land usage change is negligibly small and is only about 0.006°C per decade [1].

1.2 Increases in Atmospheric CO₂ Concentration

Carbon dioxide is an important greenhouse gas and its concentration has continuously increased since the beginning of industrial revolution around the year 1800. Accurate measurement of atmospheric CO₂ concentration at the level of sub-part per million is a very important environmental record which was started by Charles Dave Keeling in 1958. He started the experiment at Mauna Loa Observatory on the island of Hawaii in the mid-Pacific ocean [2], and since then a continuous record of CO₂ concentration has been documented. Another important record of atmospheric CO₂ concentration was started by the National Oceanic and Atmospheric Administration (NOAA) global flask sampling network in 1979 by establishing an adequate number of monitoring sites [3]. Data from the records of Mauna Loa Observatory and the global average are in close agreement, which show that CO₂ concentration is continuously increasing in the atmosphere as can be seen in Figure 1. The growth rate of CO₂ was less than 1 part per million per year (ppm yr^{-1}) in 1960s, and after 2000 it is more than 2 ppm yr^{-1} [4]. This accelerated growth rate of CO₂ can be attributed to the decreasing ability of ocean and terrestrial biosphere to take up more CO₂ [5, 6]. By extending the CO₂ record into the past 50 years using ice core and firn data [7], it was suggested that the increase in CO₂ was not much rapid before 1950, and in the 1940s it was almost flattened for 10 years, possibly due to World War II. Between 1950 and 1970 there was a steep increase in CO₂, might be due to large use of fossil fuel resulting into oil crises of 1973 and 1979 [8]. However increase in CO₂ does not reflect fossil fuel combustion very well, rather it is more a reflection of the world population. The past century has seen quite a close agreement between atmospheric CO₂ and world population, both increasing exponentially [9]. Careful observation of the data shows that CO₂ did not increase linearly; rather it increased

exponentially, doubling after a time period of 31 years. In case CO₂ increases continue at its current rate, it is expected that it would double in the year 2050 i.e. twice the pre-industrial value or 560 ppm [9]. According to the different emission scenarios of IPCC, CO₂ concentration could be anywhere between 540 and 970 ppm at the end of 21st century. Uncertainties in the carbon sink will widen the range to 490-1260 ppm i.e. 75-350% above the pre-industrial concentration [10].

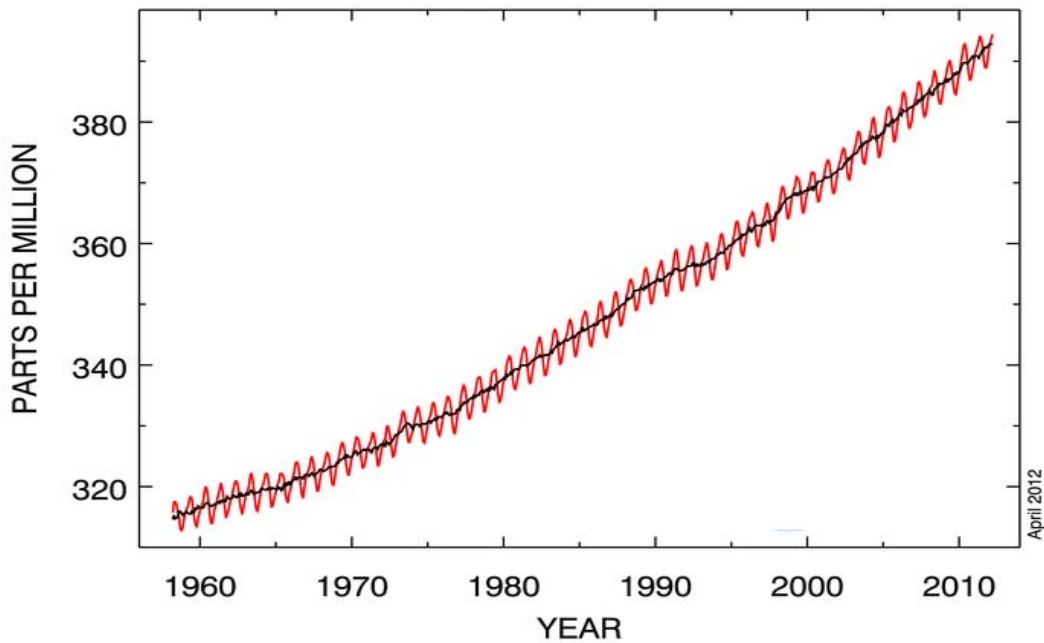


Fig. 1. Annual Increase in Atmospheric CO₂ Measured at Mauna Loa Observatory with red curve showing monthly variations and smoothed data with seasonal variations removed (<http://www.esrl.noaa.gov>).

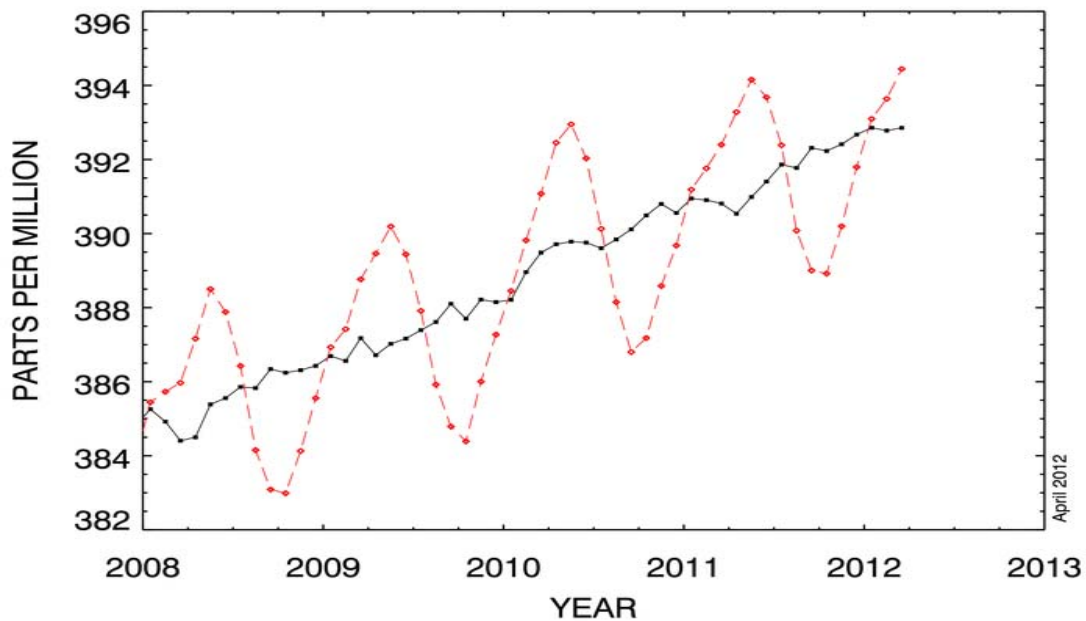


Fig. 2. Recent Monthly Mean CO₂ Measured at Mauna Loa observatory with red curve showing monthly variations and smoothed data with seasonal variations removed (<http://www.esrl.noaa.gov>).

1.3 Sources and Sinks of Atmospheric CO₂

All processes that release CO₂ to the atmosphere are called sources of CO₂ and the processes that remove it are called sinks of CO₂.

1.3.1 Natural sources and sinks

Natural sources of CO₂ are not important contributors because the CO₂ added to the atmosphere by such sources is removed by natural sinks. It is therefore called carbon cycle.

Among the natural sources, the most important is animal and plant respiration, the process in which considerable amount of CO₂ is released into the atmosphere by the breakdown of carbohydrates in the presence of oxygen in the living organisms. However

at the same time, CO₂ is removed from the atmosphere by plant photosynthesis, which stores carbon in plant biomass in the form of carbohydrates.

Another important source is ocean which releases CO₂ to the atmosphere at the sea surface but this CO₂ is absorbed back into the ocean by the process, called ocean-atmosphere exchange.

Sometimes the absorption of CO₂ by natural sinks is more than its release by natural sources and the net result is a decrease in concentration of CO₂ and the process is called carbon sequestration [1]. A very small amount of CO₂ is released to the atmosphere by volcanic eruption which releases carbon from deep rocks present in the earth crust.

1.3.2 Anthropogenic sources and sinks

Human activities release a greater amount of CO₂ to the atmosphere as compared to natural sources. Among the anthropogenic sources, the most important is the burning of fossil fuels, like coal, natural gas and petroleum. Almost all of the carbon stored in fuel is emitted in the form of CO₂. Coal is used for generation of electricity in coal based power plants as well as for commercial and residential purposes where coal is used along with oil and natural gas for cooking purposes and for keeping buildings warm. Industries also consume a huge amount of energy and release CO₂ to the atmosphere. Transportation is another important source of CO₂, mostly utilizing gasoline, diesel, CNG and jet fuel. In 2008, 40% of the fossil fuel CO₂ emissions were contributed by coal and 36% by oil [11].

Another anthropogenic source of CO₂ is deforestation, which is the permanent removal of forests, as plants are involved in carbon sequestration due to photosynthesis. Also CO₂ is added to the atmosphere either by quick emission through burning or by slow decomposition of these trees.

Fossil fuel combustion accounts for about 75% of the human caused CO₂ emissions while the rest are due to changes in land use i.e. deforestation along with burning of the biomass and change in agricultural practices [1]. Between 2000 and 2008, fossil fuel emissions increased by 20%, while emissions related to change in land use remained almost constant [11].

Anthropogenic sinks of atmospheric CO₂ include reforestation, as small and young plants consume rather greater amounts of CO₂, practices that involve collection and transport of CO₂ from large emission sources, and its injection into deep underground reservoirs.

The increase in atmospheric concentration of CO₂ at any time is determined by the balance between sources and sinks [1]. Between 1959 and 2008, out of the total annual CO₂ emissions, only 43% remained in the atmosphere, the rest being absorbed by land and in oceanic carbon sinks. With the passage of time there has been a decrease in the CO₂ uptake efficiency of the sinks which is also associated with the climate change. This is evident from the fact that in the past 50 years, the annual fraction of CO₂ emissions that remained in the atmosphere increased from 40 to 50% [11].

1.4 Environmental Effects of Increase in Atmospheric CO₂

Increase in atmospheric CO₂ will cause a number of environmental changes. Together with other greenhouse gases, it leads to increase in the earth temperature. This will affect the environment in many ways, for instance shrinking of glaciers and ice caps, rise in the sea level, changes in the distribution of plants and animals and the growing seasons. All these factors affect people, animals and plants.

1.4.1 Increase in temperature

Global mean temperature is continuously increasing and during the 20th century it has increased by $0.74 \pm 0.18^{\circ}\text{C}$, mainly due to increase in concentrations of greenhouse gases. The rate of global warming for the last half of the 20th century was almost double i.e. $0.13 \pm 0.03^{\circ}\text{C}$ per decade as compared to the period as a whole i.e. $0.07^{\circ}\text{C} \pm 0.02^{\circ}\text{C}$ per decade. 1998 and 2005 were the two warmest years on the world record since 1850, with one estimate showing 1998 to be the warmest year and two other estimates showing 2005 to be the warmest year. Land temperature has increased about twice as compared to ocean temperatures since 1979, i.e. 0.25°C per decade against 0.13°C per decade; this is due to greater heat capacity of oceans as well as greater evaporation of the ocean water. Before 1850, temperature remained stable for one to two thousand years, however since 1979, temperature has increased between 0.13 and 0.22°C per decade. It has been

estimated that during 21st century global mean temperature will further rise to 1.1-6.4°C, the uncertainty in estimates is due to uncertain buildup of gases in the atmosphere. It is also claimed that for every doubling of atmospheric CO₂, global temperature increases by 2 to 4.5°C with central official projection of 3.26°C [1].

Increase in global temperature due to greenhouse gases is a result of greenhouse effect. Earth receives solar radiation in the wavelength range of 0.2–4 µm, which is mostly in the visible region. CO₂ and other gases are transparent in this wavelength range. The light energy absorbed by earth is re-emitted in the form of heat energy to the atmosphere in the range of 4–100 µm, mostly the infrared region. CO₂ and other greenhouse gases are not transparent to infra-red radiations, they absorb this radiation, get into the excited state and emit it back to the atmosphere. This absorbance-remittance-absorbance cycle leads to increase the temperature of the atmosphere near the earth surface. CO₂ does not absorb heat directly from solar radiations but from the heat energy re-radiated by the earth surface [12].

1.4.2 Changes in cryosphere

Cryosphere, in climatic models, consists of snow, river ice, lake ice, sea ice, glaciers, ice caps, ice shelves, ice sheets, and frozen ground. Any change in the earth atmosphere has a direct effect on the cryosphere, so that they can be used as sensors for climate variability. These water bodies have undergone different variations in the past, however the present variations are associated with the rising surface air temperature. This is specially the case in northern hemisphere where the rise in temperature is almost double than the mean global temperature, from 1965 to 2005. It has been observed that the snow cover has decreased in most regions specially in spring and summer seasons, with an annual drop of 5% in the late 1980's in northern hemisphere whereas in southern hemisphere there is either very small decrease or no change in the last 40 years. The arctic sea ice also decreased and exhibited an annual mean decline of $2.7 \pm 0.6\%$ per decade since 1978. The central Arctic ice thickness has decreased up to 1 m from 1987 to 1997. Glaciers and ice caps have also decreased and a mass loss of $0.50 \pm 0.18 \text{ mm yr}^{-1}$ in Sea Level Equivalent (SLE) was observed between 1961 and 2004. Between 1991 and

2004, this loss was $0.77 \pm 0.22 \text{ mm yr}^{-1}$ SLE. Ice sheets in Greenland and Antarctica have also decreased due to increased melting and a corresponding increase in flow speed for some outlet glaciers. For Greenland ice sheets, the variation in mass was observed between -0.07 to 0.17 mm yr^{-1} SLE from 1961 to 2003, and 0.14 to 0.28 mm yr^{-1} SLE from 1993 to 2003. The change in mass of Antarctic ice sheet was found to be between -0.28 to 0.55 mm yr^{-1} SLE for 1961 to 2003, and from -0.14 to 0.55 mm yr^{-1} SLE for 1993 to 2003. The seasonal frozen grounds have also been decreased by an extent of 7% in northern hemisphere between 1901 and 2002, and in spring season the reduction has been increased up to 15% [1].

1.4.3 Sea level rise

Global mean sea level is also rising. In 20th century the average rate of sea level rise was $1.7 \pm 0.5 \text{ mm yr}^{-1}$, while this rate was $1.8 \pm 0.5 \text{ mm yr}^{-1}$ from 1961 to 2003. Rise in sea level is not uniform and it has been found that in some regions the rise in sea level is several times higher than the mean value whereas in some other places there is a decline in sea level. The rise in sea level is a result of thermal expansion in ocean due to global warming, melting of glaciers, ice caps and the Greenland and Antarctic ice sheets. Although contribution to sea level rise by different sources is not much certain, it was estimated that for the period 1961 to 2003, the average contribution of thermal expansion to sea level rise was $0.4 \pm 0.1 \text{ mm yr}^{-1}$. For the period 1993 to 2003, contributions from thermal expansion were $1.6 \pm 0. \text{ mm yr}^{-1}$ and that due to other sources were $2.8 \pm 0.7 \text{ mm yr}^{-1}$ [1].

1.4.4 Changes in precipitation, drought and monsoon

Precipitation patterns have changed with time. For northern areas it increased between 1900 and 2005, for tropics it decreased since 1970. The result is increased wet in some areas like eastern parts of North and South America, northern Europe, and northern and central Asia but increased drying in other areas like Sahel, the Mediterranean, southern Africa and parts of southern Asia. In some areas, especially at lower latitudes, there is increase in the amount of heavy precipitation although a reduction was observed in the total amount which is consistent with the warming climate and increase amount of water

vapour in the atmosphere. In northern regions, precipitation now occurs mostly as rain rather than snow. In some regions, increase in the frequency and intensity of floods and droughts has been observed.

For tropics and subtropics, droughts have become more common especially after 1970. This is considered to be a result of decreased precipitation and increased temperature which causes more evapotranspiration and more drying.

A change is also visible in the circulation of global mean monsoon. For many monsoon systems, a decadal change has been observed for monsoonal rainfall, especially across 1976-77. In East Asian monsoon systems, a change in precipitation pattern is observed, which increased in one region and decreased in the other during the last 50 years [1].

1.4.5 Changes in the extreme events

An increase in the frequency of weather-related extremes like heat waves, droughts, floods and hurricanes has been observed. In most parts of the world people come to know about it within a few hours of their occurrence only due to improved technology. This becomes a disaster as the society is not able to cope with it in most of the cases. In the past 50 years, there has been an increase in the occurrence of hot days and warm nights and decrease in the occurrence of cold days and cold nights. In mid-latitudes, during the last 50 years, there is an increase in the amount of heavy precipitation, although the mean precipitation is not changing in these areas. Since the mid-1970s, there has been an increase in destruction caused by hurricanes due to longer storm duration together with greater storm intensity. It has been found that although global warming does not create the hurricanes but it increases its strength and duration. Due to the warmer oceans, tropical storms pick more energy and become more powerful [1].

1.5 Increase in Atmospheric CO₂ and Agriculture

Agriculture is sustaining all kinds of life on earth, as animals cannot produce their own food and are dependent on agriculture directly or indirectly. With increase in population, demand for increased food production has increased. According to an estimate world population will increase from the present 7 billion to 8.7-11.3 billion in 2050 [13]. In

order to meet the needs of this massive population, crop production has to be increased by 50% in the next few decades [14]. However it is very difficult to predict the global food production in the new scenario of changing climate. Any change in the concentration of atmospheric CO₂ will affect the plants through changes in temperature and precipitation patterns [15, 16]. Since industrial revolution, CO₂ concentration in the air is increasing at a greater rate and today it is about 38% more than its pre-industrial value and will continue to increase further. How far is the agriculture affected by atmospheric CO₂, is not only an issue of future but the present crops too are growing in a much different environment compared to the pre-industrial age [17]. Understanding the effect of enhanced atmospheric CO₂ upon agriculture not only helps to know how plants will adapt themselves to the changing environment but is also important in predicting the extent to which they are already affected. Such studies started 30 years back and resulted in some detailed reviews [15, 18].

1.5.1 Enhanced atmospheric CO₂ and photosynthesis

The effect of enhanced atmospheric CO₂ on plant photosynthesis is evident as the plants can sense it directly through stomatal conductance and utilize it. Only photosynthetic organs of plants along with some reproductive organs are in direct contact with the atmosphere, the other organs being covered by a thick cuticle. In higher plants even the leaves are also covered with a thick cuticle, so that only guard cells of stomata will directly sense any change in concentrations of atmospheric CO₂, and this forms the basis for increase in crop yield with increasing atmospheric CO₂ [19].

Different metabolic steps involved in photosynthesis are believed to be affected by changes in concentrations of atmospheric CO₂, however they are not completely understood. Of them Rubisco is considered to have the potential to meaningfully respond to increasing atmospheric CO₂ concentration [15]. Rubisco is ribulose-1,5-biphosphate carboxylase/oxygenase, an enzyme present in C₃ plants. The C₃ plants name is derived from the mechanism involved in the Calvin cycle of photosynthesis in which one molecule of CO₂ combines with one molecule of ribulose-1,5-biphosphate and gives two molecules of 3-phosphoglycerate, which is a three carbon compound thus giving name C₃

to these plants. And this process is catalyzed by Rubisco. Other class of plants is called C₄ plants, in which CO₂ (HCO₃⁻) combines with phosphoenolpyruvate, a three carbon compound and yields oxaloacetate, a four carbon compound, thus giving the name C₄ to these plants. A series of enzymes are used to catalyze this process in C₄ plants. Rubisco also involves in photorespiration which is greatly minimized in C₄ plants. Photorespiration results in the loss of some of CO₂ fixed by Calvin cycle [20].

As CO₂ is the major component in the process of photosynthesis, increase in its concentration is expected to increase photosynthesis. Theoretical analysis suggests that there might be a 38% increase in photosynthetic rates when atmospheric CO₂ increases from 380 ppm to 550 ppm [21]. In practice, the increase in photosynthetic rate is not that much high. A smaller than expected increase in photosynthesis might be attributed to the accumulation of carbohydrates in the leaves which result in the down regulation of photosynthetic capacity [22, 23]. Overall, an increase in photosynthesis with increase in atmospheric CO₂ has been observed for both C₃ and C₄ plants, though increase for C₃ is greater compared to C₄ plants [20]. On the average, photosynthesis for C₃ increases by 34% and for C₄ by 10% when grown under elevated CO₂ concentration [15]. An increase of 39% in leaf photosynthesis has been reported for soybean in a meta-analysis, which summarized the results of 111 studies [24].

1.5.2 Enhanced atmospheric CO₂ and crop yield

A positive effect of enhanced atmospheric CO₂ on crop yield is obvious as increase in CO₂ concentration increases the rate of photosynthesis which leads to a greater accumulation of carbohydrates in plant leaves and results in a greater yield and increased biomass [20]. However the extent to which yield and biomass are affected is different for different crops and even for the different varieties of same crop.

Seventeen different cultivars of rice were grown under elevated atmospheric CO₂ and their response varied between 30% and 400% [25]. An increase of 31% in plant biomass and 25% in seed biomass with 19% increase in number of flowers and 18% increase in number of fruits was observed for a total of 79 different crops with elevated CO₂. Among these, the greatest increase in number of seeds was found for rice which was 42% as

compared to 29% for soybean, 15% for wheat and 5% for maize [26]. For two varieties of rice, grain yield of one increased by 30% and that of other increased by 13% when grown under the same level of atmospheric CO₂ [27].

Increases in biomass and yield were observed for a number of crops like potato with greater number of tubers and 40% increase in the tuber yield when grown at 660 ppm of CO₂ [28], sugarcane with 50% increase in biomass when CO₂ increased from 380 to 740 ppm [29], sour orange trees with 70% increase in fruit production as well as in biomass during 17 years of study [30]. Such results lead to the conclusion that increase in atmospheric CO₂ has a positive impact on the plant growth, yield and biomass mainly due to increases in photosynthetic rates.

1.5.3 Enhanced atmospheric CO₂ and respiration

Effect of enhanced atmospheric CO₂ on plant respiration is not well documented. The uncertainty is due to the fact that the mechanism responsible for the effect of enhanced atmospheric CO₂ upon the plant respiration have not been developed [31]. Different studies report different results for the effect of CO₂ on respiration, for instance a decrease by about 18%, an increase by 37% and even no change [31, 32].

1.5.4 Enhanced atmospheric CO₂ and transpiration

In transpiration, water vapors move from leaf to atmosphere through stomata. This is the same path traveled by CO₂ during photosynthesis, but in the opposite direction. Elevated CO₂ should increase stomatal opening and conductance resulting in increased uptake of CO₂ as well as increased transpiration rates [33]. However for both C₃ and C₄ plants, it has been found that at elevated CO₂, stomatal conductance decreases consistently, leading to decrease in the rate of transpiration [34, 35]. This decreases the use of water and increases water use efficiency [15]. A 40% decrease in stomatal conductance has been recorded for soybean when grown under elevated levels of CO₂ [24].

1.5.5 Enhanced atmospheric CO₂ and food quality

Enhanced atmospheric CO₂ increases the quantity of food in terms of increasing yield as well as biomass, however, its effect on the quality of food is not much understood. Due to increasing global population and widespread malnutrition, the quality of food has become as important as its quantity. In the words of Goldman “with the burgeoning consumer interest in foods that optimize health, attention is shifting from concerns over quantity alone to concerns over the constituents of foods that may promote health.”[36]. As plants are the basic sources of food, any change in their nutritional composition can affect all the population on the earth, a phenomenon similar to the present problem of hidden hunger (malnutrition), which badly affects the health and economy of over half of the world population [37]. Unfortunately very little is known about the effect of enhanced atmospheric CO₂ on the quality of the edible products of most of the food and forage crops. Moreover, the information that is available is quite contradictory with conflicting research results, and it is very difficult to draw firm conclusions on how the ongoing increase in atmospheric CO₂ will affect the food quality for both human and animal nutrition. Therefore more research is needed in this field to ascertain the quality of grains, vegetables and fruits in the future CO₂ rich atmosphere.

1.6 Objectives of the Present Study

- The main objective of the present work was to investigate experimentally the impact of enhanced atmospheric CO₂ on quality of selected dietary vegetables. Vegetables were grown in two CO₂ environments and studied for the induced chemical changes.
- The objectives also included opening research to develop climate resistant varieties of food plants.

LITERATURE REVIEW

Chapter 2

LITERATURE REVIEW

Increase in concentration of atmospheric CO₂ is known to increase the growth and yield of plants, however its effect on the nutritional quality of the crops and vegetables is not well studied. Most of the experimental work available is on grain crops like wheat, rice, barley, maize and some on potatoes. Very little is known about vegetables consumed in our daily diet. A detailed review of the available literature is given here.

The impact of elevated atmospheric CO₂ on nutritional and baking quality of wheat has been extensively investigated. Thompson and Woodward studied the influence of elevated atmospheric CO₂ upon the grain yield and quality of wheat. They observed a 30% increase in grain yield while grain nitrogen decreased by 28%. The reduction in grain nitrogen concentration with elevated CO₂ was large enough to affect the bread making processes [38]. Van Vuuren, *et al.*, observed increase in root biomass, increased potassium and phosphorus uptake by wheat roots when grown at elevated level of CO₂ [39]. Fangmeier, *et al.*, observed that elevated CO₂ altered nutrient concentration in different organs of wheat plant, i.e. reduction in N, Ca, S, Mg and Zn in leaves while P was unaffected, reduction in N, P, K, S, Ca, Mg, Fe, and Zn and increase in Mn in shoots, and reduction in all nutrients in grain. Uptake of nutrients by wheat plants increased for K, Mn, Mg and P, did not change for N, S, Zn and Fe and reduced for Ca under elevated CO₂ [40]. In another study, these workers observed that CO₂ enrichment decreased all the elements analyzed (K, Ca, S, Mg, Zn, Mn) in the wheat leaves except P and Fe, while in grains only N, Ca, Fe and S were decreased. Reduction in nitrogen content of grains lowered the baking quality of wheat [41]. Pleijel, *et al.*, found that grain yield of wheat was increased but grain protein was decreased by increased CO₂ concentration [42].

Sinclair, *et al.*, carried out a 4-year research program with wheat (*Triticum aestivum* L.) grown at different CO₂ concentrations and evaluated its effect on leaf nitrogen. Leaf nitrogen was decreased to a smaller extent with elevated CO₂ and to a larger extent when elevated CO₂ was accompanied with a low nitrogen treatment, showing that forage nutritive value can be decreased by future changes in the global environment [43].

Agrawal and Deepak reported that CO₂ treatment decreased the rate of respiration and stomatal conductance in wheat and increased total phenolics, starch and total soluble sugars [44]. Wu, *et al.*, observed that elevated CO₂ increased carbohydrates and crude starch while decreased the mineral elemental nutrient contents (P, N, Zn and K), crude protein and lysine content of wheat grain [45]. Wieser, *et al.*, studied the impact of enhanced atmospheric CO₂ on the quantitative protein composition of wheat. Elevated CO₂ decreased crude protein and all protein types and fractions including glutenin macropolymer, single gluten protein and Osborne fractions significantly except albumins and globulins, thus causing a reduction in the baking quality [46].

Högy and Fangmeier reviewed the impact of increased atmospheric CO₂ concentration on the quality of wheat grain (*Triticum aestivum* L.). Although elevated CO₂ increased the wheat productivity, it altered its chemical composition in most of the studies. Protein content was decreased with a change in amino acid composition while starch content was increased. Concentration of different micro and macro elements was also changed with CO₂ enrichment [47]. Porteausb, *et al.*, studied the chemical composition and nutritive value of wheat grains and straw grown under enhanced concentrations of CO₂ and observed that carbon dioxide enrichment did not affect the C and total ash content, reduced the N content and increased the C:N ratio for both straw and wheat. The concentrations of both non-structural and structural carbohydrates were not affected except the neutral detergent fiber, which decreased slightly under low nitrogen supply. In grains, non-structural carbohydrates including water soluble carbohydrates and starch increased, while structural carbohydrates remained unchanged except the hemicellulose, which increased under elevated CO₂ [48].

Högy, *et al.*, investigated the impact of CO₂ enrichment on yield, biomass and grain metabolites in spring wheat and observed that some of the metabolites were increased while others were decreased with elevated CO₂. Most of the amino acids including leucine, o-acetyl-L-homoserine, L-homoserine, arginine, ornithine and citrulline, norleucine, proline, L-aspartate, tyrosine, L-cysteine, alpha-ketoaminobutyrate, the polyamine putrescine and D/L-diaminopimelate were decreased significantly except polyamine spermidine which increased under enhanced CO₂. Elevated CO₂ decreased

gluconate-6-P and increased ribose-5-P among sugars and its derivatives. Sugar alcohols including glycerol-2-P and myo-inositol-P were reduced while organic acids like glucuronic acid and pyruvate content were increased significantly by elevated CO₂ [49]. Erbs, *et al.*, observed that elevated CO₂ decreased the grain protein concentration by 4-13% and grain sulphur content by 5% in wheat. Other quality parameters like kernel hardness, hagberg falling number, water extract viscosity and activity of α -amylase were also altered by elevated levels of CO₂ [50].

Response of potato to elevated CO₂ was found quite inconsistent in different studies. Wheeler, *et al.*, studied the proximate nutritional composition of potato (*Solanum tuberosum*) under different CO₂ partial pressures. Elevated CO₂ was observed to increase protein content of potato stem, decrease carbohydrates of potato leaf and stem, and did not affect the quality of potato tuber [51]. This was against the findings of Donnelly, *et al.*, who observed a decrease in nitrogen and increase in starch content of potato tuber grown under elevated CO₂. A decrease in citric acid and total glycoalkaloid content while an increase in vitamin C was also observed [52]. Decrease in citric acid, glycoalkaloid and nitrate content and increase in starch content of potato, when grown under enriched CO₂ conditions, was also observed by Vorne, *et al.* However he did not notice any change in vitamin C content of potato tubers [53]. While Högy and Fangmeier found a decrease in ascorbic acid content of potato, accompanied by an increase in fructose, glucose and total reducing carbohydrates concentration, and decreasing protein, amino acids and citric acid concentration. Among the macro (Ca, K, Mg, S and P), micro (Zn, Mn and Fe) and trace (Cd, B and Al) elements, only K and Ca were decreased significantly where as a non-significant decrease was observed on the average for all other elements [54]. Fangmeier, *et al.*, observed that increased atmospheric CO₂ decreased nutrient element concentration including macronutrients (P, N, Ca, Mg and K) and micronutrients (Fe, Zn and Mn). At maximum leaf area, potassium and nitrogen decreased both in tubers and aboveground biomass while calcium decreased only in tubers. At maturity, iron, manganese and nitrogen decreased significantly in aboveground biomass, and magnesium, potassium and nitrogen decreased in tubers reducing the quality of potato tuber [55].

Nutritional quality of soybean was observed to alter under enhanced atmospheric CO₂. Mulchi, *et al.*, found that glucose and fructose remained unchanged while starch and sucrose content increased significantly by high CO₂ concentration. Grain protein content reduced and oil content increased significantly with elevated CO₂ [56]. Jurgonski, *et al.*, observed no change in protein N, total N and amino acid contents of seeds, while leaf amino acids, leaf protein nitrogen and total dietary fiber of leaves were decreased when grown in elevated CO₂ atmosphere [57]. Wheeler, *et al.*, observed no significant trend in proximate composition of soybean except protein content of leaf and crude fiber content of stem, leaf and seed. These parameters increased with elevated CO₂ [51]. Deepak and Agrawal observed an increase in crop yield, plant growth and foliar starch content of soybean grown under elevated CO₂ [58]. An increase in dry matter accumulation, grain yield, and a decrease in coarse fat and raw protein content of soybean with elevated CO₂ was also observed by Bai, *et al.* [59].

Several reports are available on the response of rice to elevated CO₂. Dong, *et al.*, found that elevated CO₂ increased the percentage of chalky grain of rice, gel consistency and gelatinization temperature, reduced the protein content and did not affect amylase content significantly [60]. A similar decrease in protein with elevated CO₂ and no effect on amylase content of rice was also observed by Terao, *et al.* They observed a decrease in surface hardness but found no significant effect on sensory properties like deliciousness, aroma, appearance, hardness, stickiness and palatability index [61]. Yang, *et al.*, observed that elevated CO₂ enhanced the cooking and eating quality of rice by decreasing amylose content, increasing the peak viscosity and breakdown but reduced the nutritional quality by decreasing protein content [62]. A decrease in protein content and no effect on polypeptide profile of rice by elevated CO₂ was also observed by Fukayama, *et al.* [63].

The nutritional quality of barley is known to be affected by elevated CO₂. Thompson and Woodward observed increase in grain yield but a decrease in grain nitrogen of barley, thus affecting the bread making process [38]. A reduction in protein and sulphur content in barley grains, when grown at elevated levels of CO₂ was also reported by Erbs, *et al.* Other quality parameters like kernel hardness, hagerberg falling number, water extract

viscosity and activity of b-amylase were also affected by enhanced atmospheric CO₂ [50].

Orange trees and fruits also respond to elevated CO₂. Idso, *et al.*, conducted a twelve years experiment of CO₂ enrichment upon vitamin C concentration of sour orange trees. An increase of 75% in CO₂ concentration doubled the fruit production and increased vitamin C by 7%. At times production was more than double, however size of fruit decreased. When increase in fruit production was less than double, fruit size was larger and increase in vitamin C was less than 7%. In the last five years, increase in concentration of vitamin C was about $5 \pm 1\%$ on the average [64]. Idso, *et al.*, in another study, observed a decrease in the leaf chlorophyll and nitrogen of sour orange trees when grown in CO₂ enriched atmosphere. However the decrease was compensated by increased fruit productivity of CO₂ enriched trees [65]. Kimball, *et al.*, investigated the impact of CO₂ enrichment on sour orange trees (*Citrus aurantium* L.) for seventeen years. The experiment started in 1987, four trees were grown in ambient CO₂ and other four in elevated CO₂ (300 $\mu\text{mol mol}^{-1}$ above the ambient) in open top plastic chambers. Seventeen years study showed a 70% increase in biomass of all trees in the form of greater number of fruits and branches, thicker branches and trunks. However CO₂ enrichment did not produce any change in the elemental composition of the biomass [30]. Keutgen and Chen found that exposure of citrus leaves to elevated CO₂ at and above 600 ppm caused changes in macronutrient and carbohydrate content. Macronutrients decreased while carbohydrate content increased in mature leaves. No affect was seen in young leaves [66].

Few reports available on the effect of enhanced CO₂ on tomato present quite conflicting results. Wheeler, *et al.*, observed that elevated CO₂ did not affect the proximate composition of tomato fruit, showing no effect on protein, carbohydrate and elemental composition including Mg, K, and P. Only Ca increased at elevated CO₂ [67]. On the other hand Islam, *et al.*, observed an increase in carbohydrates including glucose, fructose, and sucrose. An Increase in ascorbic acid and decrease in organic acids including oxalic, malic and citric acids was also observed in the same study [68]. Li, *et*

al., observed that the uptake of nutrients including N, C, P and K increased in tomato seedlings when grown under CO₂ enriched atmosphere [69].

Effect of enhanced CO₂ on leafy vegetables has not been studied extensively. Few available reports include limited parameters. Jain, *et al.*, observed that nutritional composition of two leafy vegetables, fenugreek and spinach, was altered by elevated CO₂ with reduced Fe, Mg and N contents and increased Ca and C contents [70]. McKeehen, *et al.*, studied the impact of CO₂ enrichment on proximate and mineral contents of lettuce leaves and radish roots and leaves. With CO₂ enrichment, nitrate-N level of lettuce leaves decreased, ash content of radish leaves and roots and that of lettuce leaves decreased while starch content of lettuce increased significantly. No effect was observed on any other parameter in these plant parts [71].

Several reports on the effect of CO₂ enrichment on chemical composition of different brassica species are available. Reddy, *et al.*, found that total nitrogen and total phenolics decreased significantly in oilseed rape but remained unaffected in cabbage with elevated CO₂. In both plants the concentration of leaf glucosinolate remained unaffected in enhanced CO₂. In oilseed rape, the observed significant changes in individual glucosinolate concentrations were not consistent among cultivars [72]. Himanen, *et al.*, observed a decrease in leaf glucosinolate concentrations of oilseed rape grown under enhanced atmospheric CO₂ [73]. Högy, *et al.*, conducted quite a comprehensive study on the seed quality and energy traits of oilseed rape grown at elevated CO₂. Carbon dioxide enrichment increased yield and productivity of aboveground biomass for seeds and straw but did not affect energy content per dry weight. Elevated CO₂ decreased slightly the concentration of manganese and zinc but significantly that of boron, sulphur and cadmium in seeds. CO₂ enrichment decreased significantly total protein concentration, however on productin basis; this change was slightly compensated by increase in seed yield. Amino acids were reduced and carbon/nitrogen ratio increased with elevated CO₂. Composition of fatty acid was also altered with increase in some components (oleic acid) and decrease in others (linolenic acid, nervonic acid and the group of essential fatty acids). Only progoitrin, among glucosinolates was decreased by elevated CO₂ [74].

Elevated CO₂ also affected woody and herbaceous plants. Wullschleger, *et al.*, investigated the impact of enhanced atmospheric CO₂ on the chlorophyll content, carbon exchange rate and diurnal carbohydrate status of white oak and yellow-poplar. Elevated CO₂ reduced total chlorophyll content and increased carbon exchange rate for both plants. Morning starch was increased while evening sucrose was decreased which increased the starch/sucrose ratio of the plants [75]. Overdieck studied the nutrient concentration of plants grown under elevated CO₂. Two woody plants; *Fagus sylvatica* L. and *Acer pseudo-platanus* L. and four herbaceous; *Festuca pratensis*, *Trifolium pratense* L., *Trifolium repens* L., and *Lolium perenne* L. were included in the study. Plants grown at ambient and elevated CO₂ were analyzed for P, N, Ca, K, Mn, Mg, Zn and Fe, total absolute nutrient concentration per individual and of the whole stands of all the six species. Nutrient concentration (N, Ca, K, Mg) of all the six species were found to decrease while P remained unaffected. Fe and Mn were found to decrease for tree species. Zn decreased significantly in the plants of *Festuca pratensis* and *Trifolium pratense*. Although there was a decrease of about 20% in elemental concentration of some of the species, the total absolute concentration of elements per individual increased up to 25% for the whole vegetation. This was expected due to higher accumulation of dry matter at elevated levels of CO₂ [76]. Liu, *et al.*, studied carbohydrate and biomass acclimation of two tree species, Norway spruce (*Picea abies* L.) and European beech (*Fagus sylvatica* L.) at elevated levels of CO₂. Total carbohydrate concentration including starch and sugar increased for spruce plant and decreased for beech at elevated CO₂ [77].

Fodder quality of grass species is also observed to be affected by enhanced concentration of atmospheric CO₂. Pal, *et al.*, observed that in berseem plants, plant height, leaf size and dry and fresh mass of shoots increased while soluble proteins, leaf nitrogen, calcium, iron and activity of nitrate reductase decreased when grown at elevated CO₂. Results revealed that although forage production was increased, its nutritional quality was adversely affected by elevated CO₂ [78]. Barbehenn, *et al.*, also observed a reduction in nutritional quality of C₃ and C₄ grasses grown in elevated levels of CO₂. Starch, sugar and fructan content of C₃ grasses increased and protein content decreased under CO₂ enriched conditions. Effect of elevated CO₂ on protein levels of C₄ was much smaller

than C₃ grasses [79]. Hattas, *et al.*, investigated the impact of elevated CO₂ on the leaf phytochemical constituents of fynbos shrubs and subtropical grasses. He observed that elevated levels of CO₂ did not affect polyphenolics and tannins in the grass and fynbos species. Foliar nitrogen content was decreased only for few species of fynbos. It was concluded that different species of fynbos respond differently to elevated levels of CO₂, with a reduction in the forage quality of some species [80]. Hunt, *et al.*, observed a decrease in chlorophyll and protein content and increase in carbohydrate content of *Lolium perenne* L. caused by elevated levels of CO₂ [81]. A decrease in protein content of clover with elevated CO₂ was also observed by Gleadow, *et al.* [82]. Schenk, *et al.*, also reported the elevated CO₂ to alter nutritive value and mineral profile of white clover and perennial ryegrass. Elevated CO₂ decreased Na and K, did not affect P, and increased Ca, thus increasing Ca/P ratio beyond the required value of animal nutrition. Crude fiber content decreased and crude protein first decreased and then increased in the latter stages of the experiment [83].

Many individual results are reported for the effect of elevated CO₂ on other crops and vegetables. Wu, *et al.*, revealed that elevated CO₂ did not affect proximate composition, mineral elemental concentration, amino acid or fatty acid profile of peanuts [84]. Bindi, *et al.*, observed an increase in biomass, sugar and acid content of grapevine grown at enhanced CO₂ levels [85]. Srivastava, *et al.*, documented increase in nitrogen content of harvested leaves of mungbean grown under CO₂ enriched conditions, however, nitrogen content on a percentage basis was decreased [86]. Agüera, *et al.*, investigated the effect of different CO₂ concentrations on cucumber. They observed that concentration of soluble sugars and starch increased and that of nitrate content decreased with the rising levels of CO₂ [87]. Marinari, *et al.*, studied the effect of increased atmospheric CO₂ concentration on nutrient elemental composition of poplar leaves. These workers observed that CO₂ enrichment increased magnesium and reduced nitrogen concentration in leaves of three poplar species, *P. alba*, *P. nigra* and *P. x euramericana* [88]. Erice, *et al.*, investigated the impact of elevated CO₂ on vegetative storage protein and nitrogen accumulation for the plants of alfalfa (*Medicago sativa* L.). Results revealed that elevated CO₂ increased the biomass production, did not affect total soluble protein while it increased vegetative storage protein content in taproot of alfalfa [89]. Levine and Paré studied the impact of

enhanced CO₂ on the antioxidant capacity of scallions and observed that biomass accumulation was increased while total antioxidant activity was decreased in plants at elevated CO₂ [90]. Manderscheid, *et al.*, investigated the effects of CO₂ on yield and composition of sugar beet and observed that elevated CO₂ increased the water soluble carbohydrate in petiole but did not change the sucrose, sodium and potassium content of beet [91].

Many reviews are available in literature which illustrate the effect of elevated CO₂ on the nutritional quality of different crop plants. Mortensen reviewed the effect of CO₂ enrichment upon the greenhouse plants including forest plants, vegetables, cut flowers and pot plants. Enhanced CO₂ increased the yield of all plants by increasing plant height, dry weight, number of leaves, number of flowers and number of branches. The optimal concentration of CO₂ to enhance the yield and growth of plants in greenhouses was between 700 and 900 $\mu\text{l l}^{-1}$ while concentrations above 1000 $\mu\text{l l}^{-1}$ caused leaf injuries and growth reductions [92]. Conroy studied nutritional quality of plants grown under elevated concentrations of atmospheric CO₂. Increase in CO₂ concentrations along with the availability of phosphorus and nitrogen in soil increased biomass production and photosynthesis. Nitrogen concentration of the foliage, roots and grains decreased at elevated CO₂ level, even in the presence of soil nitrogen, thus deteriorating the nutrient value and baking quality of grains [93].

Idso and Idso reviewed the impact of CO₂ enrichment on those constituent of plants which are associated with human and animal health and found that increased CO₂ concentration, reduced nitrogen and protein contents in both human-sustaining cereal grains and animal-sustaining forage. Such reduction could be overcome by supplying extra nitrogen to the soil, in which the plants grow. CO₂ enrichment was found to increase vitamin C in some of the fruits and vegetables. It also increased concentrations of some disease fighting substances. Mineral uptake was also observed to increase which helped to overcome the reduction of the elements in plants [94]. Loladze argued that elevated CO₂ should change the elemental composition of plants with decreased mineral to carbon ratio by applying stoichiometric theory. According to him plants will face a different environment with greater concentration of CO₂ and this must affect elemental

balance of plants leading to malnutrition of important nutrients [37]. Long, *et al.*, reviewed the effect of rising atmospheric CO₂ upon plants and compared the results of chamber studies and Free Air Carbon dioxide Enrichment (FACE) studies. It was concluded that both lead to similar results, however quantitative differences were found in some studies. Overall studies showed that C₃ plants respond to increased CO₂ via increase in photosynthetic activity and decrease in transpiration rate. Increase in CO₂ results in down regulation of Rubisco, which constitutes about 20-25% of leaf nitrogen, thus decreasing leaf nitrogen and total protein content of the leaves. The reduction was slightly higher in chamber studies as compared to FACE studies [15]. Erda, *et al.*, investigated the effect of climate change on yield and quality of China crops including wheat, rice and maize, with the help of regional climate change model. Modelling suggested that the change in climate without CO₂ fertilization would reduce the yield of wheat, maize and rice by about 37% in the coming 20-80 years. CO₂ enrichment to 550 ppm would increase the biomass production and yield by about 5-15%, however it might lower the grain quality by affecting the nutritional level of China crops [95].

Taub, *et al.*, examined the impact of CO₂ on the protein content of food crops with the help of meta-analysis techniques which covered the results of 228 experiments performed on wheat, barley, rice, potato and soybean. For each crop protein concentration was decreased when grown at elevated CO₂ compared to ambient. The reduction was quantitatively different for different crops i.e. 14% for potato, 10-14% for wheat, barley and rice, and 1.4% for soybean. The effect was more significant under low soil nitrogen as compared to high soil nitrogen. In different experiments the magnitude of reduction in protein was different even for the same crops due to differences in experimental conditions [96]. Leakey, *et al.*, reviewed the impact of CO₂ enrichment on plant carbon, nitrogen and water relations. It was concluded that exposure to elevated CO₂ for long time increased the net primary production and photosynthetic carbon gain with down regulation of Rubisco activity. It also increased nitrogen use efficiency resulting in decrease of plant nitrogen. Water use efficiency was also increased. Although elevated CO₂ increased the yield of crops, however the increase was much smaller than expected [22]. DaMatta, *et al.*, reviewed the effects of temperature and CO₂ enrichment on the food quality and physiology of crops. It was concluded that crops would show a faster

growth. Productivity and water use efficiency of the crops were increased with elevated CO₂, but it altered the food quality of crops, with reduced concentration of protein and mineral nutrient and altered composition of lipid [20]. Moretti *et al.*, reviewed the impact of climate change on the quality of fruits and vegetables and concluded that elevated CO₂ caused a reduction of nutritional quality of potatoes but did not affect the quality of grape and wine [97].

Most of the research findings available in literature are on major grain crops. Data from similar studies on vegetables is scarce. Since dietary portions of vegetables (fruit, leaf, root, seed or flower as the case may be) are exposed to the atmosphere for comparatively short period of time. Therefore, it is important that chemical changes during this exposure period are investigated.

MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

3.1 Experimental Design

3.1.1 Plant material and growth

Plants selected for the study were different kinds of dietary vegetable crops commonly used in human diet both as a main meal or consumed raw in the form of salad. A list of these vegetables along with the names of varieties is given in the Table 3.1. For some vegetables, more than one variety was used to study the response of these different varieties to elevated CO₂. Two varieties were selected each for tomato and okra, five for chilies, four for onions and one each for carrot, cauliflower, cucumber, eggplant, pea, radish, spinach and turnip. Seeds of vegetables were grown in their respective seasons from May 2008 to January 2010, covering a total of three growing seasons. The dates of sowing the seeds are given in Table. 3.2. Seeds were grown in earthen pots, having a diameter of 30cm and depth of 45cm, and were kept in greenhouses. Plants were properly irrigated as required, normally equal amounts of water was applied to control and sample plants.

Table 3.1. List of dietary vegetables and their varieties used in the study

Vegetable	Variety	Edible part analyzed in the study
Tomato (<i>Lycopersicon esculentum</i>)	i. Astra ii. Eureka	fruit
Chili (<i>Capsicum annum</i>)	i. 99PE-1689 ii. Magama iii. Angrika iv. Best Choice v. Diana	fruit
Onion (<i>Allium cepa</i>)	i. Rosita ii. Red Brown iii. Dark Red iv. T1-172	bulb
Okra (<i>Abelmoschus esculentus</i>)	i. Arkanamika ii. Sabz Pari	fruit
Cucumber (<i>Cucumis sativus</i>)	Dvnasty	fruit
Spinach (<i>Spinacia oleracea</i>)	Local	leaves
Carrot (<i>Daucus carota</i>)	T-1-111	root
Pea (<i>Pisum sativum</i>)	10691	seeds
Cauliflower (<i>Brassica oleracea</i>)	Snow Grace	flower
Radish (<i>Raphanus sativus</i>)	Mino	root
Turnip (<i>Brassica rapa</i>)	Grabe	root
Eggplant (<i>Solanum melongena</i>)	Dilnashin	fruit

Table 3.2. Date of growing and final harvesting of vegetables

Vegetable Crop	Date of sowing	Date of final harvesting
Tomato	15-05-2008	26-11-2008
Chilli	15-05-2008	11-11-2008
Okra	15-05-2008	08-10-2008
Pea	24-12-2008	11-05-2009
Cucumber	24-12-2008	13-05-2009
Onion	24-12-2008	18-05-2009
Radish	24-12-2008	06-05-2009
Turnip	24-12-2008	06-05-2009
Eggplant	18-09-2009	21-01-2010
Cauliflower	18-09-2009	21-01-2010
Spinach	24-09-2009	21-12-2009
Carrot	28-09-2009	13-01-2010

3.1.2 Greenhouses

Plants were grown in 9×12 feet greenhouses located in the fields of Barani Agriculture Research Institution (BARI), Chakwal, Pakistan. Greenhouses were constructed on levelled ground surface in a position to receive maximum sunlight during daytime. Walls and roofs of the greenhouses were made from highly transparent glass. Solar energy was the source of heating. Frame of greenhouses was made from wood. Concrete blocks were used in the foundation to provide maximum strength.

3.1.3 CO₂ Monitoring

Two CO₂ partial pressures 400 $\mu\text{mol mol}^{-1}$ (Ambient) and 1000 $\mu\text{mol mol}^{-1}$ (Elevated) were maintained in the greenhouses. In experimental greenhouse pure CO₂ was released. CO₂ cylinders were purchased from the local market and fixed outside the greenhouse. Carbon dioxide was released through perforated plastic pipes from cylinder into the greenhouses at the level of plant canopy. The height of CO₂ supply pipe from the ground level was constantly adjusted to the plant canopy. Carbon dioxide was applied from the

time of seed germination to the final harvest of fruits. The concentration of CO₂ was measured regularly using a Gas Analyzer with multi IR detectors (MX-42 A Oldham, France) in both the control and experimental greenhouses. CO₂ concentration was cross checked regularly with a handy NIR sensor (TSI's IAQ-CALC™ Indoor Air Quality Meters 7515, USA).

3.1.4 Temperature measurement

Temperature of greenhouse was measured continuously and recorded. Both glass and digital thermometers were used, and were located at different positions in greenhouses. Temperature records are given in Table 3.3. Average temperature of the first growing season, from May to Nov 2008, was 23.2°C with a maximum temperature of 34.1°C and minimum of 14.2°C. Average temperature for the second growing season, from December 2008 to May 2009, was 19.9°C with a maximum of 32.0°C and minimum of 8.0°C. Average temperature of the third growing season, from September 2009 to January 2010, was 19.9°C with a maximum of 30.3°C and minimum of 7.8°C.

Table 3.3. Temperature record (°C) of the greenhouse

Date	Temperature (Max. – Min.)											
	May, 2008	Jun, 2008	Jul, 2008	Aug, 2008	Sep, 2008	Oct, 2008	Nov, 2008	Jan, 2009	Feb, 2009	Mar, 2009	Apr, 2009	May, 2009
1		32-17	32-19	29-19	30-17	27-17	27-18	20-9	24-13	24-13		32-20
2		31-16	32-18	30-20		26-16	26-16	23-10	24-12	24-14	26-15	30-19
3		31-17	29-17	31-19		29-17	26-17	18-8	22-11	25-15	27-16	30-19
4		32-16	29-19	31-20		30-17	26-15	20-10	22-11	26-15	26-15	31-19
5		33-17	30-19	28-17	30-17	29-17	26-15	20-10	24-13	26-14	27-16	28-18
6		33-17	32-20	31-18	30-16	29-17	26-16	23-11	24-13	26-14	26-15	31-19
7		30-16	30-18	32-19	31-16	28-17	26-15	19-9	23-12	27-15	26-16	32-21
8		30-16	30-20	32-19	30-17	26-15	26-16	20-10	20-10	26-15	24-14	30-20
9		31-17	31-20	30-19	30-16	28-16	26-15	22-11	20-10	26-16	26-15	31-20
10		31-13	32-21	31-19	29-17	27-15	25-14	23-12	19-10	26-15	26-16	30-19
11		31-16	31-19	33-20	28-15	27-16	26-14	24-12	20-11	27-16	27-17	30-19
12		32-17	33-21	29-18	28-15	26-15	25-13	24-12	23-12	26-16	27-16	32-21
13		30-18	33-21	31-17	26-15	27-17	24-13	23-11	17-8	27-16	28-16	30-20
14		32-19	33-21	32-18	27-15	27-16	23-13	24-12	21-11	27-16	26-16	32-20
15	34-27	30-17	33-22	32-18	28-16	26-17	22-10	23-12	22-12	27-17	27-17	32-21
16	34-18	31-18	34-21	30-17	29-17	26-17	22-10	24-13	22-11	28-17	28-17	
17	31-18	32-18	30-18	30-17	28-16	26-16	22-09	23-12	23-12	29-17	28-18	
18	34-19	32-18	31-18	31-18	28-16	26-17	21-09	23-12	23-12	28-16	27-17	
19	33-19	27-17	31-19		27-16	26-17	22-10	23-13	23-12	27-16	29-18	
20	32-18	32-16	30-15		27-15	26-17	21-09	23-13	23-13	24-14	30-19	
21	32-17	28-17	29-16		27-15	27-16	22-10	23-14	23-12	24-13	29-18	
22	31-18	31-18	30-17	31-19	27-15	27-17	21-10	23-14	21-11	27-16	31-19	
23	32-18	30-19	30-18	30-20	27-16	27-17	20-09	26-15	24-12	27-17	31-20	
24	31-19	34-21	31-19	31-19	27-16	27-17	21-08	26-15	24-12	22-13	31-20	
25	30-18	33-20	31-20	31-20	27-16	27-16	21-09	26-15	25-13	24-14	30-19	
26	29-17	33-19	31-19	31-19	27-15	28-17		23-15	25-13	27-17	30-19	
27	30-16	33-20	31-19	31-19	29-16			25-15	24-13	26-16	31-20	
28	30-16	32-19	31-19	32-20	29-17			24-14	24-13	24-14	32-21	
29	32-18	31-20	31-18	31-19				24-15		24-13	31-20	
30	31-18	32-19	31-19	28-18				26-15				
31	29-17		31-19	30-18								

3.1.5 Soil analysis

Local soil of sandy clay loam texture was used in the experiment. Details of physical and chemical characteristics of soil are given in Table 3.4. Analysis of soil was carried out just before filling in pots for growing plants.

Table. 3.4. Physical and chemical characteristics of soil used in experiment

Parameter	Unit	Concentration
Electrical Conductivity	d S/m	0.65
pH	-	7.9
Organic matter	g/kg	5.6
Total nitrogen (N)	%	0.032
Available P	mg/kg	11.4
Extractable K	mg/kg	148
EDTA Zn	mg/kg	1.8
EDTA Cu	mg/kg	2.4
EDTA Fe	mg/kg	54
EDTA Mn	mg/kg	42
Textural Class	-	Sandy clay loam

3.1.6 Harvest and sampling

Vegetables were harvested at maturity, final date of harvesting of each vegetable is given in Table 3.2. Tomato and chili were harvested at two stages of maturity, premature when green and mature when red. Vegetables were picked manually, soil particles attached were washed with distilled water to remove any contamination and then air dried. Air dried vegetables were cut into slices or pieces as required, spread on shallow trays and dried in oven at 105°C to constant weight to completely remove the moisture content [98]. Dried samples were ground to fine powder and stored in zip-locked polyethylene bags and transported to University of Peshwar, Peshawar for chemical and nutritional analysis. Fresh samples were also used for analysis of some quality parameters.

3.2 Chemical Analysis

3.2.1 Fruit number and fresh weight

Fruit number was calculated manually as total number of fruits per plant. In root vegetables like carrot, turnip and radish and onion bulb, and in cauliflower there are only one root, bulb and flower per plant respectively. In case of leafy vegetables like spinach, this measurement could not be made. In peas, number of pods per plant was calculated. Plants of eggplant died during the experiment, so total number of fruits per plant could not be calculated.

Fresh weight of samples was measured after removal of dirt particles by washing with water and air drying. A digital balance was used and results reported as fresh weight per plant. In case of spinach, leaves harvested from on pot were weighed and reported.

3.2.2 Protein determination

Protein was determined by combustion method (AOAC Method 993.13) [98]. Samples were combusted at high temperature in pure oxygen, nitrogen in the sample was released in the form of oxides and was reduced to molecular nitrogen, separated from other gases and detected by thermal conductivity detector. Protein was determined by multiplying nitrogen content with 6.25, appropriate numerical factor for vegetables.

Elemental Analyser (Vario EL III CHNS-O GmbH) in CHNS mode was used for analysis of protein. Following steps were involved in the determination of protein in the sample.

i) Sample loading

About 2 mg of dried and completely homogenized sample was weighed in a small tin vessel and the weight was recorded into the system software. Tin vessel was closed tightly to remove any trapped air. Sample in tin vessel was introduced into the auto sampler tray of the system.

ii) Combustion of sample

Sample was dropped into the combustion tube automatically where complete combustion was carried out in a highly oxygenated atmosphere at a temperature of

1150°C. The use of tin foil further increased the temperature to about 1800°C, to ensure complete combustion. Combustion gases were moved towards copper tube by helium carrier gas where nitrogen oxides were reduced to nitrogen.

iii) Separation and detection of nitrogen

Besides nitrogen oxides, the combustion gases contain CO₂, H₂O and SO₂. Thermal conductivity detector cannot distinguish between these gases, so they were removed from nitrogen by adsorption traps. Nitrogen was moved directly to the detector and was analyzed. The system software converted the nitrogen content to the corresponding protein content by multiplying with 6.25 numerical factor, already fed to the software.

3.2.3 Sugars determination

Sugars were determined by Lane-Eynon general volumetric method (AOAC Method 923.09) [98]. A known volume of boiling Cu (II) was titrated against reducing sugars using methylene blue indicator with red color end point, the volume of sugar solution was noted. Lead acetate was used for clarification of solution and excess lead from the solution was removed with potassium oxalate. Non-reducing sugars present in the sugar solution did not react with Cu (II), they were first converted to reducing sugars by heating with citric acid and titrated to give value for total sugars. Non-reducing sugars were calculated from the difference of total and reducing sugars.

i) Reagents

- a) Copper sulphate solution (69.29g CuSO₄.5H₂O/L H₂O)
- b) Potassium sodium tartrate tetrahydrate (346g KNaC₄H₄O₆.4H₂O+100g NaOH/L H₂O)
- c) Fehling solution (mixing equal volumes of a and b immediately before use)
- d) Methylene blue indicator: 1% (1g C₁₆H₁₈N₃SCl.2H₂O/100mL H₂O)
- e) Neutral lead acetate solution: 45% (225g Pb(CH₃COO)₂.3H₂O/500mL H₂O)
- f) Potassium oxalate solution: 22% (110g K₂C₂O₄.H₂O/500mL H₂O)
- g) Standard sugar solution (9.50g pure sucrose/100 H₂O+5mL HCl, allowed to stand for three days and then made the volume to 1L with H₂O)

ii) *Determination of amount of potassium oxalate solution required to precipitate lead*

Volume of potassium oxalate solution required to precipitate lead from 2mL solution of lead acetate, used as clarifying agent, was determined by mixing 2mL of lead acetate to different portions of potassium oxalate solution (1.6, 1.7, 1.8, 1.9, 2.0 and 2.1mL) and 25mL H₂O. Solution was filtered and to the filtrate, a few drops of potassium oxalate were added. The filtrate still having some lead, produced a precipitate and those having no lead did not give any precipitate. That value of potassium oxalate was noted and used in the experiment.

iii) *Neutralization and titration of standard sugar solution*

50mL of standard sugar solution was mixed with 100 mL of H₂O in a beaker, 1-2 drops of phenolphthalein indicator was added and the solution was neutralized with drop wise addition of 20% NaOH till a light pink colour was obtained. Excess NaOH was removed with 1N HCl added drop wise to the solution till the pink colour disappeared completely. The neutralized solution was diluted to 200mL with distilled water. 10mL of Fehling solution was titrated against this solution, method followed is discussed in the section iv. Volume of sugar solution used was noted and compared to the standard chart provided (Table 3.5). The tabulated values were corrected for errors accordingly.

iv) *Standard method of titration*

Sugar solution was taken in burette and 10mL of Fehling solution (5mL solution a + 5mL solution b) was taken in Erlenmeyer flask. Almost entire sugar solution required to reduce the Fehling solution was added to the flask. The mixture was boiled for two minutes, 2 drops of methylene blue indicator were added and the titration was continued by adding 2 to 3 drops of sugar solution to the boiling mixture at 10 seconds intervals. At the end point, the indicator decolorized completely and the solution turned to brick red color of Cu₂O precipitate.

v) *Sugar determination procedure*

10g of homogenized vegetable sample was taken in a beaker containing about 500mL of water. The contents of the beaker were heated gently avoiding boiling with occasional stirring. It was filtered and neutralized with 0.1N NaOH and then

with 0.1N HCl using phenolphthalein indicator. 2mL of lead acetate solution was added and allowed to stand for 10 minutes. Excess lead was removed with potassium oxalate. The solution was filtered again and the volume was made to 500mL with distilled water in a volumetric flask.

a) Reducing sugars

The clarified solution was used directly for the determination of reducing sugars. The solution was taken in a burette and titrated against Fehling solution by the standard method of titration.

Reducing sugars were calculated by the following formula:

$$\% \text{ reducing sugars} = \frac{\text{factor}}{\text{titer}} \times \frac{100}{1000} \times \text{dilution}$$

Where

Titer is the volume of sugar solution used from the burette and

Factor is the value corresponding to the titer from Table 3.5.

b) Total sugars

For total sugars determination, 100mL of the clarified solution was taken in a beaker with 5g citric acid and boiled for 10 minutes. The solution was cooled, neutralized in the same way as standard sugar solution and diluted to 250mL with water in a volumetric flask. It was titrated against Fehling solution by following the standard method of titration.

Total sugars were also determined by the following formula:

$$\% \text{ total sugars} = \frac{\text{factor}}{\text{titer}} \times \frac{100}{1000} \times \text{dilution}$$

c) Non-reducing sugars

Non-reducing sugars were determined from the difference of total and reducing sugars using the following formula

$$\% \text{ non-reducing sugars} = (\% \text{ total sugars} - \% \text{ reducing sugars}) \times 0.95$$

Table 3.5. Factors for 10mL of Fehling solution used against sugar solution (AOAC Table 930.44) [98]

Titer (mL)	Invert sugar no.	Titer (mL)	Invert sugar no.
15	50.5	33	51.7
16	50.6	34	51.7
17	50.7	35	51.8
18	50.8	36	51.8
19	50.8	37	51.9
20	50.9	38	51.9
21	51.0	39	52.0
22	51.0	40	52.0
23	51.1	41	52.1
24	51.2	42	52.1
25	51.2	43	52.2
26	51.3	44	52.2
27	51.4	45	52.3
28	51.4	46	52.3
29	51.5	47	52.4
30	51.5	48	52.4
31	51.6	49	52.5
32	51.6	50	52.5

3.2.4 Ascorbic acid/Vitamin C determination

Ascorbic acid was determined by 2,6-dichloroindophenol titrimetric method (AOAC Method 967.21) [98]. Ascorbic acid (AA) is a reducing agent and in the titration, it reduced blue color dye, 2,6-dichloroindophenol, an oxidation reduction indicator, to a colorless compound. End point of the titration was rose pink. Metaphosphoric acid was used to extract ascorbic acid from vegetable sample and to avoid autoxidation of ascorbic acid.

i) Reagents

- a) Metaphosphoric acid solution: 3% (30g HPO₃/L H₂O), the solution was kept refrigerated and not used after more than 3 days.
- b) 2,6-Dichloroindophenol solution (62.5mg dye/250mL H₂O), the solution was stored in refrigerator.
- c) Standard ascorbic acid solution: 1mg/mL (100mg standard ascorbic acid/100ml HPO₃ solution), fresh solution was prepared each time.
- d) Working standard, (10mL standard ascorbic acid solution/100mL HPO₃ solution)

ii) Standardization of dye

5mL working standard solution+5mL HPO₃ solution was titrated against the dye solution to rose pink color end point which persisted for at least 5 seconds. Micro burette was used for titration. Dye factor was calculated as follows

$$Dye\ factor = \frac{conc.\ of\ AA\ solution}{volume\ used}$$

Concentration of ascorbic acid solution (working standard) used in this case was 0.5mg (1mg/mL × 10mL/100mL × 5mL).

iii) Procedure

10g of fresh vegetable was extracted with about 40mL of metaphosphoric acid solution using pestle and mortar. The solution was filtered and volume was made to 50mL with HPO₃ solution in a volumetric flask. Sample solution was prepared fresh just before titration. 5mL of sample solution was taken, diluted to 10mL with metaphosphoric acid solution and titrated against the dye using a micro burette with a rose pink end point. Amount of ascorbic acid present in the sample was calculated using the following formula:

$$mg\ of\ AA/100g\ of\ sample = \frac{titer \times dye\ factor \times 100}{weight\ of\ sample\ taken}$$

Where titer is the volume of dye used in titration.

3.2.5 Total titratable acidity determination

Total titratable acidity was determined by AOAC method 925.53 [98]. Acids were extracted from fresh samples with freshly distilled water and titrated against standard alkali with phenolphthalein end point. Colored solutions were diluted with freshly distilled water before analysis.

0.1M NaOH solution (1g/250mL H₂O) was prepared and standardized against potassium hydrogen phthalate (KHP). This solution was taken in a burette. 10g of fresh sample was extracted with about 80mL of freshly distilled water using a kitchen blender. The solution was filtered, transferred to 100mL volumetric flask and diluted up to the mark. 10mL of this solution was taken in Erlenmeyer flask, 0.3mL of phenolphthalein was added and titrated against NaOH solution to a light pink color end point. For recognition of end point, two samples were run at the same time for color comparison. In case of colored solutions, 10mL of the solution was further diluted to 100mL with freshly boiled water and titrated. Percent total titratable acidity was calculated using the following formula:

$$\begin{aligned} & \text{Total titratable acidity (\%)} \\ &= \frac{\text{Molarity of alkali} \times \text{acid factor} \times \text{titer} \times 100}{\text{vol. of sample taken for titration}} \\ & \times \frac{\text{vol. of sample solution}}{\text{wt. of sample taken}} \end{aligned}$$

Acid factor for different acids is given here: citric acid: 0.070, malic acid: 0.067, tartaric acid: 0.075, acetic acid: 0.060 and oxalic acid: 0.045.

3.2.6 Crude fat determination

Crude fat was determined by soxhlet extraction method (AOAC Method 920.39) [98]. Fats were extracted using hexane in a soxhlet extractor. Around 5g of dried and powdered sample was taken in a thimble, closed with fat free filter paper and placed in soxhlet extractor. Solvent vessel of the extractor was filled with *n*-hexane, 3-4 anti-bumping chips were added and extraction was carried out at a temperature of 60°C, below the boiling point of *n*-hexane. Extraction was continued for 8-12 hours depending

on the nature of the sample. Round bottom flask of the extractor was attached to a rotary to distill the solvent. Fats with some solvent were transferred to an already tared beaker, the solvent still remaining in the fats was further evaporated and weight of the extracted fats was determined. All the apparatus including thimble, extractor, extractor vessel and beaker were dried in oven and cooled in desiccator before using in experiment.

3.2.7 Crude fiber determination

Crude fiber was determined according AOAC method 962.09 [98]. Defatted sample was digested with boiling H_2SO_4 (1.25% solution) and filtered. The residue of the filtrate was digested with boiling NaOH (1.25% solution) and filtered. The residue was dried, ignited and the loss in weight was recorded as crude fiber. Boiling acid digested carbohydrates and boiling alkali digested proteins, the remaining residue was largely cellulose, a non-assimilable carbohydrate.

i) Reagents and chemicals

- a) Sulphuric acid solution: 0.255N (1.25% by dissolving 12.5g H_2SO_4 /L H_2O , specific gravity 1.84), solution was standardized against standard alkali and concentration was adjusted to $\pm 0.01\text{N}$.
- b) Sodium hydroxide solution: 0.313N (1.25% by dissolving 12.5g NaOH /L H_2O), solution was standardized against KHP and concentration was adjusted to $\pm 0.01\text{N}$.
- c) Ethyl alcohol: 95%
- d) n-hexane

ii) Procedure

About 2g of dry powdered sample was taken, fats were extracted as described in section 3.2.6. The defatted sample was transferred to a digestion flask, 0.5g asbestos and 200mL of 1.25% H_2SO_4 solution were added to the sample. The flask was fitted with condenser and heated so that sulphuric acid started boiling within 1-2 minutes. Boiling was continued for 30 minutes. Sample particles were prevented from adhering to the glass surface by shaking after every few minutes. The contents of the flask were poured immediately to a filtration cloth, the residue on the cloth

was washed with boiling water till all the acid was removed and transferred again to the digestion flask with 200mL of NaOH. The contents were heated to boiling within 1-2 minutes and kept boiling for 30 minutes with shaking at intervals. Contents of the flask were filtered through Gooch crucible fitted with asbestos mat, first washed with boiling water and then with 15mL ethanol. Insoluble material in crucible was dried at 105°C to a constant weight and the weight was recorded. Crucible along with contents were ignited in a muffle furnace at 500-550°C, cooled in desiccator and weighed. Percent crude fiber was determined by the following formula:

$$\text{Crude fiber (\%)} = \frac{\text{wt. of dried insoluble matter} - \text{wt. of ash}}{\text{wt. of sample}} \times 100$$

3.2.8 Ash determination

Ash content was determined according to AOAC dry ashing method (Method 923.03) [98]. Volatile content of the dried sample was removed by charring. The organic content was removed by burning in air at high temperature to CO₂, N₂ and H₂O. The remaining residue, composed of minerals, was calculated as ash.

Around 5g of the dried and powdered sample was taken in a porcelain crucible which was already dried and weighed. Sample was charred on a low flame and then heated in muffle furnace at 550°C for 5 hours. The ash obtained was cooled in desiccator, moistened, dried and re-ashed till a constant weight was obtained. Ash was cooled in desiccator, weighed and the amount determined by the following formula:

$$\text{Ash (\%)} = \frac{\text{weight of sample after ashing}}{\text{weight of sample}} \times 100$$

3.2.9 Elemental analysis

i) Combustion method

Elements in the sample (C, H, N and S) were determined by combustion method (AOAC Method 993.13) [98]. About 2mg of dried and homogenized sample was packed in a

small tin vessel and introduced into elemental analyser (Vario EL III CHNS-O Elemental Analyser GmbH). Complete combustion of the sample was carried out at high temperature in oxygenated atmosphere. The elements were converted to their corresponding oxides and moved by carrier gas through copper tubes where nitrogen oxides were reduced and passed directly to the detector while CO₂, H₂O and SO₂ were trapped in adsorption columns. Thermal desorption of the gas traps and subsequent detection occurred when nitrogen analysis was complete. Desorption of the gas traps occurred in such a way to prevent mixing of different gases and ensure complete separation.

ii) *Wet digestion method*

Elements Ca, Mg, K, Zn, Mn, Pb, Ni, Cr, Cd, Fe and Cu were analysed by wet digestion method [99]. About 0.5mg of dried powdered sample was digested with 10mL acid mixture (HNO₃:H₂SO₄:HClO₄ in ratio of 5:1:1) at 80°C. The digest was filtered with Whatman No. 42 filter paper, and diluted to 50mL with distilled water. For macro-elements (Ca, Mg and K) a further dilution of 2mL sample solution to 25mL with distilled water was carried out. For Ca and Mg determination, Lanthanum oxide was added as ionization suppressant. Lanthanum oxide solution was prepared by dissolving 27.7g of La₂O₃ in 49.7mL HCl and diluting the solution to 500mL with distilled water. Plant samples were introduced into atomic absorption spectrophotometer (AAS- Perkin Elmer, Analyst 700) for elemental analysis. Each element was determined at its specific wavelength using specific hollow cathode lamp as radiation source. The instrument was equipped with standard burner, air acetylene flame and a solid state detector. The results were reported as percent for macro-elements and ppm for micro-elements.

3.2.10 *Fatty acids analysis*

Oil was extracted from the vegetable samples and the fatty acids present were derivatized to corresponding methyl esters (having relatively lower boiling points than fatty acids) and injected to GC-column. Fatty acid methyl esters (FAMES) were separated, detected and analyzed (AOAC Method 991.39) [98].

i) Reagents and chemicals

- a) Boron trifluoride solution: 10% in methanol
- b) Methanolic sodium hydroxide solution: 0.5 N (2.0 g NaOH/100mL CH₃OH)
- c) Methanol: HPLC grade
- d) Hexane: HPLC grade
- e) Sodium chloride: saturated solution (36 gram NaCl/100mL H₂O)
- f) Tridecanoic acid methyl ester: internal standard (13.7 mg/mL hexane)

ii) Sample preparation

About 25mg of oil sample, 0.1mL of internal standard and 1.5mL of methanolic sodium hydroxide solution were added to 20mL test tube. Weight of the oil was introduced into the system software. The test tube was sealed with Teflon-lined cap and heated in boiling water bath for 5 minutes. The hydrolyzed sample was cooled, 2.5mL of boron trifluoride solution was added, sealed and heated in boiling water bath for 30 minutes with occasional shaking. The esterified solution was cooled and 5mL of saturated sodium chloride solution was added to decrease the solubility of FAMES and facilitate the extraction with hexane. The solution was extracted twice with hexane (1 mL each). The hexane extract was filtered through 0.45µm membrane filter and injected into GC-MS using auto injection system.

iii) Instrument and operating conditions

GC-MS (Shimadzu Model QP 2010 plus) equipped with treated polyethylene glycol column (30 m × 0.35 mm, 0.25 µm, Technochroma) was used for separation and detection of FAMES. 1µL of sample solution was injected to the system by auto injection system. Helium was used as carrier gas and the instrument was operated at 70 KPa pressure with a split ratio of 1:50. Injector, ion source and interface temperature were maintained at 240°C, 250°C and 240°C respectively. Column oven operating conditions are given in the Table 3.6. Total GC program time was 45.67 minutes with solvent cut time of 1.6 minutes, MS start time of 1.65 minutes and MS end time of 45 minutes. Acquisition mode of the instrument was set at scan with 85-380 M/Z. Peaks recorded on the chromatogram were analyzed by the instrument and the results were recorded as percentage concentration or ppm of the corresponding fatty acids.

Table. 3.6. Column oven programming of GC-MS

Rate (°C/min)	Temperature (°C)	Hold (minutes)
-	50	1
15	150	0
2.5	175	5
2.5	220	5

3.3 Statistical Analysis

For triplicate experimental data, mean and standard deviations were calculated using Microsoft Excel 2010, the results are thus expressed as mean $\pm$ standard deviation throughout the results and discussion section.

Data was subjected to student's *t* test for independent samples using software program SPSS 16.0 (SPSS Inc., USA) and the value of probability level (*P*) was evaluated. The *P*-level was indicated significant at $P \leq 0.05$, non-significant at $P > 0.1$ and trend at $0.05 < P \leq 0.1$. The *P*-level is mentioned with all the results.

The fatty acids analysis was in singlicates due to non-availability of enough sample material. So no statistical analysis was applied on fatty acid data of samples.

RESULTS AND DISCUSSION

Chapter 4

RESULTS AND DISCUSSION

4.1 Effect of Enhanced CO₂ on Tomato (*Lycopersicon esculentum*) Fruit

Tomato (*Lycopersicon esculentum*) is one of the most important vegetables used worldwide in a number of ways. It is either used as fresh vegetable in the form of salad or processed into products like soup or ketchup. It is also blended into juice which is used as a drink in many parts of the world. The quality of tomato fruit is assessed by its chemical composition including dry matter, sugars, acidity and volatile compounds [100]. Tomato is also an important source of minerals and vitamin C [101].

In the present study the effect of enhanced atmospheric CO₂ on physico-chemical changes in tomato was evaluated. The proximate, elemental and fatty acid composition, vitamin C and total titratable acidity of two varieties of tomato at two stages of maturity was investigated. The results revealed interesting trends in changes in the nutritional parameters of tomato fruits.

4.1.1 Effect on yield

Elevated CO₂ increased the yield of tomato fruit by increasing the number of fruits per plant as well as fresh weight of fruit per plant as shown in Table. 4.1. Fruit number has been increased by 68.42% and 59.09% while fresh weight of fruit per plant increased by 83.61% and 74.27% for Eureka and Astra respectively when grown under elevated CO₂. Increase in number of fruits per plant is significant at 95% confidence level ($P \leq 0.05$) and increase in fresh weight of fruit per plant is significant at 99% confidence level ($P \leq 0.01$). Increase in yield with elevated CO₂ can be explained on the basis of increased photosynthesis. Rate of photosynthesis is directly related to concentration of CO₂ in the atmosphere and any enhancement in CO₂ will increase the photosynthetic activity and hence the productivity of food crops and vegetables. Özçelik and Akilli reported 43% increase in tomato fruit when grown at 1000 $\mu\text{mol mol}^{-1}$ [102]. Increase in photosynthetic rate, biomass, fruit fresh and dry weight, plant height, stem thickness, number of roots

and shoots of tomato with elevated CO₂ has been observed in many earlier studies [69, 103-105].

Table 4.1. Yield of tomato fruits grown at two levels of atmospheric CO₂

Parameter	Tomato Variety	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
No. of fruits/plant					
	Eureka	6.33 ± 1.155	10.67 ± 2.081	+68.42	0.034
	Astra	7.33 ± 1.527	11.67 ± 2.082	+59.09	0.044
Fresh Weight (g/plant)					
	Eureka	73.22 ± 3.76	134.43 ± 4.81	+83.61	<0.001
	Astra	40.58 ±3.029	70.72 ± 3.421	+74.27	<0.001

4.1.2 Effect on proximate composition

The effect of elevated CO₂ on proximate composition of tomato has been summarized in Table. 4.2. Enhanced CO₂ caused changes in the proximate composition of tomato fruits by decreasing protein, vitamin C, fats and ash content and increasing sugars and fibre contents of both varieties of tomatoes at both stages of maturity.

Protein content of tomato fruits in our experiment was comparable to the values reported by Guil-Guerrero and Reboloso-Fuentes [101]. Protein content of Eureka was 12.23 ± 0.405% and Astra was 15.36 ± 0.750% which was reduced significantly by elevated CO₂. Reduction was 13.64% and 18.27% for Eureka and Astra respectively and is significant at 95% confidence level ($P \leq 0.05$) (Table 4.2). The reduction in protein content with elevated CO₂ has been observed for many grains and other crops like wheat, barley, potato, soybean and maize [96] but the results are different for experiments. Li, *et al.*, observed decrease in nitrogen and hence protein content of roots while increase in nitrogen content of stem and leaves of tomato [69]. Wheeler, *et al.*, observed no change in protein content of tomato fruit grown under CO₂ enriched atmosphere [67]. Different hypotheses have been presented to explain the decrease in nitrogen and protein content of

plants when grown at elevated CO₂. The most important is dilution mechanism, which indicates that elevated CO₂ results in a greater photosynthetic activity and greater biomass. Increased biomass dilutes all the other elements except those used in photosynthesis (C, H and O) as the uptake of other elements do not increase at the same rate as that of photosynthesis [37]. Another hypothesis suggests the decrease in nitrogen uptake by leaves as a result of an increase in water use efficiency and a decrease in transpiration rate which collectively decrease the uptake of water from soil, resulting in reduced uptake of nitrogen along with water from soil [106]. Reduced plant demand for nitrogen by increasing nitrogen use efficiency of the plants also leads to reduction in nitrogen and protein content of plants. Another factor which decreases the nitrogen content of plants is the down regulation of photosynthetic enzyme “Rubisco” a major nitrogen containing enzyme in green leaves of C₃ plants. Decrease in nitrogen content of leaves reduces the nitrogen and protein content of roots, tubers, flowers and stems of plants as it mostly comes by translocation from leaves [107]. There are some mechanisms which increase the nitrogen content of plants and these include the change in mycorrhizal status and increased root capacity for nitrogen uptake due to increased root area with elevated CO₂. However the net effect is a decrease in nitrogen and hence protein content in most of the plants [106].

Sugars and fibre content of tomato fruits increased with elevated CO₂, as seen in Table 4.2. Sugars present in tomato were mostly reducing sugars, i.e. glucose and fructose, with small quantity of non-reducing sugars. This is in agreement with previous experiments conducted on tomato [68]. Total sugar content of Eureka was 40.37 ± 0.930 and Astra was $33.31 \pm 1.101\%$. These results support the statement of Suárez, *et al.*, that nutritional composition of tomato depends to some extent on the variety being cultivated [108]. Green premature stage of Eureka contained lesser amount of sugars, i.e. $34.46 \pm 0.998\%$ as compared to red mature Eureka fruit which is in agreement with the results of Dalal, *et al.*, that sugar content of tomato increases with maturity of the fruit [109].

Elevated CO₂ increased total sugars of tomato fruits (Table 4.2). The increase was 16.12% for Astra, 20.85% for Eureka and 14.16% for Eureka premature. Reducing sugars increased by 15.64% for Astra, 22.69% for Eureka and 12.41% for premature stage of

Eureka. Non-reducing sugars also increased for Astra and for both stages of Eureka. Increase in total sugars and reducing sugars is significant at 95% confidence level ($P \leq 0.05$) for both varieties at two stages of maturity, but increase in non-reducing sugars is non-significant ($P > 0.1$). Increase in the amount of sugar with elevated CO₂ in our work is in agreement with the results of Islam, *et al.*, where reducing sugars-glucose and fructose increased with elevated CO₂ and no significant effect on non-reducing sugars-sucrose was observed [68].

Fiber content of tomato in our experiment was smaller than previously reported by Suárez, *et al.* [108], and it increased with enhanced CO₂ concentration. As summarized in Table 4.2, for Astra the increase was 5.25% and for Eureka it was 4.40%. For premature stage of Eureka, fiber content increased, however the increase in this case was non-significant. For mature stages of Astra and Eureka, increase in the fiber content with elevated CO₂ was significant at 95% confidence level ($P \leq 0.05$). Fiber content for a number of crops has been found to increase with elevated CO₂, however no specific trend has been previously observed for tomato [67]. Increase in sugar and fiber content with enhanced atmospheric CO₂ is expected because any increase in CO₂ leads to increased rate of photosynthesis causing the plants to accumulate greater amount of carbohydrates and carbon based secondary compounds.

Tomato fruits grown in our greenhouses contained a large amount of vitamin C and the quantity is comparable to that mentioned by Guil-Guerrero and Rebollosa-Fuentes [101]. Vitamin C content in our experiment increased with maturity of the tomato fruit. This is in accordance with the results of Islam, *et al.*, and Dalal, *et al.*, who stated the ascorbic acid content of red stage of tomato was higher than premature green stage [68, 109]. However elevated CO₂ decreased ascorbic acid content by 20.02% and 24.72% for Eureka and Astra respectively (Table 4.2). The decrease was significant at 99% confidence level ($P \leq 0.01$) for both varieties. The decrease in ascorbic acid is smaller for premature green stage of Eureka, i.e. 9.59%, significant at 95% confidence level ($P \leq 0.05$). The reason for greater impact on mature fruit could be the longer time spent in CO₂ rich atmosphere compared to the green stage. Some earlier experiments conducted on tomato fruits reported increase in ascorbic acid when grown in CO₂ enriched

atmosphere [68]. The reason for reduction of ascorbic acid can be the nutritional dilution caused by higher amount of biomass produced at elevated CO₂. Another reason may be the fact that additional carbon was funnelled to the plant biomass and not to the antioxidant compounds including ascorbic acid [90]. Tomato is a good source of ascorbic acid and any decrease due to enhanced concentration of CO₂ will lower the nutritional quality of tomato.

Total titratable acidity of tomato fruits was higher for red stage than green premature stage (Table 4.2), results similar to that reported by Dalal, *et al.* [109]. However the values of percent total titratable acidity in our experiment were higher. Total titratable acidity decreased slightly with atmospheric CO₂ enrichment, the decrease was non-significant statistically ($P>0.1$). No results are reported earlier on the effect of elevated CO₂ on total titratable acidity of tomato, although organic acid content has been reported to decrease significantly when grown at higher concentrations of CO₂ [68].

Fat content of tomato in our experiment was slightly lower, Eureka has higher content than Astra and red stage of Eureka contained more fats than the green premature stage (Table 4.2). The varietal differences in fat contents of tomato has already been reported in literature [101]. Elevated CO₂ decreased the fat content of tomato. The decrease was 11.52% for Astra, 13.71% for Eureka and 9.95% for premature stage of Eureka. The decrease was significant at 95% confidence level ($P\leq0.05$) for both varieties.

Ash content of tomato in the present work was different than the previously reported results [101, 108]. Ash content of green stage was smaller than red stage and decreased with elevated CO₂ (Table 4.2). Reduction was 11.40% for Astra and 8.83% for Eureka, and was significant at 95% confidence level ($P\leq0.05$). The ash content of green stage of Eureka tended to decrease by 5.41% ($0.05<P\leq0.1$).

The reduction in fat and ash content with enhanced CO₂ was slightly smaller for premature stage of Eureka as compared to mature stage and this may be due to the reason that premature fruit has spent considerably less time in enhanced CO₂ environment as compared to mature fruit and hence the effect is less pronounced. No significant reduction in either fat or ash content of tomato has been observed in earlier studies [67].

Table 4.2. Proximate composition of tomato fruits grown at two levels of atmospheric CO₂

Tomato Variety	Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
Eureka					
	Protein (%)	12.23 ±0.405	10.56 ± 0.250	-13.64	0.004
	Total Sugars (%)	40.37 ± 0.930	48.79 ± 1.049	+20.85	<0.001
	Reducing Sugars (%)	36.16 ± 1.075	44.37 ± 1.093	+22.69	0.001
	Non-reducing Sugars (%)	3.99 ± 1.747	6.63 ± 1.083	+66.03	ns
	Fiber (%)	10.83 ± 0.164	11.31 ± 0.246	+4.40	0.049
	Vitamin C (mg of AA/100 gram)	101.29 ± 3.151	81.01 ± 2.549	-20.02	0.001
	Total Titratable Acidity (%)	0.57 ± 0.035	0.48 ± 0.032	-6.76	ns
	Fat (%)	3.31 ± 0.247	2.85 ± 0.091	-13.71	0.041
	Ash (%)	9.14 ± 0.391	8.33 ± 0.309	-8.83	0.049
Eureka premature					
	Total Sugars (%)	34.46 ± 0.998	39.34 ± 1.011	+14.16	0.004
	Reducing Sugars (%)	29.59 ± 1.401	33.27 ± 1.088	+12.41	0.022
	Non-reducing Sugars (%)	5.22 ± 3.013	5.78 ± 1.978	+10.72	ns
	Fiber (%)	10.11 ± 0.105	10.49 ± 0.281	+3.72	(0.087)
	Vitamin C (mg of AA/100 gram)	63.59 ± 3.395	57.49 ± 2.443	-9.59	0.037
	Total Titratable Acidity (%)	0.57 ± 0.051	0.54 ± 0.030	-4.71	ns
	Fat (%)	2.21 ± 0.115	1.99 ± 0.030	-9.95	0.028
	Ash (%)	8.17 ± 0.204	7.72 ± 0.266	-5.41	(0.092)
Astra					
	Protein (%)	15.36 ± 0.750	12.55 ± 1.200	-18.27	0.026

	Total Sugars (%)	33.31 ± 1.101	38.68 ± 1.424	+16.12	0.007
	Reducing Sugars (%)	30.56 ± 0.435	35.34 ± 1.312	+15.64	0.002
	Non-reducing Sugars (%)	2.62 ± 0.929	3.18 ± 0.278	+21.43	ns
	Fiber (%)	9.58 ± 0.276	10.08 ± 0.114	+5.25	0.043
	Vitamin C (mg of AA/100 gram)	71.44 ± 3.065	53.78 ± 3.841	-24.72	0.003
	Total Titratable Acidity (%)	0.49 ± 0.032	0.46 ± 0.021	-4.79	ns
	Fat (%)	1.99 ± 0.110	1.77 ± 0.075	-11.52	0.040
	Ash (%)	10.99 ± 0.120	9.74 ± 0.355	-11.40	0.004

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.1.3 Effect on elemental composition

Elemental composition of tomato fruits is given in Table 4.3. Experiments results are comparable to nutritional values available in nutrient databases of United States Department of Agriculture (USDA) [110] and McCance and Widdowson's [111]. The values of Ca, Mg, Zn and Cu in present work are greater than the values of McCance and Widdowson's but lower than USDA. The amount of K and Fe is lower but that of Mn is higher than the values of both databases. The differences in our values and the values of two databases could be a result of change in variety, geographical region, method of cultivation and sampling. Suárez, *et al.*, have reported that these factors affect the nutrient and chemical composition of tomato fruits [108].

The impact of elevated CO₂ on elemental composition of tomato fruit is given in Table 4.3. The amount of C, H, Ca, Fe and Cu increased in two varieties significantly at 95% confidence level ($P\leq 0.05$), S tended to increase ($0.05<P\leq 0.1$) while the rest of the elements, N, Mg, Zn, Mn and heavy metal Pb, Ni, Cr and Cd decreased significantly ($P\leq 0.05$). Potassium remained unaltered ($P>0.1$) for both varieties of tomato. The extent of increase and decrease was different for the two varieties. Increase in C and H with elevated CO₂ is due to increase in photosynthetic activity which increases carbon based

compounds like carbohydrates. As C and H are main elements in carbohydrates, any increase in latter will increase the former. Since plants are growing in a different environment with increased biomass, a change in elemental composition of plants is understandable. It is expected that larger plants growing in CO₂ rich environment should require higher amounts of elements, so the mineral concentration of plants should increase. However the case is not so. Most of the elements have rather decreased in tomato fruit. Different mechanisms have been suggested by the earlier workers to explain the change in elemental composition with elevated CO₂ [106]. These include:

- Nutrient dilution caused by an increased plant size, i.e., the plant size increases but the concentration of elements does not increase in the same proportion which decreases the percent composition of the elements.
- Increased water use efficiency which decreases the water uptake and hence the uptake of elements by the plants from the soil.
- Increased root size which increases the uptake of elements from the soil, and hence the concentration of elements in plants should increase.
- A change in mycorrhizal status, i.e. increase in the colonization of mycorrhizal fungi around the plant roots, which increases the root efficiency for nutrient uptake.

All these mechanisms operate at the same time and net result can be either increase or decrease in the elemental composition of the plants as can be seen in the present results for tomato (Table 4.3). This is in accordance with the findings of Loladze, according to him elemental concentration of the plants should alter with mostly decreased status, however an increase or no change is also a possibility. He reported a decrease in N, P, Fe and Zn but increase in Ca content of rice and decrease in every measured element except K in wheat with elevated CO₂ [37]. In earlier experiment conducted on tomato, K, P and Mg were not changed by elevated CO₂ while Ca content of two varieties of tomato increased. Calcium content was thought to be related with water movement in plants and so increased in tissues where transpiration rate is low like fruits and tuber [67]. The idea that elevated CO₂ increases the uptake of elements from soil is supported by the results of Li, *et al.*, who observed increased uptake of N, K, P and C from hydroponic solution by

tomato seeds, showing increased demand of these nutrients [69]. The results of Overdieck reveal that elemental concentration of herbaceous and woody plants grown under CO₂ enriched atmosphere decreased but absolute total concentration of the plant tissues increased, supporting the idea of greater uptake of elements from the soil but overall affect was reduction due to dilution effect caused by bigger size of plants [76]. A non-significant increase in Fe, Mn and S has been observed for potato caused by enhanced CO₂ [54].

It is important to notice that the two varieties studied have responded differently to elevated CO₂. It is evident from Table 4.3 that change in elemental composition is all the way different with enhanced CO₂. The difference can be seen in all the elements, however it is very much pronounced for C:N ratio, Mg, Zn and Fe.

Table 4.3. Elemental composition of tomato fruits grown at two levels of atmospheric CO₂

Tomato Variety	Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
		400 (ambient)	1000		
Eureka	Macro-elements (%)				
	C	32.71 ± 1.242	43.55 ± 0.218	+33.14	<0.001
	N	1.96 ± 0.065	1.69 ± 0.040	-14.43	0.004
	H	4.77 ± 0.107	5.74 ± 0.122	+17.61	<0.001
	S	0.53 ± 0.007	0.57 ± 0.024	+6.58	(0.072)
	Ca	0.14 ± 0.000	0.15 ± 0.000	+4.81	<0.001
	Mg	0.18 ± 0.003	0.14 ± 0.008	-22.82	0.001
	K	0.46 ± 0.001	0.46 ± 0.000	-0.07	ns
	C/N ratio	16.72 ± 0.389	25.78 ± 0.344	+54.23	<0.001
	Micro-elements (μg g ⁻¹)				
	Zn	15.41 ± 0.083	13.25 ± 0.023	-14.02	<0.001
	Mn	43.00 ± 0.104	41.18 ± 0.244	-4.23	<0.001
	Fe	34.34 ± 0.111	388.60 ± 0.122	+13.16	<0.001
	Pb	0.59 ± 0.059	0.46 ± 0.034	-21.93	0.029
	Ni	5.03 ± 0.122	2.46 ± 0.092	-51.06	<0.001

	Cu	2.78 ± 0.020	3.51 ± 0.012	+26.14	<0.001
	Cr	2.55 ± 0.058	18.20 ± 0.120	-28.72	0.001
	Cd	0.27 ± 0.003	0.25 ± 0.001	-5.99	0.001
Astra	Macro-elements (%)				
	C	31.66 ± 2.120	44.41 ± 2.020	+40.27	0.002
	N	2.46 ± 0.120	2.01 ± 0.192	-18.27	0.026
	H	4.32 ± 0.200	5.52 ± 0.219	+27.77	0.002
	S	0.37 ± 0.013	0.39 ± 0.004	+6.50	(0.095)
	Ca	0.13 ± 0.000	0.14 ± 0.000	+3.85	<0.001
	Mg	0.18 ± 0.004	0.17 ± 0.001	-5.48	0.014
	K	0.46 ± 0.001	0.46 ± 0.000	-0.40	(0.085)
	C/N ratio	12.92 ± 1.264	22.32 ± 3.154	+72.74	0.009
	Micro-elements (µg g ⁻¹)				
	Zn	19.63 ± 0.163	14.06 ± 0.080	-28.36	<0.001
	Mn	43.15 ± 0.120	39.80 ± 0.092	-7.77	<0.001
	Fe	37.36 ± 0.144	38.49 ± 0.120	+3.03	<0.001
	Pb	0.61 ± 0.073	0.42 ± 0.045	-31.28	0.018
	Ni	5.04 ± 0.317	1.97 ± 0.219	-60.85	<0.001
	Cu	3.03 ± 0.042	3.57 ± 0.050	+17.80	<0.001
	Cr	2.27 ± 0.042	1.79 ± 0.168	-21.41	0.031
	Cd	0.27 ± 0.009	0.25 ± 0.003	-9.38	0.010

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.1.4 Effect on fatty acid composition

Fatty acid composition and effect of CO₂ on fatty acid composition of tomato varieties is given in Table 4.4. Concentration of majority of fatty acids was higher in Eureka variety as compared to Astra and this is also reflected by the fat content of the two varieties (Table 4.2). Major fatty acid in our varieties was linoleic acid followed by linolenic acid, palmitic acid, and oleic acid. This profile is somewhat similar to the profile of Guil-Guerrero and Rebollosa-Fuentes [101] except the linolenic acid, which was present in smaller quantity in their varieties. Other fatty acids were present in minor amount in tomato oil in the present work. Fatty acids have mostly decreased or remained unaffected

by elevated CO₂ in our experiment. Major fatty acids, linoleic and oleic, decreased, palmitic acid increased in both varieties and linolenic acid decreased in Astra and increased in Eureka. Stearic acid decreased in Eureka and increased very slightly in Astra. Among the minor fatty acids, myristic acid, margaric acid, arachidic acid and tetracosanoic acid increased slightly for Eureka and remained unaltered for Astra. Pentadecanoic acid and behenic acid increased slightly for both varieties. Elaidic acid, octadecanedienoic acid and tricosanoic acid decreased slightly for Astra and increased for Eureka. Although the decrease or increase was about 50% and in some cases even 100%, but as the analysis were not carried out in triplicate and no statistics is applied on the results, it is difficult to draw any solid conclusion for these minor fatty acids.

The decrease in fatty acid of tomato fruits with elevated CO₂ is in accordance with the reduction in fat content of tomato fruits under the same condition as shown in Table 4.2. To our knowledge, no results on the effect of enhanced atmospheric CO₂ upon the fatty acid composition of tomato have been reported to date, though reported for some other crops. Previous studies on other crops report no change in fatty acid composition under CO₂ enriched conditions. In the reviews of DaMatta, *et al.*, and Idso and Idso, it is reported that oil and fatty acid composition of wheat, maize and soybean remains unaltered under by elevated CO₂ [20, 94]. Similarly no significant influence of CO₂ enrichment has been observed on the fatty acid profile of peanut oil [84]. The only reduction in fatty acid composition with elevated CO₂ has been observed for oilseed rape [74]. The reduction in important fatty acids like linoleic and linolenic acid is of great concern, as these are essential fatty acids used as fuel and also in biological processes in human body [112]. Human body is unable to synthesize these fatty acids directly, so any reduction in fatty acids will lower the nutritional quality of tomato fruits.

Table 4.4. Fatty acid composition of tomato fruits grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	Astra		Eureka	
	CO ₂ concentration (μmol mol ⁻¹)			
	400 (ambient)	1000	400 (ambient)	1000
C14:0; Myristic acid	0.001	0.001	0.003	0.004
C15:0; Pentadecanoic acid	0.000	0.001	0.001	0.002
C16:0; Palmitic acid	0.046	0.047	0.114	0.126
C17:0; Margaric acid	0.001	0.001	0.002	0.003
C18:0; Stearic acid	0.010	0.013	0.035	0.028
C18:1c; Oleic acid	0.016	0.005	0.076	0.056
C18:1n9t; Elaidic acid	0.002	0.001	0.007	0.007
C18:2c; Linoleic acid	0.128	0.007	0.471	0.410
C18:2t; Ocatadecadienoic acid	0.002	0.000	0.000	0.004
C18:3n3; Linolenic acid	0.063	0.001	0.233	0.315
C20:0; Arachidic acid	0.003	0.003	0.007	0.008
C22:0; Behenic acid	0.001	0.002	0.004	0.005
C23:0; Tricosanoic acid	0.001	0.000	0.002	0.004
C24:0; Tetracosanoic acid	0.002	0.002	0.005	0.007

4.2 Effect of Enhanced CO₂ on Chili (*Capsicum annuum*) Fruit

Chili (*Capsicum annuum*) is an important vegetable which is used worldwide as vegetable, spices and even as medicine in some countries. It is consumed either raw or cooked. It is eaten as salad, fried, mixed with other vegetables, soups and salsas. It is dried, powdered and stored for long periods of time. Capsicum is also important from nutritional point of view, containing high levels of vitamins including A, C, E and B complex, aromas and natural colours [113]. In medicine, it is used as analgesic and circulatory stimulant. Capsicum spray can be used as a non-lethal weapon to disperse

people and was used by native Americans against invaders and nowadays by police force in many countries [114].

The purpose of the present experiment was to evaluate the effect of enhanced atmospheric CO₂ on the nutritional composition of different varieties of chili (*Capsicum annuum*) at two stages of maturity i.e. a premature green stage and a mature red stage. It was found that increase in atmospheric CO₂ caused a nutritional imbalance for all varieties of capsicum at both stages of maturity. Since data on these particular local cultivars is not available, a point to point comparison is not possible, but the trend of change is compared with other plants.

4.2.1 Effect on yield

Elevated CO₂ increased the number of capsicum fruits produced per plant as well as fresh weight of the capsicum fruits, thus increasing the yield of capsicum. Average increase in number of fruits per plant was 41.21%. For Magama and Angrika the increase was significant at 95% confidence level ($P \leq 0.05$) and was 34.67% and 50.00% respectively. For 99PE-1689, Best Choice and Diana the increase in number of fruits followed a trend ($0.05 < P \leq 0.1$) and was 39.47%, 46.67% and 35.29% respectively. Total fresh weight of capsicum fruits increased for all varieties significantly at 95% confidence level ($P \leq 0.05$), increase was 53.65%, 51.41%, 59.55%, 45.89% and 43.80% for 99PE-1689, Magama, Angrika, Best Choice and Diana respectively (Table 4.5). Increase in number of fruits/plant and fruits' fresh weight was quantitatively different for the five varieties of capsicum, however, qualitatively the response of all varieties to the elevated CO₂ was same. The results are in agreement with the previous experiments where growth, yield and root/shoot ratio of capsicum plants increased with the enhanced atmospheric CO₂ [115, 116]. This is quite expected as any increase in concentration of atmospheric CO₂, a raw material for photosynthesis, will increase the rate of photosynthesis, thus increasing the edible biomass, the final product of photosynthesis.

Table 4.5. Yield of capsicum fruits grown at two levels of atmospheric CO₂

Parameter	Capsicum Variety	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
No. of fruits/plant					
	99PE-1689	9.50 ± 2.082	13.25 ± 2.754	+39.47	(0.073)
	Magama	6.50 ± 1.291	8.75 ± 0.957	+34.61	0.031
	Angrika	3.00 ± 0.816	4.50 ±0.577	+50.00	0.024
	Best Choice	3.75 ±0.957	5.50 ± 1.291	+46.67	(0.072)
	Diana	4.25 ± 0.957	5.75 ± 0.957	+35.29	(0.069)
Fresh Weight (g/plant)					
	99PE-1689	45.93 ± 6.117	70.58 ± 4.413	+53.65	0.001
	Magama	20.57 ± 3.619	34.17 ± 4.741	+51.41	0.008
	Angrika	14.75 ± 2.130	23.54 ± 3.399	+59.55	0.005
	Best Choice	19.14 ± 2.624	27.92 ± 3.570	+45.89	0.007
	Diana	21.47 ± 3.117	30.87 ± 2.946	+43.80	0.005

number in parenthesis indicate trend ($0.05 < P \leq 0.1$)

4.2.2 Effect on proximate composition

Elevated CO₂ affected the proximate composition of capsicum fruits by decreasing protein, fat, ash and vitamin C and increasing sugar and fat content for all varieties including green stages (Table 4.6).

Protein content was determined for only three varieties i.e. 99PE-1689, Magama and Best Choice due to non-availability of the Angrika, Diana and green stages of capsicum which was already consumed in the rest of analysis. Highest amount of protein was present in the Magama which was 21.01 ± 0.731 and lowest amount was present in 99PE-1689 which was 12.29 ± 0.718 . Protein content of Best Choice was 15.85 ± 0.756 . A similar variation in the protein content of different varieties of capsicum was also reported by Cook, *et al.*, [117]. Elevated CO₂ decreased the protein content of capsicum fruit significantly at 95% confidence level ($P \leq 0.05$) (Table 4.6). Highest reduction was observed for Best Choice i.e., 31.62%. Lowest reduction was observed for Magama, having the greater amount of protein, i. e. 25.10%. No such reduction in protein has been

previously reported for capsicum, however observed for other crops like rice, potato, and soybean [54, 96]. The reduction in protein content with elevated CO₂ is considered to be due to nutritional dilution caused by increased non-structural carbohydrates because of increased photosynthesis [118]. Increased water use efficiency and decreased rate of transpiration at elevated CO₂ reduces the nitrogen uptake by plant roots and as a result decreases the protein content of the plant [93]. Lower protein content at enhanced CO₂ badly affects nutritional quality of capsicum.

Sugars in capsicum are mostly reducing sugars i.e. glucose and fructose, with small amount of non-reducing sugars i.e. sucrose (Table 4.6). The amount of total sugars were 38.69% $\pm$ 0.851, 29.02% $\pm$ 0.810, 21.38% $\pm$ 0.916, 28.57% $\pm$ 0.605 and 27.41% $\pm$ 1.346 for 99PE-1689, Magama, Angrika, Best Choice and Diana respectively. Reducing sugars were 32.80% $\pm$ 1.635 for 99PE-1689, 21.11% $\pm$ 0.816 for Magama, 12.51% $\pm$ 0.981 for Angarika, 24.37% $\pm$ 1.043 for Best Choice and 17.93% $\pm$ 0.874 for Diana variety. Non-reducing sugars in 99PE-1689, Magama, Angrika, Best Choice and Diana were 5.59% $\pm$ 1.356, 7.52% $\pm$ 0.722, 8.43% $\pm$ 0.393, 3.99% $\pm$ 0.663 and 9.01% $\pm$ 1.005 respectively. The amount of total, reducing and non-reducing sugars in premature green stage of 99PE-1689 was 22.22% $\pm$ 0.737, 14.37% $\pm$ 0.548 and 7.46% $\pm$ 1.212 respectively. It can be seen that the sugar content of mature red stage of 99PE-1689 was higher than the corresponding green stage. This supports the findings of Martinez, *et al.*, who proposed greater accumulation of sugars and carbohydrates in the cell walls of mature capsicum fruits compared to premature fruits due to changes in the action of cell wall degrading enzymes [119].

Sugar content of capsicum increased in the elevated atmospheric CO₂ (Table 4.6). In 99PE-1689, increase in total sugars was 12.85%, 13.38% in reducing sugars and 9.90% in non-reducing sugars. Just like the ambient values of sugars, the increase in sugar with elevated CO₂ was also higher for red stage than the green stage of 99PE-1689. For green stage, total sugars increased by 9.66% reducing sugars by 12.64% and non-reducing by 4.20%. For Magama, increase in total sugar was 13.59%, reducing sugar was 15.35% and non-reducing was 2.32%. Increase in total sugars for Angrika was 12.75 %, reducing sugars was 18.01% and non-reducing sugars was 8.68% in elevated CO₂. For Best

Choice, the observed increase in total sugar with elevated CO₂ was 13.86%, reducing sugar was 14.15% and non-reducing was 12.15%. Total sugars of Diana increased by 11.83%, reducing sugars increased by 14.37% and non-reducing sugars by 7.03% with elevated CO₂. Increase in total and reducing sugar was significant at 95% confidence level ($P \leq 0.05$) for all varieties of capsicum fruits including the green stage. Increase in non-reducing sugars was non-significant ($P > 0.1$), except that for Angrika, which tended to increase ($0.05 < P \leq 0.1$).

Fiber content of capsicum was higher and was almost similar for green and red stages of capsicum varieties, results being in agreement with the previous results [119]. Elevated CO₂ increased the fiber content of red stages significantly at 95% confidence level ($P \leq 0.05$) and for green stages it tended to increase the fiber content ($0.05 < P \leq 0.1$). Increase was 7.97%, 8.75%, 9.03%, 6.09% and 7.10% for red mature stages of 99PE-1689, Magama, Angrika, Best Choice and Diana respectively. Increase in fiber content for green stage of 99PE-1689, Magama and Angrika was 4.71%, 3.19% and 4.05% respectively. Although the fiber content of red and green stages was not much different, the effect of elevated CO₂ on fiber content was much pronounced for red stage as compared to green stage.

Effect of enhanced CO₂ on carbohydrate, sugar and fiber contents of capsicum is not reported earlier, however increase was observed for other crops and vegetables like tomato [68] and potato [54]. Increase in sugar and fiber concentration is expected as enhanced atmospheric CO₂ leads to increased photosynthetic rate, resulting in greater amount of plant non-structural carbohydrates leading to relatively more carbon based compounds. The fact that sugar and fiber content of red stage was affected more than green stage can be due to greater time spend by red stage in elevated CO₂ environment as compared to green stage.

Vitamin C is a very important constituent of capsicum and in the present study its amount increased with maturity of the fruit. This is in accordance with the previous experiment conducted on capsicum [119]. Vitamin C content of two varieties, i.e., 99PE-1689 and Angrika, fall within the limits of Recommended Dietary Allowance (RDA) (60-90

mg/day). Vitamin C content of the rest of the three varieties, Magama, Best Choice and Diana, and the green stage of capsicum was slightly below the limit. The difference in ascorbic acid content of different varieties and the two stages of the same variety is in accordance with the previous results [120].

Enhanced atmospheric CO₂ decreased vitamin C of all varieties significantly at 95% confidence level ($P \leq 0.05$). The decrease was 13.81%, 15.66%, 13.03%, 14.57% and 11.84% for red stages of 99PE-1689, Magama, Angrika, Best Choice and Diana respectively. For green stages of 99PE-1689, Magama and Angrika, the reduction was 9.37%, 12.12% and 8.98% respectively. The reduction for red stage was slightly more than the green stage of the corresponding variety. As a result of CO₂ enhancement, Ascorbic acid content of 99PE-1689 decreased below the RDA value, for Angrika the reduced value was still within the RDA limits. Effect of elevated CO₂ on ascorbic acid content of capsicum is not reported earlier, although it has been studied for other crops and vegetables. Nutritional dilution due to greater biomass and funneling of additional carbon to plant biomass rather than antioxidant activity can be the reasons for a decrease ascorbic acid in at elevated CO₂. Capsicum is considered an important source of ascorbic acid and can be compared to the other recognized sources of ascorbic acid [121]. The decrease in ascorbic acid content of capsicum due to elevated CO₂ has adversely affected its nutritional quality.

Total titratable acidity of red stage of capsicum was slightly higher than premature green stage, a result similar to the previous findings [119]. Total titratable acidity decreased slightly with elevated CO₂, non-significantly at ($P > 0.1$) (Table 4.6). Total titratable acidity of 99PE-1689 red stage was $0.35 \pm 0.035\%$ and that of green stage was $0.27 \pm 0.025\%$. A decrease of 11.54% and 9.76% was observed in the total titratable acidity of 99PE-1689 red and green respectively. Acidity of Magama red, Magama green, Angrika red, Angrika green, Best Choice and Diana was $0.33 \pm 0.030\%$, $0.26 \pm 0.030\%$, $0.32 \pm 0.015\%$, $0.25 \pm 0.038\%$, $0.20 \pm 0.026\%$ and $0.20 \pm 0.030\%$ and it decreased by 10.10%, 7.69%, 10.31%, 6.58%, 11.67% and 8.33% respectively, however the decrease was non-significant for all varieties. No results have been reported earlier on the effect of elevated CO₂ on total titratable acidity of capsicum fruit.

Fat content of capsicum fruit was slightly low and it increased with the maturity of the fruit, may be due to higher carotenoid pigment in mature red stage, a result in agreement with Martinez, *et al.* [119]. Fat content of capsicum fruit decreased when grown under enhanced CO₂ conditions (Table 4.6). Reduction in fat content was 10.45%, 11.36%, 13.61%, 9.69% and 8.98% for 99PE-1689, Magama, Angrika, Best Choice and Diana respectively. Fat content of green stage of 99PE-1689, Magama and Angrika decreased by 9.95%, 8.07% and 9.81% ($P>0.1$) respectively. The reduction was significant for red mature fruit at 95% confidence level ($P\leq 0.05$) and was non-significant ($P>0.1$) for green premature fruit except 99PE-1689 which followed a trend ($0.05<P\leq 0.1$). The reduction in fat content of the green stage was slightly less as compared to red stage of the corresponding variety. This may be explained as the red stage has spent a little more time in the greenhouse gas chamber as compared to green stage and as a result red stage is affected more than the green stage.

Ash content of capsicum was in range of 8.20% to 10.64% and that of green stage was lower than the red stage, similar results are reported by Martinez, *et al.* [119]. This may be due to the movement of inorganic ions from other parts to active growth region of the plant during ripening [122]. Ash content of capsicum fruits decreased with elevated CO₂, the reduction being significant ($P\leq 0.05$) and higher for red stages being in the range of 7.55 to 12.37%, as compared to green stages where the reduction was non-significant ($P>0.1$) for 99PE-1689 and Angrika, and trend ($0.05<P\leq 0.1$) for Magama. The reduction in ash content of green fruits ranged between 4.61 and 6.04%. The effect of elevated CO₂ on fiber and ash content of capsicum fruit has not been reported earlier. The reduction is mainly considered a result of nutritional dilution caused by higher amounts of non-structural carbohydrates at enhanced level of CO₂.

Table 4.6. Proximate composition of capsicum fruits grown at two levels of atmospheric CO₂

Capsicum Variety	Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
99PE-1689 Red					
	Protein (%)	12.29 ± 0.718	8.99 ± 1.456	-26.84	0.024
	Total Sugars (%)	38.69 ± 0.851	43.66 ± 1.261	+12.85	0.005
	Reducing Sugars (%)	32.80 ± 1.635	37.19 ± 1.074	+13.38	0.018
	Non-reducing Sugars (%)	5.59 ± 1.356	6.150 ± 0.267	+9.90	ns
	Fiber (%)	13.39 ± 0.221	14.46 ± 0.188	+7.97	0.003
	Vitamin C (mg of AA/100 gram)	63.31 ± 1.161	54.56 ± 1.197	-13.81	0.001
	Total Titratable Acidity (%)	0.35 ± 0.035	0.31 ± 0.0351	-11.54	ns
	Fat (%)	5.81 ± 0.153	5.20 ± 0.220	-10.45	0.017
	Ash (%)	8.89 ± 0.200	7.79 ± 0.255	-12.37	0.004
99PE-1689 Green					
	Total Sugars (%)	22.22 ± 0.737	24.37 ± 0.693	+9.66	0.021
	Reducing Sugars (%)	14.37 ± 0.548	16.187 ± 0.259	+12.64	0.007
	Non-reducing Sugars (%)	7.46 ± 1.212	7.77 ± 0.899	+4.20	ns
	Fiber (%)	13.95 ± 0.291	14.61 ± 0.313	+4.71	(0.056)
	Vitamin C (mg of AA/100 gram)	41.50 ± 0.627	37.61 ± 1.246	-9.37	0.008
	Total Titratable Acidity (%)	0.27 ± 0.025	0.25 ± 0.021	-9.76	ns
	Fat (%)	4.39 ± 0.200	3.95 ± 0.255	-9.95	(0.080)
	Ash (%)	8.10 ± 0.225	7.73 ± 0.220	-4.61	ns
Magama Red					

	Protein (%)	21.01 ± 0.731	15.74 ± 1.337	-25.10	0.004
	Total Sugars (%)	29.02 ± 0.810	32.97 ± 0.780	+13.59	0.004
	Reducing Sugars (%)	21.11 ± 0.816	24.35 ± 0.991	+15.35	0.012
	Non-reducing Sugars (%)	7.52 ± 0.722	7.69 ± 0.683	+2.32	ns
	Fiber (%)	14.48 ± 0.255	15.75 ± 0.207	+8.75	0.003
	Vitamin C (mg of AA/100 gram)	49.58 ± 1.206	41.82 ± 1.140	-15.66	0.001
	Total Titratable Acidity (%)	0.33 ± 0.030	0.30 ± 0.021	-10.10	ns
	Fat (%)	6.63 ± 0.227	5.88 ± 0.195	-11.36	0.012
	Ash (%)	10.53 ± 0.236	9.44 ± 0.240	-10.35	0.005
Magama Green					
	Fiber (%)	14.84 ± 0.203	15.31 ± 0.242	+3.19	(0.069)
	Vitamin C (mg of AA/100 gram)	42.79 ± 0.795	37.60 ± 1.207	-12.12	0.003
	Total Titratable Acidity (%)	0.26 ± 0.030	0.24 ± 0.020	-7.69	ns
	Fat (%)	3.18 ± 0.205	2.92 ± 0.191	-8.07	ns
	Ash (%)	9.94 ± 0.250	9.34 ± 0.300	-6.04	(0.056)
Angrika Red					
	Total Sugars (%)	21.38 ± 0.916	24.11 ± 0.115	+12.75	0.007
	Reducing Sugars (%)	12.51 ± 0.981	14.76 ± 0.754	+18.01	0.034
	Non-reducing Sugars (%)	8.43 ± 0.393	9.16 ± 0.429	+8.68	(0.095)
	Fiber (%)	15.68 ± 0.445	17.10 ± 0.373	+9.03	0.013
	Vitamin C (mg of AA/100 gram)	69.86 ± 1.666	60.75 ± 1.303	-13.03	0.002
	Total Titratable Acidity (%)	0.32 ± 0.015	0.29 ± 0.020	-10.31	(0.083)
	Fat (%)	5.12 ± 0.280	4.42 ± 0.171	-13.61	0.021

	Ash (%)	10.63 ± 0.290	9.83 ± 0.185	-7.55	0.016
Angrika Green					
	Fiber (%)	16.71 ± 0.299	17.39 ± 0.362	+4.05	(0.067)
	Vitamin C (mg of AA/100 gram)	62.08 ± 1.086	56.51 ± 0.765	-8.98	0.002
	Total Titratable Acidity (%)	0.25 ± 0.038	0.24 ± 0.0252	-6.58	ns
	Fat (%)	2.17 ± 0.201	1.96 ± 0.098	-9.81	ns
	Ash (%)	9.26 ± 0.143	8.78 ± 0.375	-5.15	ns
Best Choice					
	Protein (%)	15.85 ± 0.756	10.84 ± 0.887	-31.62	0.002
	Total Sugars (%)	28.57 ± 0.605	32.53 ± 0.526	+13.86	0.001
	Reducing Sugars (%)	24.37 ± 1.043	27.82 ± 0.791	+14.15	0.010
	Non-reducing Sugars (%)	3.99 ± 0.663	4.47 ± 0.252	+12.15	ns
	Fiber (%)	15.22 ± 0.210	16.14 ± 0.222	+6.09	0.006
	Vitamin C (mg of AA/100 gram)	49.40 ± 1.581	42.20 ± 1.318	-14.57	0.004
	Total Titratable Acidity (%)	0.20 ± 0.026	0.18 ± 0.030	-11.67	ns
	Fat (%)	6.81 ± 0.220	6.15 ± 0.160	-9.69	0.014
	Ash (%)	8.24 ± 0.260	7.27 ± 0.308	-11.77	0.014
Diana					
	Total Sugars (%)	27.41 ± 1.346	30.65 ± 0.694	+11.83	0.021
	Reducing Sugars (%)	17.93 ± 0.874	20.51 ± 0.577	+14.37	0.013
	Non-reducing Sugars (%)	9.01 ± 1.005	9.64 ± 0.724	+7.03	ns
	Fiber (%)	15.31 ± 0.261	16.40 ± 0.452	+7.10	0.023
	Vitamin C (mg of AA/100 gram)	50.07 ± 1.327	44.14 ± 1.255	-11.84	0.005
	Total Titratable	0.20 ± 0.030	0.18 ± 0.015	-8.33	ns

	Acidity (%)				
	Fat (%)	7.20 ± 0.250	6.55 ± 0.230	-8.98	0.030
	Ash (%)	8.20 ± 0.300	7.58 ± 0.158	-7.64	0.033

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq0.1$)

4.2.3 Effect on elemental composition

Elemental composition of capsicum fruits and effect of elevated CO₂ on elemental composition is given in Table 4.7. Concentration of different elements in the present study is comparable to values available in nutrient databases of United States Department of Agriculture (USDA) [110] and McCance and Widdowson's [111]. Most of the elements like N, K, Zn, Mg and Mn studied in present work are in slightly higher concentration compared to these databases. Fe and Cu are higher than McCance and Widdowson's values and lower than USDA values. These differences might be due to differences in agricultural and fertilization practices [120].

Elemental composition of capsicum varieties was disturbed with elevated CO₂. Macro elements C and H increased significantly ($P\leq0.05$), Ca, Mg and N decreased significantly ($P\leq0.05$), while no change was observed for S and K. Amongst the microelements Zn and Pb decreased, Fe and Mn increased significantly while no significant effect was observed for Cu, Cr and Ni, as the decrease observed was non-significant. Reduction in Cu and Cr followed a trend ($0.05<P\leq0.1$) in 99PE-1689 and Best Choice varieties. Carbon and hydrogen are the main constituents of carbohydrates and increase in C and H may be due to increase in carbohydrates concentration with enhanced atmospheric CO₂. Reduction in Nitrogen and other elements could be a result of dilution caused by increase in non- structural carbohydrates, or a decrease in the uptake of minerals due to reduced transpiration from leaves with elevated CO₂ [31, 93]. Increase in elements can be explained by increased uptake of minerals from soil due to increased root size and change in mycorrhizal status at elevated CO₂ [106]. Reduction in heavy metals like Pb and increase in important nutrients like Fe and Mn with elevated CO₂ is favourable, but at the same time reduction in N and Zn could adversely affect the nutritional quality of capsicum fruit.

Table 4.7. Elemental composition of capsicum fruits grown at two levels of atmospheric CO₂

Capsicum Variety	Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
		400 (ambient)	1000		
99PE-1689	Macro-elements (%)				
	C	38.32 ± 0.540	46.63 ± 0.54	+21.69	<0.001
	N	1.97 ±0.115	1.44 ±0.233	-26.84	0.024
	H	5.24 ±0.103	6.29 ± 0.202	+19.98	0.001
	S	0.43 ± 0.042	0.42 ± 0.036	-2.35	ns
	Ca	0.23 ± 0.001	0.22 ± 0.003	-5.75	0.003
	Mg	0.32 ± 0.002	0.31 ± 0.001	-2.34	0.007
	K	2.95 ± 0.011	2.97 ±0.009	+0.51	ns
	C/N ratio	19.54 ± 1.419	33.03 ± 5.799	+69.05	0.017
	Micro-elements (μg g ⁻¹)				
	Zn	32.07 ± 0.416	29.87 ± 0.503	-6.86	0.004
	Mn	33.32 ± 0.072	33.77 ± 0.130	+1.36	0.006
	Fe	48.59 ± 0.094	50.71 ± 0.011	+4.36	<0.001
	Pb	0.12 ± 0.006	0.10 ± 0.006	-18.11	0.012
	Ni	1.13 ± 0.110	1.10 ± 0.092	-2.37	ns
	Cu	6.68 ± 0.050	6.55 ± 0.076	-2.09	(0.056)
	Cr	1.35 ± 0.046	1.29 ± 0.058	-4.43	ns
Magama	Macro-elements (%)				
	C	30.54 ±0.340	37.57 ± 0.440	+23.02	0.000
	N	3.36 ± 0.117	2.52 ± 0.214	-25.10	0.004
	H	3.35 ± 0.198	4.02 ± 0.159	+20.06	0.010
	S	0.43 ±0.022	0.40 ± 0.026	-7.85	ns
	Ca	0.39 ± 0.004	0.36 ± 0.011	-6.62	0.019
	Mg	0.33 ± 0.002	0.32 ± 0.002	-4.21	0.001
	K	3.49 ± 0.021	3.51 ± 0.0116	+0.62	ns
	C/N ratio	9.09 ± 0.418	14.98 ± 1.103	+64.76	0.001
	Micro-elements (μg g ⁻¹)				
	Zn	41.35 ± 0.955	39.17 ± 0.361	-5.29	0.021

	Mn	35.51 ± 0.064	37.20 ± 0.092	+4.77	<0.001
	Fe	43.05 ± 0.056	46.37 ± 0.095	+7.72	<0.001
	Pb	0.26 ± 0.006	0.22 ± 0.006	-14.23	0.002
	Ni	1.74 ± 0.065	1.69 ± 0.083	-2.99	ns
	Cu	5.52 ± 0.079	5.44 ± 0.031	-1.51	ns
	Cr	1.68 ± 0.079	1.62 ± 0.083	-3.11	ns
Angrika	Macro-elements (%)				
	Ca	0.23 ± 0.006	0.21 ± 0.005	-7.88	0.016
	Mg	0.37 ± 0.001	0.35 ± 0.003	-4.82	0.001
	K	3.42 ± 0.018	3.43 ± 0.022	+0.56	ns
	Micro-elements (µg g ⁻¹)				
	Zn	33.33 ± 0.945	30.93 ± 0.702	-7.20	0.024
	Mn	36.87 ± 0.090	38.74 ± 0.035	+5.08	<0.001
	Fe	43.21 ± 0.031	44.67 ± 0.042	+3.36	<0.001
	Pb	0.17 ± 0.008	0.15 ± 0.007	-13.67	0.021
	Ni	1.33 ± 0.090	1.30 ± 0.092	-2.01	ns
	Cu	4.07 ± 0.061	3.99 ± 0.050	-2.13	ns
	Cr	1.15 ± 0.083	1.09 ± 0.011	-5.20	ns
Best Choice	Macro-elements (%)				
	C	39.91 ± 0.330	49.16 ± 0.570	+23.18	<0.001
	N	2.54 ± 0.121	1.73 ± 0.142	-31.62	0.002
	H	5.60 ± 0.153	6.42 ± 0.183	+14.65	0.004
	S	0.40 ± 0.027	0.38 ± 0.025	-5.24	ns
	Ca	0.40 ± 0.004	0.38 ± 0.006	-3.86	0.022
	Mg	0.43 ± 0.002	0.41 ± 0.004	-5.85	0.001
	K	3.15 ± 0.023	3.18 ± 0.011	+0.71	ns
	C/N ratio	15.77 ± 0.883	28.50 ± 2.671	+80.75	0.001
	Micro-elements (µg g ⁻¹)				
	Zn	43.47 ± 1.301	39.87 ± 1.101	-8.28	0.022
	Mn	34.25 ± 0.070	35.80 ± 0.092	+4.53	<0.001
	Fe	49.27 ± 0.031	53.62 ± 0.060	+8.82	<0.001
	Pb	0.14 ± 0.006	0.12 ± 0.005	-15.25	0.009
	Ni	1.83 ± 0.090	1.81 ± 0.090	-1.09	ns

	Cu	3.11 ± 0.070	3.05 ± 0.061	-1.93	ns
	Cr	1.59 ± 0.030	1.52 ± 0.035	-4.20	(0.067)
Diana	Macro-elements (%)				
	Ca	0.44 ± 0.008	0.41 ± 0.008	-5.83	0.017
	Mg	0.43 ± 0.004	0.42 ± 0.002	-3.30	0.005
	K	3.21 ± 0.053	3.23 ± 0.035	+0.68	ns
	Micro-elements (µg g ⁻¹)				
	Zn	31.07 ± 0.808	27.93 ± 0.757	-10.09	0.008
	Mn	40.31 ± 0.083	42.61 ± 0.101	+5.69	<0.001
	Fe	42.55 ± 0.030	43.86 ± 0.040	+3.07	<0.001
	Pb	0.18 ± 0.007	0.15 ± 0.007	-16.92	0.006
	Ni	1.43 ± 0.081	1.38 ± 0.020	-3.72	ns
	Cu	4.96 ± 0.092	4.81 ± 0.101	-3.09	ns
	Cr	1.48 ± 0.072	1.42 ± 0.035	-4.05	ns

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.2.4 Effect on fatty acid composition

Fatty acids were determined for two varieties of capsicum, 99PE-1689 and Best Choice and are given in Table 4.8. The major fatty acids found in capsicum were palmitic, linoleic and linolenic acid. These results are in agreement with previous work of Cook, *et al.* [117], who determined chemical composition of 13 varieties of capsicum and found that linoleic and linolenic acid were major fatty acids. Although the response of different fatty acids to enhanced CO₂ was not same, a general decreasing trend was observed. Enhanced atmospheric CO₂ affected all the three major fatty acids of capsicum. Palmitic and linoleic acids decreased for both varieties while linolenic acid decreased for one variety and increased for the other. Linoleic and linolenic acids are essential fatty acids, as they are not only used as fuel but also play important role in biological processes in human body. Human body is unable to synthesize them and they must be taken through diet [112]. Elaidic acid also decreased for one variety and increased for the other. Only myristic acid increased for both varieties, the rest of fatty acids including stearic acid, oleic acid, octadecadienoic acid, arachidic acid, behenic acid and tetracosanoic acid decreased for both varieties. Concentration of fatty acids including pentadecanoic acid,

palmitoleic acid, margaric acid and tricosanoic acid remained almost unaffected by enhanced concentration of atmospheric CO₂ for both varieties of capsicum. Decrease in concentration of fatty acids by elevated CO₂ is also in agreement with the reduction in crude fat content of capsicum varieties (Table 4.6). The decrease in fatty acids, especially essential fatty acids at elevated CO₂ understood to adversely affect the quality of capsicum fruit.

Table 4.8. Fatty acid composition of capsicum fruits grown at two levels of CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	99PE-1689		Best Choice	
	CO ₂ concentration (μmol mol ⁻¹)			
	400 (ambient)	1000	400 (ambient)	1000
C12:0; Lauric acid	0.007	0.009	0.002	0.002
C14:0; Myristic acid	0.036	0.045	0.020	0.038
C15:0; Pentadecanoic acid	0.002	0.002	0.002	0.003
C16:0; Palmitic acid	0.231	0.197	0.391	0.345
C16:1c; Palmitoleic acid	0.007	0.006	0.006	0.005
C17:0; Margaric acid	0.006	0.005	0.007	0.007
C18:0; Stearic acid	0.077	0.052	0.073	0.067
C18:1c; Oleic acid	0.067	0.041	0.068	0.058
C18:1n9t; Elaidic acid	0.011	0.008	0.007	0.011
C18:2c; Linoleic acid	0.850	0.738	1.147	0.833
C18:2t; Ocatadecadienoic acid	0.012	0.009	0.023	0.011
C18:3n3; Linolenic acid	0.312	0.188	0.203	0.490
C20:0; Arachidic acid	0.026	0.017	0.029	0.022
C22:0; Behenic acid	0.020	0.014	0.026	0.016
C23:0; Tricosanoic acid	0.004	0.002	0.004	0.003
C24:0; Tetracosanoic acid	0.019	0.014	0.019	0.012

4.3 Effect of Enhanced CO₂ on Onion (*Allium cepa*) Bulb

Onion (*Allium cepa*) is used worldwide either raw or cooked and is important due to its characteristic flavour and medicinal uses. It is used fresh as salad in fast food like burgers and sandwiches, and cooked with many other vegetables to enhance their flavour. Dried and powdered onion is used in salad, pickle, sauce, soup and meat. Onion contains high amount of protein, sugars, calcium, magnesium, potassium and antioxidants [123]. In medicine, it is used in diseases like headache, tumors, bites and heart problems [124].

The objective of the present study was to evaluate the effects of enhanced atmospheric CO₂ on the proximate, elemental and fatty acid composition of four varieties of onion. The nutritional quality of onion was disturbed by enhanced atmospheric CO₂. To the best of our knowledge, report of comprehensive study on the effect of elevated CO₂ on onion is not available.

4.3.1 Effect on yield

As onion plants contain only one bulb per plant, only fresh weight of bulb was determined and reported. Elevated CO₂ increased the fresh weight of onion bulbs significantly at 95% confidence level ($P \leq 0.05$) (Table 4.9). Fresh weight/bulb increased by 80.94%, 126.7%, 113.3% and 84.97% for Rosita, Red Brown, Dark Red and T1-172 varieties respectively. Increase in biomass of onion with increased concentration of atmospheric CO₂ is in agreement with previous experiments [125, 126]. Levine & Paré observed maximum increase in the biomass of green onions (scallions) at 1200 $\mu\text{mol mol}^{-1}$, when it was grown at 400, 1200 and 4000 $\mu\text{mol mol}^{-1}$ of CO₂ [90]. Increase in biomass of crops and vegetables are quite expected due to higher rate of photosynthesis at elevated CO₂ which results in greater yield and biomass [20].

Table 4.9. Yield in terms of fresh weight of onion bulbs grown at two levels of atmospheric CO₂

Onion Variety	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Rosita	22.36 $\pm$ 5.094	40.46 $\pm$ 5.509	+80.94	0.014
Red Brown	26.31 $\pm$ 4.051	59.66 $\pm$ 5.639	+126.70	0.001
Dark Red	30.18 $\pm$ 4.845	64.39 $\pm$ 5.470	+113.30	0.001
T1-172	15.79 $\pm$ 3.793	29.20 $\pm$ 4.619	+84.97	0.018

4.3.2 Effect on proximate composition

Elevated CO₂ affected the chemical quality of onion bulbs by altering the proximate composition with reduced protein, fats and vitamin C and increased sugars and fiber (Table 4.10).

Protein content and effect of elevated CO₂ on protein content of onion bulb are summarized in Table 4.10. The amount of protein in onion in our experiment is more than reported by Mota, *et al.*, and Thompson, *et al.* [127, 128] in their experiments, however, it is close to the value of protein in onion reported by USDA National Nutrient Database for Standard Reference [110]. In our experiment, the protein content was different in all four varieties of onion which strengthen the statement that nutritional quality of onion varies from one variety to another.

Protein content of all four varieties decreased significantly at 95% confidence level ($P \leq 0.05$) with elevated CO₂. The decrease was 17.84%, 19.53%, 14.52% and 12.01% for Rosita, Red Brown, Dark Red and T1-172 respectively. Not only the ambient protein level of the varieties differed from each other but also the extent to which it is affected by elevated CO₂ was different. The greatest decrease in protein was observed for Red Brown variety (19.53%) and smallest decrease in T1-172 (12.01%). Reduction in protein content of onion with elevated CO₂ has not reported earlier. Decrease in protein is considered a result of dilution caused by greater assimilation of carbon based secondary compounds

like carbohydrates due to enhanced rate of photosynthesis [118]. Decreased rate of transpiration due to partial stomatal closure is also reported to reduce nitrogen uptake by roots and hence in the plant [35, 93, 129]. Ainsworth and Long suggested that decrease of protein with enhanced CO₂ is due to the decrease of Rubisco concentration in the green leaves [35]. Rubisco is an important enzyme which contains about 58% of protein present in the green leaves [130]. Decrease in nitrogen content of leaf leads to a decrease in nitrogen content of seeds and tubers, as it mostly comes by translocation from leaves [107]. Reduction in protein will adversely affect the nutritional quality of onion particularly in a population searching for protein rich food. Due to greater increase in fresh weight, total amount of protein per onion bulb increased at elevated levels of CO₂, however amount of protein per 100 gram of onion bulb decreased, which means extra amount of the vegetable has to be taken in order to get the same amount of protein.

Sugar content was determined for one variety of onion i.e., Rosita. Sugar content of onion (Rosita) in our experiment was comparable to the value of total sugars reported by Mota, *et al.* in dried onion samples [127]. The exact amount of sugar content in our study is different from the reported values of Kahane, *et al.*, [131] but proportion of reducing and non-reducing sugars are comparable. The difference can be due to varietal change and the conditions under which the onions were grown. Amount of sugars were increased with increased concentration of atmospheric CO₂ (Table 4.10). Total sugars in Rosita were $41.95 \pm 0.552\%$ at ambient conditions which increased significantly ($P \leq 0.05$) to $46.66 \pm 1.036\%$ with an increase of 11.24%. Reducing sugars (glucose + fructose) were $32.45 \pm 1.246\%$ in onion bulbs which increased significantly ($P \leq 0.05$) to $36.79 \pm 0.619\%$ at elevated CO₂ with an increase of 13.37%. Non-reducing sugars (sucrose) increased by 3.98% non-significantly ($P > 0.1$) from $9.02 \pm 0.674\%$ to $9.38 \pm 1.053\%$ at elevated CO₂. Increase in amount of sugar with elevated CO₂ is not reported earlier for onion, however observed for other crops like tomato [68] and potato [54]. Increase in sugars can be explained as a result of increased photosynthesis due to elevated CO₂, leading to a greater accumulation of carbon and carbon based compounds like carbohydrate and sugars [20]. The same explanation also implies to increased fiber content of onion grown at elevated CO₂, which was 10.74, 15.82, 14.23 and 13.44% for Rosita, Red Brown, Dark Red and

T1-172 respectively. The increase in fiber was significant at 95% confidence level ($P \leq 0.05$) (Table 4.10).

Vitamin C content of onion bulb are given in Table 4.10 and is slightly smaller than McCance and Widdowson's [111]. Elevated CO₂ decreased the ascorbic acid content of onion by 20.72% for Rosita, 19.10% for Red Brown, 21.64% for Dark Red and 17.14% for T1-172. The decrease was significant at 95% confidence level ($P \leq 0.05$). A reduction in total antioxidant activity of green onions and potato was observed by Levine & Paré [90] and Högy & Fangmeier [54] respectively, however this is against the results of Idso, *et al.*, [64] who observed that elevated CO₂ increases the concentration of ascorbic acid in sour orange juices. Nutritional dilution due to greater biomass and funneling of additional carbon to plant biomass rather than antioxidant activity can be the reasons of reduced ascorbic acid at elevated CO₂. Reduction in ascorbic acid content of onion with elevated CO₂ reduces the nutritional quality of onions.

Total titratable acidity of onion was not affected significantly by enhancement in CO₂ concentration, however non-significant ($P > 0.1$) reduction of 7.76, 8.51, 6.52 and 5.60% was observed for Rosita, Red Brown, Dark Red and T1-172 respectively (Table 4.10). The effect of elevated CO₂ on total titratable acidity has not studied earlier for onion. Islam, *et al.*, [68] observed a significant reduction in organic acid content of tomato while Högy & Fangmeier [54] observed a significant reduction in citric acid but a non-significant increase in malic acid concentration of potato with elevated CO₂.

Fat content of onion in the present study was higher than those reported by Mota, *et al.*, [127]. Fat content of onion bulbs was negatively related with elevated CO₂ (Table 4.10). The decrease was 13.95% for Rosita, 12.77% for Red Brown, 10.75% for Dark Red and 14.50% for T1-172 with increase in concentration of CO₂ from 400 to 1000 $\mu\text{mol mol}^{-1}$. Reduction in fat content was significant at 95% confidence level ($P \leq 0.05$). Increase in biomass of onion bulbs at elevated CO₂ resulting in nutritional dilution can be a reason for decreased fat content.

Ash content of onion in the present experiment was comparable to the ash content of onion analyzed by Ketiku [123]. A non-significant ($P > 0.1$) reduction in ash content was

observed with enhanced concentrations of CO₂; 5.33%, 6.25%, 7.63 and 4.72% for Rosita, Red Brown, Dark Red and T1-172 respectively (Table 4.10).

Table 4.10. Proximate composition of onion bulbs grown at two levels of atmospheric CO₂

Onion Variety	Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
Rosita					
	Protein (%)	12.26 ± 0.119	10.07 ± 0.163	-17.84	<0.001
	Total Sugars (%)	41.95 ± 0.552	46.66 ± 1.036	+11.24	0.002
	Reducing Sugars (%)	32.45 ± 1.246	36.79 ± 0.619	+13.37	0.006
	Non-reducing Sugars (%)	9.02 ± 0.674	9.38 ± 1.053	+3.98	ns
	Fiber (%)	7.45 ± 0.240	8.25 ± 0.183	+10.74	0.010
	Vitamin C (mg of AA/100 gram)	3.72 ± 0.101	2.95 ± 0.116	-20.72	0.001
	Total Titratable Acidity (%)	0.24 ± 0.020	0.23 ± 0.038	-7.76	ns
	Fat (%)	1.56 ± 0.036	1.34 ± 0.040	-13.95	0.002
	Ash (%)	6.00 ± 0.255	5.68 ± 0.226	-5.33	ns
Red Brown					
	Protein (%)	14.02 ± 0.094	11.28 ± 0.087	-19.53	<0.001
	Fiber (%)	8.62 ± 0.170	9.98 ± 0.252	+15.82	0.001
	Vitamin C (mg of AA/100 gram)	3.01 ± 0.155	2.43 ± 0.102	-19.10	0.006
	Total Titratable Acidity (%)	0.50 ± 0.018	0.45 ± 0.066	-8.51	ns
	Fat (%)	1.43 ± 0.050	1.24 ± 0.069	-12.77	0.021
	Ash (%)	8.85 ± 0.420	8.30 ± 0.559	-6.25	ns
Dark Red					
	Protein (%)	17.52 ± 0.113	14.97 ± 0.106	-14.52	<0.001
	Vitamin C (mg	4.53 ± 0.155	3.55 ± 0.102	-21.64	0.001

	of AA/100 gram)				
	Total Titratable Acidity (%)	0.49 ± 0.037	0.45 ± 0.048	-6.52	ns
	Fiber (%)	6.70 ± 0.145	7.65 ± 0.102	+14.23	0.001
	Fat (%)	1.43 ± 0.050	1.27 ± 0.031	-10.75	0.011
	Ash (%)	7.21 ± 0.337	6.66 ± 0.316	-7.63	ns
T1-172					
	Protein (%)	12.54 ± 0.119	11.04 ± 0.094	-12.01	<0.001
	Fiber (%)	7.76 ± 0.241	8.81 ± 0.201	+13.44	0.005
	Vitamin C (mg of AA/100 gram)	3.55 ± 0.101	2.94 ± 0.101	-17.14	0.002
	Total Titratable Acidity (%)	0.26 ± 0.037	0.25 ± 0.026	-5.60	ns
	Fat (%)	1.75 ± 0.081	1.49 ± 0.080	-14.50	0.018
	Ash (%)	11.290.310	10.76 ± 0.325	-4.72	ns

ns: non-significant ($P>0.1$)

4.3.3 Effect on elemental composition

Results of the impact of elevated CO₂ on elemental composition of onion varieties are given in Table 4.11. It has been found that the concentration of N, Ca, Mn and Fe is higher, and that of K, Zn and Cu is lower than corresponding reference values reported in USDA [110] and McCance and Widdowson's [111]. Mg concentration is higher than USDA value and lower than McCance and Widdowson's standard Mg values. The concentration of elements in present study showed a variation in different varieties. This is in accordance with the finding of Bibak, *et al.*, who stated that elemental concentration of onion depends upon cultivar and geographical region where they are grown [132].

Elevated CO₂ altered the elemental composition of onion bulbs by increasing C and Zn and decreasing N, Mn, Fe, Pb and Cr significantly ($P<0.05$). No significant change was observed in H, S, Ca, K, Mg, Ni and Cd concentration ($P>0.1$) while Cu tended to decrease ($0.05<P\leq 0.1$) with elevated CO₂ (Table 4.11). Carbon increased by 16.45%, 17.69%, 19.10% and 21.36% Rosita, Red Brown, Dark Red and T1-172 respectively.

Increase in Zn was 9.55% for Rosita, 7.98% for Red Brown, 11.12% for Dark Red and 12.59% for T1-172. Nitrogen concentration was negatively correlated with CO₂. Due to increase in C and decrease in N, C:N ratio increased significantly in all onion varieties. Observed reduction for Mn was 3.64%, 5.60%, 7.72% and 6.07% for Rosita, Red Brown, Dark Red and T1-172 respectively. Reduction in Fe was 5.79% for Rosita, 4.65% for Red Brown, 6.80% for Dark Red and 5.30% for T1-172. Concentration of Pb decreased by 7.53%, 8.44%, 8.98% and 11.29% for Rosita, Red Brown, Dark Red and T1-172 respectively. Decrease in concentration of Cr in Rosita, Red Brown, Dark Red and T1-172 was 7.50%, 6.64%, 5.61% and 4.63% respectively. Hydrogen was not correlated with CO₂ in case of Rosita and Dark Red while it tended to decrease for Red Brown and T1-172 by 5.42% and 4.77% respectively. Copper tended to decrease for Rosita, Dark Red and T1-172 by 2.07%, 2.30% and 2.05% respectively. A reduction of 1.07% in Cu was also observed for Red Brown, however that was non-significant statistically ($P>0.01$). Elements including S, Ca, K, Mg, Ni and Cd were not correlated significantly with increase in concentration of CO₂. Carbon is the main constituent of carbohydrates and any increase in concentration of carbohydrates with elevated CO₂ will automatically increase the concentration of carbon in onion bulb. Decrease in Nitrogen, Manganese, Iron, Lead and Chromium can be explained as a result of dilution of these elements with increased carbohydrates. Decrease in transpiration followed by reduction in uptake of mineral from soil by plants may also be responsible for reduction in elemental composition [93]. A reduction in elemental composition for a number of crops with elevated CO₂ has been reported by Loladze [37]. Increase in Zn and decrease in heavy metals like Pb and Cr with elevated CO₂ are beneficial from nutritional point of view, however it happens at the cost of other nutritionally important minerals like N, Mn and Fe which lowers the nutritional quality of onion bulbs.

Table 4.11. Elemental composition of onion bulbs grown at two levels of atmospheric CO₂

Onion Variety	Parameter	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
Rosita	Macro-elements (%)				
	C	31.31 $\pm$ 0.030	36.46 $\pm$ 0.010	+16.45	<0.001
	N	1.96 $\pm$ 0.019	1.61 $\pm$ 0.026	-17.84	<0.001
	H	5.83 $\pm$ 0.060	5.76 $\pm$ 0.017	-1.13	ns
	S	0.60 $\pm$ 0.021	0.63 $\pm$ 0.018	+4.85	ns
	Ca	0.26 $\pm$ 0.001	0.26 $\pm$ 0.000	-0.023	ns
	Mg	0.22 $\pm$ 0.002	0.22 $\pm$ 0.001	+1.20	ns
	K	0.46 $\pm$ 0.001	0.46 $\pm$ 0.001	-0.086	ns
	C/N	15.96 $\pm$ 0.139	22.62 $\pm$ 0.371	+41.75	<0.001
	Micro-elements ($\mu\text{g g}^{-1}$)				
	Zn	12.64 $\pm$ 0.035	13.85 $\pm$ 0.050	+9.55	<0.001
	Mn	33.79 $\pm$ 0.050	32.51 $\pm$ 0.064	-3.79	<0.001
	Fe	47.09 $\pm$ 0.070	44.37 $\pm$ 0.050	-5.79	<0.001
	Pb	0.29 $\pm$ 0.005	0.27 $\pm$ 0.009	-7.53	0.023
	Ni	2.02 $\pm$ 0.087	1.93 $\pm$ 0.090	-4.29	ns
	Cu	1.61 $\pm$ 0.011	1.58 $\pm$ 0.020	-2.07	(0.067)
	Cr	1.60 $\pm$ 0.053	1.48 $\pm$ 0.035	-7.50	0.030
	Cd	0.22 $\pm$ 0.005	0.21 $\pm$ 0.006	-2.45	ns
Red Brown	Macro-elements (%)				
	C	35.21 $\pm$ 0.050	41.44 $\pm$ 0.020	+17.69	<0.001
	N	2.24 $\pm$ 0.015	1.81 $\pm$ 0.014	-19.53	<0.001
	H	5.92 $\pm$ 0.140	5.60 $\pm$ 0.194	-5.42	(0.081)
	S	0.78 $\pm$ 0.033	0.83 $\pm$ 0.029	+6.24	ns
	Ca	0.61 $\pm$ 0.004	0.60 $\pm$ 0.002	-0.384	ns
	Mg	0.26 $\pm$ 0.001	0.26 $\pm$ 0.002	+1.19	ns
	K	0.32 $\pm$ 0.003	0.32 $\pm$ 0.003	-0.364	ns
	C/N	15.70 $\pm$ 0.083	22.96 $\pm$ 0.167	+46.25	<0.001
	Micro-elements ($\mu\text{g g}^{-1}$)				
	Zn	10.35 $\pm$ 0.031	10.85 $\pm$ 0.128	+4.90	0.003

	Mn	45.51 ± 0.081	42.96 ± 0.020	-5.60	<0.001
	Fe	55.67 ± 0.061	53.09 ± 0.064	-4.65	<0.001
	Pb	0.31 ± 0.002	0.28 ± 0.001	-8.44	0.012
	Ni	1.93 ± 0.050	1.83 ± 0.099	-4.84	ns
	Cu	6.23 ± 0.042	6.17 ± 0.046	-1.07	ns
	Cr	1.51 ± 0.042	1.41 ± 0.023	-6.64	0.022
Dark Red	Macro-elements (%)				
	C	34.45 ± 0.020	41.03 ± 0.030	+19.10	<0.001
	N	2.80 ± 0.018	2.40 ± 0.017	-14.52	<0.001
	H	5.86 ± 0.145	5.63 ± 0.136	-3.87	ns
	S	0.64 ± 0.025	0.67 ± 0.021	+5.81	ns
	Ca	0.21 ± 0.000	0.20 ± 0.000	-0.065	ns
	Mg	0.25 ± 0.002	0.25 ± 0.001	+1.01	ns
	K	0.46 ± 0.002	0.46 ± 0.001	-0.274	ns
	C/N	12.29 ± 0.086	17.12 ± 0.109	+39.33	<0.001
	Micro-elements (µg g ⁻¹)				
	Zn	10.09 ± 0.023	10.99 ± 0.081	+8.92	<0.001
	Mn	28.93 ± 0.064	26.72 ± 0.087	-7.64	<0.001
	Fe	53.71 ± 0.070	50.05 ± 0.050	-6.80	<0.001
	Pb	0.51 ± 0.006	0.47 ± 0.009	-8.98	0.002
	Ni	1.08 ± 0.111	1.05 ± 0.120	-3.09	ns
	Cu	2.31 ± 0.023	2.26 ± 0.035	-2.30	(0.091)
	Cr	2.73 ± 0.042	2.58 ± 0.053	-5.61	0.017
	Cd	0.22 ± 0.007	0.21 ± 0.006	-3.94	ns
T1-172	Macro-elements (%)				
	C	32.82 ± 0.020	39.83 ± 0.030	+21.36	<0.001
	N	2.01 ± 0.019	1.77 ± 0.015	-12.01	<0.001
	H	5.70 ± 0.167	5.43 ± 0.114	-4.77	(0.080)
	S	0.92 ± 0.028	0.96 ± 0.024	+3.90	ns
	Ca	0.22 ± 0.003	0.21 ± 0.002	-0.740	ns
	Mg	0.23 ± 0.002	0.23 ± 0.002	+1.21	ns
	K	0.46 ± 0.001	0.46 ± 0.001	-0.359	ns
	C/N	16.34 ± 0.150	22.55 ± 0.174	+38.00	<0.001

	Micro-elements ($\mu\text{g g}^{-1}$)				
	Zn	15.03 ± 0.142	16.93 ± 0.050	+12.59	<0.001
	Mn	29.07 ± 0.081	27.30 ± 0.035	-6.10	<0.001
	Fe	47.19 ± 0.083	44.69 ± 0.081	-5.30	<0.001
	Pb	0.53 ± 0.006	0.47 ± 0.003	-11.29	<0.001
	Ni	1.61 ± 0.090	1.52 ± 0.040	-5.79	ns
	Cu	3.25 ± 0.030	3.18 ± 0.040	-2.05	(0.083)
	Cr	3.03 ± 0.011	2.89 ± 0.031	-4.63	0.002
	Cd	0.23 ± 0.007	0.22 ± 0.007	-3.45	ns

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.3.4 Effect on fatty acid composition

Fatty acids are present in very small concentration in onion, the major among them are palmitic acid and linoleic acid. This is in agreement with the result of Tsiaganis, *et al.* [133]. Fatty acid composition of onion was not affected to a greater extent by elevated CO_2 (Table 4.12). Different fatty acids responded differently to elevated CO_2 , even same fatty acids responded differently in different varieties, like palmitic acid increased slightly for Rosita, decreased for Red Brown and T1-172 and did not change in Dark Red. Most of the fatty acids followed the same trend for all varieties like linoleic acid, linolenic acid, arachidic acid and behenic acid decreased and myristic acid, pentadecanoic acid, margaric acid and stearic acid remained unaffected invariably. Myristic acid, pentadecanoic acid, margaric acid and stearic acid remained unaffected for Rosita, Red Brown and Dark Red and decreased slightly for T1-172. Reduction in fatty acid will cause lowering of nutritional quality of onion by elevated CO_2 . A similar reduction in fatty acid composition of oilseed rape was observed by Högy *et al.* [74]. Reduction in fatty acids is in accordance with the decrease in fat content of onion varieties grown in CO_2 enriched atmosphere.

Table 4.12. Fatty acid composition of onion bulbs grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	Rosita		Red Brown		Dark Red		T1-172	
	AMB	ELE	AMB	ELE	AMB	ELE	AMB	ELE
C14:0; Myristic acid	0.003	0.003	0.001	0.000	0.001	0.001	0.004	0.001
C15:0; Pentadecanoic acid	0.005	0.005	0.002	0.002	0.001	0.001	0.007	0.002
C16:0; Palmitic acid	0.054	0.059	0.020	0.012	0.015	0.015	0.082	0.024
C17:0; Margarinic acid	0.002	0.002	0.001	0.001	0.001	0.001	0.005	0.001
C18:0; Stearic acid	0.008	0.009	0.002	0.001	0.002	0.001	0.009	0.002
C18:1c; Oleic acid	0.034	0.008	0.007	0.010	0.011	0.000	0.008	0.002
C18:2c; Linoleic acid	0.199	0.011	0.036	0.019	0.040	0.002	0.002	0.000
C18:3n3; Linolenic acid	0.012	0.000	0.008	0.002	0.006	0.000	-	-
C20:0; Arachidic acid	0.011	0.008	0.013	0.007	0.015	0.010	0.057	0.013
C21:0; Heneicosanoic acid	-	-	0.001	0.001	0.001	0.001	0.005	0.001
C22:0; Behenic acid	0.015	0.011	0.014	0.010	0.013	0.010	0.055	0.017
C23:0; Tricosanoic acid	0.003	0.000	0.002	0.001	0.001	0.000	0.007	0.002
C24:0; Tetracosanoic acid	0.013	0.008	0.004	0.005	0.003	0.003	0.019	0.009

AMB: Ambient concentration (400 $\mu\text{mol mol}^{-1}$) and ELE: Elevated concentrations (1000 $\mu\text{mol mol}^{-1}$)

4.4 Effect of Enhanced CO₂ on Okra (*Abelmoschus esculentus*) Fruit

Okra (*Abelmoschus esculentus*), one of the most popular vegetables in Asian countries, is also used in many other countries of the world. It is an important constituent of human diet, used in different methods. It is cooked alone or mixed with other vegetables or with meat. Due to its mucilaginous property, it is used in cooking soups and stews in many tropical countries [134]. Roasted and ground seeds of okra are used as substituent for coffee [135]. Okra is used for medicinal purposes in some countries [136]. Okra is very important from nutritional point of view in terms of high carbohydrates, mostly fibrous, high proteins and high seed oils [135].

In the present study the effect of enhanced atmospheric CO₂ was studied on the yield, nutritional, elemental and fatty acid composition of two varieties of okra. Nutritional

quality of okra was altered by elevated levels of CO₂ in terms of reduced protein, vitamin C and most of the minerals and increased sugars and fibers. To our knowledge, this is the first comprehensive study on the response of okra to elevated CO₂, so a line by line comparison cannot be made.

4.4.1 Effect on yield

Elevated CO₂ increased the yield of okra varieties in terms of number of fruits per plant and fresh weight per plant (Table 4.13). Increase in number of fruits per plant was 100% and 150% for Arkanamika and Sabz Pari respectively. Increase in Fresh weight per plant was 64.15% for Arkanamika and 169.50% for Sabz Pari. The increase was significant on 95% confidence level ($P \leq 0.05$) for both varieties. It can be seen that in terms of yield, Sabz Pari is affected more as compared to Arkanamika variety. Increase in yield of okra with elevated CO₂ is in agreement with the result of Sionit, *et al.* [137]. They studied the yield and growth of okra at different levels of CO₂ between 350 and 1000 ppm and observed maximum growth and yield at 1000 ppm of CO₂. Increase in yield of vegetables with elevated CO₂ is not unexpected as CO₂ is the main component of photosynthesis and increase in its concentration will result in greater build-up of biomass.

Table 4.13. Yield of okra fruits grown at two levels of atmospheric CO₂

Parameter	Okra Variety	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
		400 (ambient)	1000		
No. of fruits/plant					
	Arkanamika	1.67 ± 0.577	3.33 ± 0.577	+100.00	0.024
	Sabz Pari	1.33 ± 0.577	3.33 ± 0.577	+150.00	0.013
Fresh Weight (g/plant)					
	Arkanamika	23.46 ± 2.756	38.50 ± 3.310	+64.15	0.004
	Sabz Pari	22.43 ± 3.560	60.44 ± 4.550	+169.5	<0.001

4.4.2 Effect on proximate composition

Proximate composition of okra has been affected by elevated CO₂ with reduced proteins, vitamin C and fats and increased sugars and fibers (Table 4.14).

Protein content and effect of elevated CO₂ on protein content of okra are given in Table 4.14. Protein content of Sabz Pari is higher than Arkanamika and is comparable to the values reported by Longe, *et al.* [138], however it is much higher than that reported by Camciuc, *et al.* [139]. Protein content of Sabz Pari is also comparable to the value of USDA [110] and McCance and Widdowson's [111], however that of Arkanamika is much smaller than these values.

Elevated CO₂ reduced the protein content of Arkanamika by 23.95% and that of Sabz Pari by 18.24%. The decrease in protein content with elevated CO₂ was statistically significant at 95% confidence level ($P \leq 0.05$). Protein content of Arkanamika, already having smaller amount of protein, was affected more by elevated CO₂ compared to Sabz Pari. The effect of elevated CO₂ on protein content of okra has not studied earlier. However a reduction has been reported for other crops and vegetables like rice, maize, soybean, wheat and potato [15, 47, 54, 96]. Reduction in protein with elevated CO₂ is difficult to explain by a single mechanism. Mostly it is considered to be the result of increased biomass which leads to dilution of many nutrients including protein. Decreased uptake of nitrogen by roots due to reduced transpiration is another factor which decreases protein content of vegetables. Down-regulation of Rubisco at elevated CO₂ is also thought to be a reason for decreased protein content [106].

Sugar content and effect of elevated CO₂ on sugar content of okra are given in Table 4.14. Total sugar contents are $14.08 \pm 0.464\%$ and $11.98 \pm 0.696\%$ for Arkanamika and Sabz Pari respectively. Most of the sugars in okra are non-reducing sugars i.e. $11.76 \pm 0.643\%$ and $10.11 \pm 0.423\%$ in Arkanamika and Sabz Pari respectively. Reducing sugars are present in smaller amount i.e. $1.70 \pm 0.571\%$ in Arkanamika and $1.33 \pm 0.294\%$ in Sabz Pari. This is in agreement with findings of Zaidi, *et al.* [140]. Total sugar content of okra increased with elevated CO₂. Increase was significant at 95% confidence level ($P \leq 0.05$) and was 18.73% for Arkanamika and 19.34% for Sabz Pari. Although total

sugar content of Arkanamika was higher than that of Sabz Pari, the increase in sugars with elevated CO₂ was almost similar for the two varieties. Increase in non-reducing sugars was also significant ($P \leq 0.05$) i.e. 18.98% for Arkanamika and 19.61 for Sabz Pari. Reducing sugars also increased for both varieties, however it was non-significant statistically ($P > 0.1$).

Fiber content of okra in our experiments was higher and increased with elevated CO₂ (Table. 4.14) Fiber content of Arkanamika was $29.37 \pm 0.741\%$ and that of Sabz Pari was $29.79 \pm 0.743\%$. This was higher than the fiber content of all five varieties of okra studied by Longe, *et al.*, [138] but slightly lower than the value observed by Olaniyi, *et al.* [141]. Increase in fiber content of okra with elevated CO₂ was significant at 95% confidence level ($P \leq 0.05$) and was 8.19% for Arkanamika and 6.49% for Sabz Pari.

Effect of elevated CO₂ on carbohydrate content of okra has not reported earlier. Many other crops have shown an increase in carbohydrate content at elevated CO₂. Potato was observed to follow an increase in total sugar content by elevated CO₂ in one experiment [54]. However no affect was observed for the total sugar content of potato when grown under CO₂ enriched conditions in another experiment [52]. Increase in starch and sucrose content whereas no effect on glucose and fructose content of elevated CO₂ was observed for soybean leaves [56]. Increase in water soluble carbohydrate of sugar beet was also observed at elevated CO₂ [91]. Increase in sugar and fiber with elevated CO₂ is certain, as greater amount of biomass is produced under the said condition and the additional carbon is utilized in build-up of carbohydrates and any carbon based secondary compounds.

Vitamin C content of Arkanamika and Sabz Pari was almost similar, i.e. 32.55 ± 0.864 mg/100g FW and 34.57 ± 0.598 mg/100g FW respectively (Table. 4.14). This was smaller than that reported by Babarinde and Fabunmi [142], however it was much higher than the ascorbic acid content of okra fruit reported by Olaniyi, *et al.* [141]. Ascorbic acid content of okra decreased significantly ($P \leq 0.05$) at elevated CO₂. The decrease was 17.72% for Arkanamika and 13.66% for Sabz Pari respectively. The decrease in vitamin C content of okra with elevated CO₂ is not studied earlier. Experiments on other crops has quite contradicting results with most reporting an increase in ascorbic acid content of

crops like potato [52] and fruits like sour oranges [64]. Vorne, *et al.*, observed no change in ascorbic acid content of potato [53] while Högy and Fangmeier observed decrease in ascorbic acid content of potato under CO₂ enriched atmosphere [54]. The decrease in ascorbic acid of okra fruit with elevated CO₂ can be due to nutritional dilution as a result of increased biomass at elevated CO₂. Another possibility may be the fact that additional carbon taken up by the plants was funnelled in build-up of plant biomass rather than antioxidants like ascorbic acid. Decrease in ascorbic acid content badly affects the nutritional quality of okra. Again ascorbic acid content of Arkanamika variety was affected more as compared to Sabz pari as it was in the case of protein.

Total titratable acidity of okra in our study was $0.22 \pm 0.021\%$ and $0.18 \pm 0.020\%$ for Arkanamika and Sabz Pari respectively (Table. 4.14). This was smaller than that reported by Babarinde and Fabunmi [142]. Effect of elevated CO₂ on total titratable acidity is given in table 4.14. It can be seen that although elevated CO₂ decreased percent acidity of okra, however the decrease was non-significant statistically ($P>0.1$).

Fat content of okra was $2.59 \pm 0.111\%$ and $3.12 \pm 0.148\%$ for Arkanamika and Sabz Pari respectively (Table 4.14). Although okra seed is an important source of fats, however fat content of fruit is not much high. Fat content of okra fruit in our experiment was comparable to that reported by Longe, *et al.* [138]. Fat contents of okra decreased significantly at 95% confidence level ($P\leq 0.05$) with elevated CO₂. The decrease was 12.24% and 9.52% for Arkanamika and Sabz Pari respectively.

Ash content of okra in our experiment was $7.72 \pm 0.252\%$ and $8.24 \pm 0.295\%$ in Arkanamika and Sabz Pari respectively (Table. 4.14). This was smaller than the value of ash content of okra reported earlier by Longe, *et al.*, [138] and Babarinde and Fabunmi [142]. Ash content of okra decreased with elevated CO₂. The decrease was 7.29% for Arkanamika and 9.79% for Sabz Pari. The decrease in ash content was significant at 95% confidence level ($P\leq 0.05$). The effect of enhanced CO₂ on fat and ash content of okra has not been investigated before. The observed decrease can be due to nutritional dilution as observed for other proximate composition of okra except carbohydrates.

Table 4.14. Proximate composition of okra fruits grown at two levels of atmospheric CO₂

Okra Variety	Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
Arkanamika					
	Protein (%)	12.86 ± 1.019	9.78 ± 0.713	-23.95	0.013
	Total Sugars (%)	14.08 ± 0.464	16.71 ± 0.406	+18.73	0.002
	Reducing Sugars (%)	1.70 ± 0.571	1.98 ± 0.563	+16.90	ns
	Non-reducing Sugars (%)	11.76 ± 0.643	13.99 ± 0.575	+18.98	0.011
	Fiber (%)	29.37 ± 0.741	31.78 ± 0.753	+8.19	0.017
	Vitamin C (mg of AA/100 gram)	32.55 ± 0.864	26.78 ± 0.802	-17.72	0.001
	Total Titratable Acidity (%)	0.22 ± 0.021	0.20 ± 0.015	-11.94	ns
	Fat (%)	2.59 ± 0.111	2.27 ± 0.123	-12.24	0.029
	Ash (%)	7.72 ± 0.252	7.16 ± 0.161	-7.29	0.031
Sabz Pari					
	Protein (%)	19.02 ± 1.437	15.55 ± 1.081	-18.24	0.029
	Total Sugars (%)	11.98 ± 0.696	14.29 ± 0.529	+19.34	0.010
	Reducing Sugars (%)	1.33 ± 0.294	1.56 ± 0.227	+17.25	ns
	Non-reducing Sugars (%)	10.11 ± 0.423	12.09 ± 0.563	+19.61	0.008
	Fiber (%)	29.79 ± 0.743	31.72 ± 0.382	+6.49	0.016
	Vitamin C (mg of AA/100 gram)	34.57 ± 0.598	29.85 ± 0.654	-13.66	0.001
	Total Titratable Acidity (%)	0.18 ± 0.020	0.16 ± 0.025	-9.26	ns
	Fat (%)	3.12 ± 0.148	2.82 ± 0.101	-9.52	0.046
	Ash (%)	8.24 ± 0.295	7.44 ± 0.215	-9.79	0.019

ns: non-significant (*P*>0.1)

4.4.3 Effect on elemental composition

Elemental composition of okra and its change with elevated CO₂ is summarized in Table 4.15. The amount of almost all nutrients, i.e. N, Ca, Mg, K, Zn, Mn, Fe and Cu is lower than the standard values of these nutrients as reported in USDA [110] and McCance and Widdowson's [111]. The difference between the elemental composition of the two okra varieties studied here and the literature data has revealed that elemental composition depends not only on the variety but also on the regional and climatic conditions under which they are grown.

Elemental composition of okra was disturbed by elevated CO₂ with increased C, Ca and Fe, decreased N, Mg, Zn, Mn and Pb and no effect on H, S, K, Ni, Cu and Cr (Table 4.15). Increase in C was 31.36% for Arkanamika and 27.99% for Sabz Pari and was significant at 99% confidence level ($P \leq 0.01$). An increase of 4.16% and 5.93% in Ca and 7.75% and 5.52% in Fe was observed for Arkanamika and Sabz pari respectively. Increase in Ca and Fe was also significant at 99% confidence level ($P \leq 0.01$). Decrease in N, Mg, Zn, Mn and Pb was significant statistically ($P \leq 0.05$). Nitrogen decreased by 23.95 and 18.24, Mg by 2.61 and 3.79, Zn by 4.60 and 6.98, Mn by 16.73 and 12.65 and Pb by 15.82 and 13.87 for Arkanamika and Sabz Pari respectively. A decrease for H, S, Ni and Cu and increase for K and Cr was also observed, however it was non-significant statistically ($P > 0.1$). Chromium tended to decrease ($0.05 < P \leq 0.1$) for Arkanamika grown at elevated CO₂. Increase in C and decrease in N increased the C:N ratio for both varieties of okra. Increase in Carbon at elevated CO₂ is due to increased photosynthesis as stated earlier, and this can also be seen by increase in non-structural (sugars) as well as structural carbohydrates (fiber) in okra. Decrease in Nitrogen is also reflected by a decrease in protein. On the average elements followed a decreasing trend and this is reflected by the reduced ash content of okra at elevated CO₂. The reason for increase of some elements can be an increased uptake of these by bigger size roots of the plant at elevated CO₂. The decrease in elements is mainly due to nutritional dilution as well as reduced uptake of elements from soil due to reduced transpiration at elevated CO₂ [106]. Increase in important nutrients like Ca and Fe and decrease in toxic heavy metals like Pb at elevated CO₂ is quite favourable. However, at the same time many other important

nutrients like N, Mg, Zn and Mn are reduced which increases the chances of hidden hunger in human population.

Table 4.15. Elemental composition of okra fruits grown at two levels of atmospheric CO₂

Okra Variety	Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
		400 (ambient)	1000		
Arkanamika	Macro-elements (%)				
	C	34.25 ± 0.680	44.99 ± 0.870	+31.36	<0.001
	N	2.06 ± 0.163	1.57 ± 0.114	-23.95	0.013
	H	5.57 ± 0.147	5.30 ± 0.166	-4.76	ns
	S	0.36 ± 0.022	0.33 ± 0.041	-8.05	ns
	Ca	0.34 ± 0.002	0.35 ± 0.001	+4.16	<0.001
	Mg	0.57 ± 0.003	0.56 ± 0.002	-2.61	0.001
	K	2.18 ± 0.026	2.19 ± 0.037	+0.25	ns
	C/N ratio	16.73 ± 1.661	28.88 ± 2.666	+72.61	0.003
	Micro-elements (μg g ⁻¹)				
	Zn	55.07 ± 0.416	52.53 ± 0.416	-4.60	0.002
	Mn	30.67 ± 0.090	25.54 ± 0.092	-16.73	<0.001
	Fe	58.59 ± 0.103	63.13 ± 0.050	+7.75	<0.001
	Pb	0.13 ± 0.009	0.11± 0.005	-15.82	0.023
	Ni	2.07 ± 0.042	2.02 ± 0.092	-2.26	ns
	Cu	5.21 ± 0.170	5.10 ± 0.193	-2.05	ns
	Cr	0.33 ± 0.006	0.34 ± 0.005	+3.05	(0.094)
Sabz Pari	Macro-elements (%)				
	C	35.69 ± 0.320	45.68 ± 0.430	+27.99	<0.001
	N	3.04 ± 0.230	2.49 ± 0.173	-18.24	0.029
	H	5.84 ± 0.201	5.67 ± 0.142	-2.86	ns
	S	0.58 ± 0.028	0.55 ± 0.021	-5.48	ns
	Ca	0.26 ± 0.002	0.27 ± 0.001	+5.93	<0.001
	Mg	0.53 ± 0.009	0.51 ± 0.005	-3.79	0.025
	K	2.47 ± 0.008	2.47 ± 0.010	+0.27	ns
	C/N ratio	11.78 ± 0.998	18.41 ± 1.110	+56.31	0.002

	Micro-elements ($\mu\text{g g}^{-1}$)				
	Zn	61.13 ± 1.361	56.87 ± 0.611	-6.98	0.008
	Mn	35.19 ± 0.081	30.74 ± 0.080	-12.65	<0.001
	Fe	63.52 ± 0.053	67.03 ± 0.042	+5.52	<0.001
	Pb	0.14 ± 0.005	0.12 ± 0.005	-13.87	0.008
	Ni	1.30 ± 0.140	1.29 ± 0.122	-1.53	ns
	Cu	4.93 ± 0.180	4.87 ± 0.121	-1.22	ns
	Cr	0.109 ± 0.005	0.115 ± 0.005	+5.49	ns

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.4.4 Effect on fatty acid composition

Fatty acid composition of two okra varieties is given in Table 4.16. Fatty acid content of Sabz Pari is higher than Arkanamika and this is also reflected by the fat content of the two varieties ($3.12 \pm 0.148\%$ for Sabz Pari vs $2.59 \pm 0.111\%$ for Arkanamika). The major fatty acid in both varieties is linoleic acid. In Sabz Pari it is followed by palmitic acid and then linolenic acid whereas in Arkanamika it is followed by linolenic acid and palmitic acid. The order of the other fatty acids is the same in both varieties, i.e. oleic acid and stearic acid. The order of fatty acids in Sabz Pari is exactly the same as reported by Berry [143]. Other fatty acids present in okra are myristic acid, pentadecanoic acid, margaric acid, elaidic acid, arachidic acid, behenic acid, tricosanoic acid and tetracosanoic acid.

Fatty acids were mostly decreased by elevated CO_2 (Table 4.16). Amongst the major fatty acids linoleic and linolenic acids were reduced by elevated CO_2 for both varieties of okra. Palmitic acid was increased in Arkanamika but decreased in Sabz Pari. Oleic acid was also reduced for both varieties. Stearic acid was the only fatty acid increased by elevated CO_2 in both varieties. Rest of the fatty acids are negligibly affected or not affected at all. Since no statistical analysis was performed on fatty acid data, it is hard to say much about the significance of the results. However, the decrease in linolenic and linoleic acid concentration is quite large for both varieties (Table 4.16). Effect of elevated CO_2 on fatty acid composition of okra has not been reported earlier. Similar studies on other crops like wheat, maize, soybean and peanut oil did not show any change in fatty acid contents with elevated CO_2 [20, 84, 94]. Högy, *et al.*, is the only group which

reported changes in fatty acid composition [74]. They observed a decrease in major fatty acids of rapeseed oil. The reduction in essential fatty acids like linoleic acid and linolenic acid with elevated CO₂ will adversely affect the nutritional quality of okra.

Table 4.16. Fatty acid composition of okra fruits grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	Arkanamika		Sabz Pari	
	CO ₂ concentration (μmol mol ⁻¹)			
	400 (ambient)	1000	400 (ambient)	1000
C14:0; Myristic acid	0.002	0.002	0.004	0.005
C15:0; Pentadecanoic acid	0.001	0.001	0.002	0.002
C16:0; Palmitic acid	0.063	0.082	0.166	0.153
C17:0; Margaric acid	0.003	0.003	0.007	0.006
C18:0; Stearic acid	0.008	0.011	0.024	0.026
C18:1c; Oleic acid	0.013	0.004	0.030	0.028
C18:1n9t; Elaidic acid	0.003	0.003	0.006	0.004
C18:2c; Linoleic acid	0.087	0.022	0.197	0.161
C18:3n3; Linolenic acid	0.078	0.013	0.121	0.073
C20:0; Arachidic acid	0.004	0.005	0.011	0.012
C22:0; Behenic acid	0.006	0.007	0.015	0.014
C23:0; Tricosanoic acid	0.003	0.003	0.007	0.005
C24:0; Tetracosanoic acid	0.003	0.003	0.008	0.007

4.5 Effect of Enhanced CO₂ on Cucumber (*Cucumis sativus*) Fruit

Cucumber (*Cucumis sativus*) is an important vegetable consumed throughout the world. It originated from the tropical and sub-tropical regions and is sensitive to cold climates, but it is grown in cold regions as a greenhouse vegetable [144]. It is used fresh as salads and pickled with vinegar. It is an important source of some of the vitamins like A, C, folic acid and caffeic acid. Its water retention property makes it a cure for various skin problems like dermatitis, sunburns and swollen eyes [145].

Response of cucumber to enhanced levels of CO₂ in the atmosphere was studied in the present work. It was observed that although yield of cucumber increased but nutritional quality was disturbed with increased carbohydrates and reduced proteins and vitamin C. Elemental and fatty acid composition of cucumber was also altered. A complete comparison with literature is not possible due to non-availability of data, however comparison can be made with other crops and vegetables.

4.5.1 Effect on yield

Elevated CO₂ increased the yield of cucumber by increasing the number of fruits as well as fresh weight of fruits per plant (Table 4.17). Increase in number of fruits was 60.0% and was significant at 95% confidence level ($P \leq 0.05$). Increase in fresh weight of fruits was 43.78% and was significant at 99% confidence level ($P \leq 0.01$). Increase in biomass, fresh weight, fruit yield, fresh and dry weights of leaves and shoots has already observed for cucumber [146-148]. As stated earlier, increase in yield is due to increased photosynthesis at elevated levels of atmospheric CO₂. More of CO₂ in atmosphere, more is taken up by the plants and used in photosynthesis with a resultant increase in plant biomass and yield.

4.5.2 Effect on proximate composition

Proximate composition of cucumber and effect of elevated CO₂ on proximate composition are given in Table 4.17. Protein content of cucumber in the present work was $15.24 \pm 0.181\%$ and it was higher than the reference value of USDA [110] and lower than McCance and Widdowson's [111]. The protein content was also higher than the one reported by Ruiz and Romero [149] in their experiments. The difference in the values can be due to change in geographical regions, cultivation methods and varieties used in the studies. Elevated CO₂ decreased the protein content of cucumber by 11.15% and the decrease was significant at 99% confidence level ($P \leq 0.01$) (Table 4.17). The reduction in protein can be attributed to reduced nitrogen content of the plant at elevated CO₂. Although the nitrogen uptake by plants increases at elevated CO₂, the percent nitrogen content of the plants decreases due to increased size of the plants grown in CO₂. Another possibility is reported to be the down-regulation of Rubisco and reduced transpiration

which in turn reduces the nitrogen uptake by the plant roots [106]. Any change in roots composition is directly reflected by the other plant parts, like stem, leaves, flowers and fruits.

Total sugar content of cucumber in our study was $35.04 \pm 0.770\%$, of which $18.05 \pm 0.393\%$ was reducing sugars and $16.14 \pm 0.372\%$ was non-reducing sugars (Table 4.17). Besides the difference in the value of total sugars, the ratio of reducing and non-reducing sugars in our experiment was quite similar to the previous findings of Widders and Kwantes [150] and Du and Tachibana [151], where the combined glucose and fructose (reducing sugars) were almost equal to the sucrose content (non-reducing) of the cucumber. Sugar content of cucumber increased with elevated CO₂ (Table 4.17). Total sugars increased by 15.20%, reducing by 18.43% and non-reducing by 11.77%. Increase in sugar was significant at 95% confidence level ($P \leq 0.05$).

Fiber content of cucumber in the present study was $12.72 \pm 0.195\%$ and was increased significantly by elevated CO₂ (Table 4.17). Fiber content was slightly higher than the reference values reported in USDA. The increase was 8.41% and was significant at 95% confidence level ($P \leq 0.05$).

There are no reports of the effect of elevated CO₂ on the carbohydrate content of cucumber. Data on other crops and vegetables mostly present an increasing trend. The carbohydrate contents of tomato, potato and sugar beet have been observed to increase at elevated CO₂ [54, 68, 91]. In soybean leaves, starch and sucrose were increased while glucose and fructose were unaffected by elevated CO₂ [56]. Increase in sugars and fiber with elevated CO₂ are due to increased photosynthetic products i.e. carbohydrates.

Vitamin C content of cucumber in our experiment was 2.34 ± 0.153 mg/100g (fresh weight) which was higher than the reference value of Vitamin C mentioned in McCance and Widdowson's [111] and lower than that of USDA [110]. It was also higher than the value reported by Ruiz and Romero [149]. Ascorbic acid content decreased with elevated CO₂. The decrease was 18.57% and was significant at 95% confidence level ($P \leq 0.05$). No decrease in vitamin C content of cucumber under elevated CO₂ conditions has been observed previously. The decrease in ascorbic acid content can be a result of nutritional

dilution and use of additional carbon in biomass production rather than the antioxidants as was observed in case of scallions [90].

Total titratable acidity of cucumber in the present study was $0.222 \pm 0.032\%$ and decreased slightly with elevated CO₂ (Table 4.17). The decrease in total titratable acidity was 4.76%, however it was non-significant statistically ($P>0.1$).

Fat content of cucumber in our study was $1.67 \pm 0.061\%$ (Table 4.17). It was lower than the reference value of fat content of cucumbers mentioned in USDA [110] and McCance and Widdowson's [111]. Fat content of cucumber decreased by 8.35% under elevated CO₂ conditions. The decrease was significant at 95% confidence level ($P\leq 0.05$). Decrease in fat content with elevated CO₂ could be mainly due to nutritional dilution.

Ash content of cucumber in the present study was $14.51 \pm 0.729\%$. The value was higher than the USDA value. Elevated CO₂ decreased ash content by 4.02%, however the decrease was non-significant statistically ($P>0.1$). Impact of enhanced CO₂ on the ash content of cucumber is not reported earlier.

Table 4.17. Yield and proximate composition of cucumber fruits grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
No. of fruits/plant	5.00 ± 1.000	8.00 ± 1.000	+60.00	0.021
Fresh Weight (g/plant)	265.4 ± 6.280	381.5 ± 6.306	+43.78	<0.001
Protein (%)	15.24 ± 0.181	13.54 ± 0.150	-11.15	<0.001
Total Sugars (%)	35.04 ± 0.770	40.37 ± 1.022	+15.20	0.002
Reducing Sugars (%)	18.05 ± 0.393	21.37 ± 0.409	+18.43	0.001
Non-reducing Sugars (%)	16.14 ± 0.372	18.04 ± 0.668	+11.77	0.013
Fiber (%)	12.72 ± 0.195	13.79 ± 0.287	+8.41	0.006
Vitamin C (mg of AA/100 gram)	2.34 ± 0.153	1.91 ± 0.174	-18.57	0.031
Total Titratable Acidity (% FW)	0.22 ± 0.032	0.21 ± 0.018	-4.76	ns
Fat (%)	1.67 ± 0.061	1.53 ± 0.047	-8.35	0.035
Ash (%)	14.51 ± 0.729	13.92 ± 0.571	-4.02	ns

ns: non-significant ($P>0.1$)

4.5.3 Effect on elemental composition

Elemental composition of cucumber is given in Table 4.18. The concentration of elements in cucumber is much different than USDA [110] and McCance and Widdowson's [111]. Among the elements, N, Ca, Mg, K, Zn and Fe were lower and Mn was higher than McCance and Widdowson's. Elements like Cu, K, Zn and Fe were lower and Ca, Mg and Mn were higher than USDA. The differences in the values from the reference values and among the reference itself is due to varietal change and change in cultivation methods and geographic regions.

Elemental composition of cucumber was affected by elevated CO₂. Elements like C, H, Ca and Mg increased, N, Zn, Mn and Fe decreased while S, Pb, Ni, Cu, Cr and Cd were

not affected by elevated CO₂ (Table 4.18). Carbon increased by 22.09%, H by 9.95%, Ca by 9.88% and Mg increased by 5.92%. The increase was significant at 99% confidence level ($P \leq 0.01$). Nitrogen decreased by 11.15%, Zn by 7.96%, Mn by 3.56% and Fe by 4.86%. The reduction in these elements was also significant at 99% confidence level ($P \leq 0.01$). Very slight increase was observed for K, however it was non-significant statistically. A non-significant increase ($P > 0.1$) was also observed for Pb, Cu, Cr and Cd, while in S and Ni a non-significant decrease was observed. Increase in carbon and decrease in nitrogen increased the C:N ratio of the cucumber. Increase in carbon and hydrogen might be due to increase in photosynthesis and carbohydrates, as C and H are the main ingredients of it. It has been suggested by Loladze, that except C and H, all the other elements should decrease in the plants due to nutritional dilution [37]. In the present study, we not only see a decrease in some elements but we also observe an increase in elements other than C and H (Ca and Mg). This is not unexpected as elevated CO₂ increases the root size of plants and hence can increase the uptake of elements from the soil too. However it is very difficult to say which mechanism works for which element. Loladze in his review also observed an increase in some elements for specific crops like increase of Ca in rice and increase of K in wheat [37]. Whatever may be the reason but the fact is that elevated CO₂ caused a nutritional imbalance in cucumber fruits by increasing some elements and decreasing the others, thus increasing the chances of the phenomenon of ‘hidden hunger’ in a vast population.

Table 4.18. Elemental composition of cucumber fruits grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
	400 (ambient)	1000		
Macro-elements (%)				
C	36.57 ± 0.060	44.65 ± 0.080	+22.09	<0.001
N	2.44 ± 0.029	2.17 ± 0.024	-11.15	<0.001
H	5.19 ± 0.013	5.71 ± 0.017	+9.95	<0.001
S	0.64 ± 0.043	0.60 ± 0.037	-6.23	ns
Ca	0.35 ± 0.001	0.39 ± 0.002	+9.88	<0.001
Mg	0.79 ± 0.002	0.84 ± 0.007	+5.92	<0.001
K	0.46 ± 0.001	0.46 ± 0.001	+0.27	ns
C/N	14.99 ± 0.154	20.60 ± 0.191	+37.42	0.000
Micro-elements (μg g ⁻¹)				
Zn	21.95 ± 0.081	20.20 ± 0.060	-7.96	<0.001
Mn	31.46 ± 0.140	30.34 ± 0.191	-3.56	0.001
Fe	31.67 ± 0.094	30.13 ± 0.031	-4.86	<0.001
Pb	0.26 ± 0.001	0.28 ± 0.007	+6.39	ns
Ni	1.95 ± 0.175	1.75 ± 0.153	-10.24	ns
Cu	2.09 ± 0.070	2.16 ± 0.020	+3.51	ns
Cr	1.03 ± 0.182	1.20 ± 0.270	+16.88	ns
Cd	0.20 ± 0.011	0.21 ± 0.064	+2.99	ns

ns: non-significant ($P>0.1$)

4.5.4 Effect on Fatty acid composition

Fatty acid composition of cucumber is given in Table 4.19. The major fatty acid in cucumber fruit was linolenic acid followed by linoleic acid and palmitic acid. Other fatty acids present in small quantity were tetracosanoic acid, behenic acid, stearic acid and pentadecanoic acid. Myristic acid, margaric acid, arachidic acid and tricosanoic acid were found in very small amount in the lipids of cucumber fruit. The pattern of fatty acids in cucumber fruit lipids in our experiment is similar to the previous studies of Fishwick, *et*

al., [152] and Draper [153], who observed linolenic acid, palmitic acid and linoleic acid to be the major fatty acids in cucumber fruit lipids.

Elevated CO₂ decreased the fatty acid content of cucumber (Table 4.19). Linolenic acid, the major fatty acid, was decreased so much that it became negligibly small in cucumbers grown under CO₂ enriched atmosphere. The linoleic acid was also decreased to a great extent. On the other hand palmitic acid was increased slightly. The rest of the fatty acids were very slightly affected or remained unaffected by elevated CO₂. In previous studies on other crops and vegetables, no trend was observed for the effect of enhanced CO₂ on fatty acid composition [20, 84, 94]. Only the fatty acid concentration of oilseed rape was observed to decrease under CO₂ enriched conditions [74]. Linoleic and linolenic acids are essential fatty acids and any decrease in their concentration will badly affect the nutritional quality of cucumber. The decrease in fatty acids of cucumber fruits is in agreement with decrease in fat content of the fruit as seen in Table 4.17.

Table 4.19. Fatty acid composition of cucumber fruits grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C14:0; Myristic acid	0.005	0.006
C15:0; Pentadecanoic acid	0.011	0.011
C16:0; Palmitic acid	0.086	0.092
C17:0; Margaric acid	0.006	0.007
C18:0; Stearic acid	0.015	0.019
C18:2c; Linoleic acid	0.111	0.004
C18:3n3; Linolenic acid	0.273	-
C20:0; Arachidic acid	0.008	0.008
C22:0; Behenic acid	0.018	0.015
C23:0; Tricosanoic acid	0.009	0.008
C24:0; Tetracosanoic acid	0.025	0.027

4.6 Effect of Enhanced CO₂ on Spinach (*Spinacia oleracea*) Leaves

Spinach (*Spinacia oleracea*) is a leafy vegetable consumed throughout the world either raw as salad or cooked in a number of ways. It may be steamed, boiled, microwaved, fried or sometimes pickled. It is also frozen and canned to be stored for later use. Spinach is very important from nutrition point of view with high vitamins, proteins, ascorbic acid, folic acid, calcium, iron and potassium and small amounts of many other minerals [154]. Spinach is also important from medicinal point of view, high antioxidants makes it good for cancer prevention and high folate content makes it good to reduce the risk of cardiovascular diseases [155, 156].

In the present study the impact of enhanced atmospheric CO₂ was studied on yield and quality characteristics i.e. protein, vitamin C, fat, ash, fiber as well as elemental and fatty acid composition of spinach. Although yield of spinach grown at elevated CO₂ increased, the quality was reduced with decreased concentration of protein and vitamin C and reduced elemental and fatty acid concentration.

4.6.1 Effect on yield

In case of spinach the yield was calculated as weight of fresh leaves harvested per pot. It was observed that yield of spinach increased with elevated CO₂ (Table 4.20). The increase in fresh weight of leaves was 35.31% and was significant at 99% confidence level ($P \leq 0.01$). Increase in growth and yield of spinach with elevated CO₂ has been observed in the earlier experiments. Jain, *et al.*, observed increase in growth, leaf number, leaf area and dry weights of leaves and stems [70]. Holbrook, *et al.*, observed that fresh weight of whole spinach plant almost doubled when grown under CO₂ enriched conditions [157]. Increase in yield of spinach leaves with elevated CO₂ is mainly due to photosynthetic activity of green leaves, which increases under elevated CO₂ conditions. Increase in photosynthesis increases the fresh weight and hence yield of spinach leaves.

4.6.2 Effect on proximate composition

Proximate composition and the effect of elevated CO₂ on proximate composition of spinach are given in the Table 4.20. Protein content under ambient conditions in the

present study was $12.56 \pm 0.113\%$. The protein content of spinach in our experiment was lower than the value reported by Singh *et al.*, in their experiments [158]. However such differences are mainly due to difference in growing and cultivation methods, the region of cultivation and the variety being analysed. Elevated CO₂ decreased the protein content of spinach by 15.88%. The decrease was significant at 99% confidence level ($P \leq 0.01$) (Table 4.20). A decrease in nitrogen and hence protein content was also observed by Jain, *et al.*, in their experiments. These workers observed a 17% decrease in nitrogen and protein content of spinach at elevated CO₂ [70]. The decrease in protein content of spinach was of the same level as in grains like wheat, maize, barley and soybean [96] and also to the other vegetables studied in the present work. The decrease in protein content is due to decrease in nitrogen at elevated CO₂. In green leaves it is thought to be due to down-regulation of photosynthetic enzyme Rubisco [35]. Rubisco contains 58% of protein present in the green leaves [130] and any decrease in its concentration will directly affect the protein content of green leaves. Another reason is the nutritional dilution caused by increased non-structural carbohydrates at elevated CO₂ [118].

Fiber content of spinach in the present experiment was $5.47 \pm 0.132\%$ and was much lower than the reference values of USDA [110]. Fiber content of spinach was increased by elevated CO₂. The increase was 11.46% and was significant at 99% confidence level ($P \leq 0.01$) (Table 4.20). Increase in fiber content with increased concentration of atmospheric CO₂ is due to increased photosynthesis and increased carbon based compounds.

Vitamin C content of spinach in the present experiment was 26.88 ± 1.014 mg/100g of fresh weight. It falls within the range of ascorbic acid content in different spinach species studied by Bergquist, *et al.*, [159] i.e. 14-46 mg 100g⁻¹ FW. However it was lower than the vitamin C content in spinach observed by Singh *et al.*, [158]. Elevated CO₂ decreased the ascorbic acid content of spinach by 15.72% and the decrease was significant at 99% confidence level ($P \leq 0.01$) (Table 4.20). Effect of elevated CO₂ on vitamin C content of spinach is not studied earlier. The decrease was contrary to some studies on orange juice, tomato and potato [52, 64, 68], however it was similar to other studies conducted on potato and onion [54, 90]. Main reason for the reduction of ascorbic acid in the spinach is

likely the nutritional dilution and utilization of additional carbon in manufacture of carbohydrates rather than ascorbic acid. Decrease in ascorbic acid will lower the quality of spinach in future CO₂ rich environment.

Fat content of spinach in the present experiment was $2.45 \pm 0.110\%$ and ash content was $18.82 \pm 0.627\%$. These contents were much lower than the study of McDermid and Stuercke [160]. Fat and ash content of spinach decreased with elevated CO₂. Fat content decreased by 18.80% and ash decreased by 4.43%. The decrease in fat content was significant at 99% confidence level ($P \leq 0.01$). The reduction in ash content was non-significant statistically ($P > 0.1$). The reduction in fat with elevated CO₂ was due to nutritional dilution due to increased biomass of spinach under these conditions.

Table 4.20. Yield and proximate composition of spinach leaves grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Fresh Weight (g/pot)	140.7 ± 7.118	190.3 ± 6.878	+35.31	0.001
Protein (%)	12.56 ± 0.113	10.56 ± 0.437	-15.88	0.002
Fiber (%)	5.47 ± 0.132	6.09 ± 0.146	+11.46	0.005
Vitamin C (mg of AA/100 gram)	26.88 ± 1.014	22.65 ± 1.056	-15.72	0.007
Total Titratable Acidity (%)	0.22 ± 0.032	0.20 ± 0.048	-9.52	ns
Fat (%)	2.45 ± 0.110	1.99 ± 0.150	-18.80	0.013
Ash (%)	18.82 ± 0.627	17.98 ± 0.558	-4.43	ns

ns: non-significant ($P > 0.1$)

4.6.3 Effect on Elemental composition

Elemental composition and effect of elevated CO₂ on the elemental composition of spinach are given in Table 4.21. Concentrations of elements in spinach in the present study were much smaller than the corresponding elemental concentration in reference values. For example N, Ca, Mg, K, Zn were in lower quantities than the corresponding

values in USDA [110] and McCance and Widdowson's [111]. Mn and Cu comparable to McCance and Widdowson's but much lower than USDA. Concentration of Fe is higher than McCance and Widdowson's but lower than USDA.

Elemental composition of spinach was disturbed by elevated CO₂. Among the elements, C, H and Ca were increased in quantity, N, Zn, Mn, Fe, Pb, Ni, Cu and Cr were decreased while S, Mg, K and Cd remained unaffected. Carbon in the present study was $24.62 \pm 0.150\%$ which was increased by 19.05%. Hydrogen was $3.33 \pm 0.023\%$ and was increased by 16.34%. Calcium was $0.475 \pm 0.000\%$ and was increased by 6.51%. The increase in these three elements was significant at 99% confidence level ($P \leq 0.01$). Besides these elements, a small increase was also observed for Mg, concentration of Mg was $0.152 \pm 0.003\%$ and was increased by 0.873%, however this increase was non-significant statistically ($P > 0.1$). Nitrogen content decreased by 15.88%, Zn, Mn, Fe, Pb, Ni, Cu and Cr reduced by 19.56%, 3.32%, 19.32%, 18.71%, 13.39%, 14.60% and 7.75% respectively. The decrease in these elements was significant at 99% confidence level ($P \leq 0.01$), except the Cr which was significant at 95% confidence level ($P \leq 0.05$). A non-significant decrease of 13.73%, 0.415% and 3.61% was observed for S, K and Cd respectively. Increase in C and decrease in N increased the C:N ratio of spinach at elevated CO₂. In earlier study conducted by Jain, *et al.*, increase in C and Ca and decrease in N, Mg and Fe content of spinach leaves has been observed when grown under elevated CO₂ conditions [70]. Increase in C and H and decrease in nitrogen is in accordance with increased carbohydrates and proteins. Although ash content of spinach was not altered but elemental concentration was reduced under CO₂ enriched conditions. Reduction in elements like Mg, Fe and Zn will lower the nutritional quality of spinach at elevated CO₂.

Table 4.21. Elemental composition of spinach leaves grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Macro-elements (%)				
C	24.62 ± 0.150	29.31 ± 0.130	+19.05	<0.001
N	2.01 ± 0.018	1.69 ± 0.070	-15.88	0.002
H	3.33 ± 0.023	3.88 ± 0.019	+16.34	<0.001
S	0.47 ± 0.043	0.40 ± 0.047	-13.73	ns
Ca	0.48 ± 0.000	0.51 ± 0.001	+6.51	<0.001
Mg	0.15 ± 0.003	0.15 ± 0.001	+0.873	ns
K	0.46 ± 0.001	0.46 ± 0.003	-0.415	ns
C/N	12.25 ± 0.035	17.36 ± 0.643	+41.66	<0.001
Micro-elements (μg g ⁻¹)				
Zn	35.85 ± 0.181	28.84 ± 0.060	-19.56	<0.001
Mn	54.47 ± 0.133	52.67 ± 0.200	-3.32	<0.001
Fe	301.3 ± 0.404	243.1 ± 0.351	-19.32	<0.001
Pb	0.64 ± 0.017	0.53 ± 0.011	-18.71	<0.001
Ni	4.48 ± 0.131	3.88 ± 0.072	-13.39	0.002
Cu	3.33 ± 0.030	2.85 ± 0.140	-14.60	0.004
Cr	2.58 ± 0.092	2.38 ± 0.053	-7.75	0.031
Cd	0.24 ± 0.011	0.23 ± 0.003	-3.61	ns

ns: non-significant ($P>0.1$)

4.6.4 Effect on fatty acid composition

Fatty acid composition and effect of elevated CO₂ on fatty acid composition of spinach are given in Table 4.22. Major fatty acids in spinach were linolenic acid and palmitic acid. Linoleic acid and tetracosanoic acid were also present in considerable amounts. The other fatty acids were present in small quantity in spinach. Free fatty acids were present in very small amount in the spinach leaves. The pattern of fatty acids concentrations were similar to Constantopoulos and Kenyon [161]. Elevated CO₂ decreased the two major fatty acids of spinach leaves. Linolenic acid was reduced from 0.182% to negligibly

small amount, so that it was not even detected. Palmitic acid was reduced from 0.069% to 0.049%. Linoleic, behenic, tricosanoic and tetracosanoic acids were also reduced. None of the fatty acids was observed to increase with enhanced CO₂. Effect of elevated CO₂ is not studied earlier for spinach. The decrease in the present experiment is in accordance with the decrease in fat content of spinach. Reduction in fatty acids, especially the essential fatty acids with elevated CO₂ will lower the nutritional quality of spinach.

Table 4.22. Fatty acid composition of spinach leaves grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C14:0; Myristic acid	0.002	0.001
C15:0; Pentadecanoic acid	0.001	0.001
C16:0; Palmitic acid	0.069	0.049
C17:0; Margaric acid	0.001	0.001
C18:0; Stearic acid	0.005	0.003
C18:2c; Linoleic acid	0.029	0.002
C18:3n3; Linolenic acid	0.182	-
C20:0; Arachidic acid	0.002	0.001
C22:0; Behenic acid	0.009	0.004
C23:0; Tricosanoic acid	0.006	0.001
C24:0; Tetracosanoic acid	0.024	0.010

4.7 Effect of Enhanced CO₂ on Carrot (*Daucus carota*) Root Tuber

Carrot (*Daucus carota*) is a root vegetable which is used both as fresh as well as cooked throughout the world. It is used as salad, fried in oil, steamed or boiled and in soups. Carrot desserts like carrot cake, carrot pudding, gajjar ka halwa are very popular in different countries. Carrot juice is also marketed either alone or mixed with other fruit juices. Carrots contain high amount of dietary fibre, vitamins A, B6, C, K, folate, potassium and manganese. They contain very low level of cholesterol and fats [158].

Carrots are important from medicinal point of view as they help in promoting vision health by decreasing the risk of cataracts and glaucoma, and prevent and/or reduce the breast and lung cancer in humans and animals [162].

In the present experiment the effect of elevated CO₂ was studied on the yield and chemical composition of carrots. It was observed that protein, vitamin C, fats and fatty acid composition of carrot was decreased and elemental composition was altered by elevated CO₂.

4.7.1 Effect on yield

Effect of elevated CO₂ on the yield of carrot root is summarized in the Table 4.23. As there is only one carrot root per plant, the yield effect was studied by increase or decrease in fresh weight of carrot root. It was observed that fresh weight of root increased by 69.20% and the increase was significant at 99% confidence level ($P \leq 0.01$). The increase in carrot root with elevated CO₂ was also observed in previous experiments. Idso, *et al.*, observed 100% increase in root yield of carrot when atmospheric CO₂ concentration was doubled [163]. Idso, *et al.*, in another study observed 36% increase in root shoot ratio whereas Wheeler, *et al.*, observed 80% increase in root shoot ratio at elevated CO₂ [164, 165]. Increase in fresh weight of carrot root will increase the productivity at enhanced levels of CO₂.

4.7.2 Effect on proximate composition

Proximate composition and impact of CO₂ enrichment on proximate composition of carrot root are summarized in Table 4.23. Protein content of carrot in the present study was $14.99 \pm 0.281\%$, which was higher than the results of Bao and Chang [166] and Brunsgaard, *et al.*, [167] but much lower than Singh, *et al.* [158]. However according to Brunsgaard, *et al.*, protein content of carrot depends on variety as well as time of harvesting [167]. Elevated CO₂ decreased the protein content of carrot by 24.30%. The decrease was significant at 99% confidence level ($P \leq 0.01$). Decrease in protein content of carrot root with elevated CO₂ is not reported earlier. Data on another root vegetable, i.e. radish presented decrease in protein content of shoots but not the roots [168]. Decrease in

protein content of carrot roots may be due to decrease in protein content of green leaves. Another reason may be the dilution of nitrogen and protein in the carrot root due to increased biomass and loss of adequate uptake of nitrogen by carrot roots. The decrease in protein content of carrot roots will lower its nutritional quality under enhanced CO₂.

Fiber content of carrot roots in the present study was $9.42 \pm 0.235\%$. It was less than the fiber content of carrot roots in the study of Brunsgaard, *et al.*, [167]. Elevated CO₂ increased the fiber content of carrot roots by 15.88%. Increase was significant at 99% confidence level ($P \leq 0.01$) (Table 4.23). Increase in fiber content with elevated CO₂ is due to increase in carbon assimilation by green leaves, which is transferred to the roots as well.

Vitamin C content of carrot root in the present experiment was $3.35 \pm 0.101 \text{ mg } 100\text{g}^{-1}$. This was much lower than the ascorbic acid concentration in carrot roots reported by Singh, *et al.* [158] and slightly lower than USDA values [110]. However it was comparable to the values of ascorbic acid for carrot root reported in McCance and Widdowson's [111]. Vitamin C concentration of carrot root was decreased by elevated CO₂. The decrease was 9.09% and was significant at 95% confidence level ($P \leq 0.05$) (Table 4.23). No such reduction in ascorbic acid content of carrot root with elevated CO₂ is observed earlier. Decrease in ascorbic acid content of carrot root will lower its nutritional quality in future CO₂ enriched atmosphere.

Total titratable acidity of carrot roots was $0.349 \pm 0.032\%$. It was much lower than the value of titratable acidity reported by Phan and Hsu [169] in carrot roots. Total titratable acidity was decreased by 6.06% under elevated CO₂ conditions but the decrease was non-significant statistically ($P > 0.1$). The effect of elevated CO₂ has not been studied earlier on total titratable acidity of carrot roots, however organic acid and citric acids have been observed to decrease in tomato and potato respectively under CO₂ enriched conditions [54, 68].

Fat content of carrot root in the present study was $1.83 \pm 0.081\%$. It was higher than the lipid content of carrot root reported by Bao and Chang [166] but lower than the standard reference values of USDA [110] and McCance and Widdowson's [111]. Fat content was

decreased by elevated CO₂. The decrease was 11.68% and was significant at 95% confidence level ($P \leq 0.05$). Ash content of carrot in the present study was $9.66 \pm 0.526\%$ and was higher than the ash content of carrot root reported by Bao and Chang [166] and also higher than the standard reference values of USDA [110]. It decreased by 6.69% at elevated CO₂, however the decrease was non-significant statistically ($P > 0.1$). The reduction in fat content of carrot under enhanced CO₂ conditions is not studied earlier, however it may be due to nutritional dilution caused by enhanced biomass at elevated CO₂.

Table 4.23. Yield and proximate composition of carrot root grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Fresh Weight (g/plant)	33.41 ± 4.938	56.52 ± 5.915	+69.20	0.007
Protein (%)	14.99 ± 0.281	11.35 ± 0.106	-24.30	<0.001
Fiber (%)	9.42 ± 0.235	10.92 ± 0.363	+15.88	0.004
Vitamin C (mg of AA/100 gram)	3.35 ± 0.101	3.04 ± 0.101	-9.09	0.021
Total Titratable Acidity (%)	0.35 ± 0.032	0.33 ± 0.048	-6.06	ns
Fat (%)	1.83 ± 0.081	1.61 ± 0.103	-11.68	0.047
Ash (%)	9.66 ± 0.526	9.01 ± 0.343	-6.69	ns

ns: non-significant ($P > 0.1$)

4.7.3 Effect on elemental composition

Elemental composition and effect of elevated CO₂ on elemental composition of carrot root is given in Table 4.24. By comparing the elemental concentration of carrots to the standard reference values of USDA [110] and McCance and Widdowson's [111], it was observed that N was higher than McCance and Widdowson's, Mg and Zn were higher than USDA and lower than McCance and Widdowson's, Mn was higher while Ca, K, Fe and Cu were lower than the two reference values. It can be seen in Table 4.24 that

elemental concentration of carrot root is disturbed by elevated CO₂. Elements like C, H, Ca, Mn and Cu were increased while N, Zn, Fe and Cr were decreased with CO₂ enrichment. Some of the elements like S, Mg, K, Pb, Ni and Cd were not affected by elevated CO₂. Increase in C, H, Ca, Mn and Cu were 15.16%, 13.16%, 9.08%, 4.63% and 13.16% respectively. Increase in these elements was significant at 99% confidence level ($P \leq 0.01$). The decrease in N, Zn, Fe and Cr was also significant at 99% confidence level ($P \leq 0.01$) and was 24.30%, 17.13%, 7.78% and 26.03% respectively. Sulphur was decreased by 9.12% and Ni by 8.43%, however the decrease was non-significant statistically ($P > 0.1$). A non-significant increase was also observed for Mg, Pb and Cd. For K, the decrease was very small and non-significant as well. Increase in C and decrease in N has increased the C:N ratio by 52.10% for carrot root grown at elevated CO₂. Increase in C and H and decrease in N, Zn, Fe and Cr is in agreement with the statement of Loladze, who stated that the elements involved in photosynthesis like C, H and O should increase while the rest of the elements should decrease [37]. Increase for C and H is similar to the increase in C and H for the other vegetables and is mainly due to increase in biomass of carrot root at elevated CO₂. Decrease in elements like N, Zn, Fe and Cr is mainly due to nutritional dilution caused by increased C, H and carbohydrates. Increase in some other elements can be explained on the basis of increase in uptake from soil by increased size of roots [106]. Effect of elevated CO₂ is not studied earlier for carrot. However decrease in important elements like N and Fe will lower the nutritional quality of carrot at elevated atmospheric CO₂.

Table 4.24. Elemental composition of carrot root grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Macro-elements (%)				
C	35.82 ± 0.070	41.25 ± 0.090	+15.16	<0.001
N	2.40 ± 0.045	1.82 ± 0.017	-24.30	<0.001
H	4.69 ± 0.072	5.31 ± 0.023	+13.16	<0.001
S	0.71 ± 0.035	0.65 ± 0.041	-9.12	ns
Ca	0.17 ± 0.000	0.18 ± 0.000	+9.08	<0.001
Mg	0.17 ± 0.001	0.17 ± 0.002	+1.29	ns
K	0.46 ± 0.001	0.46 ± 0.001	-0.39	ns
C/N	14.93 ± 0.309	22.72 ± 0.262	+52.10	<0.001
Micro-elements (μg g ⁻¹)				
Zn	16.73 ± 0.092	13.87 ± 0.136	-17.13	<0.001
Mn	27.67 ± 0.117	29.01 ± 0.098	+4.63	<0.001
Fe	17.31 ± 0.042	15.97 ± 0.046	-7.78	<0.001
Pb	0.35 ± 0.003	0.36 ± 0.001	+2.49	ns
Ni	1.19 ± 0.232	1.09 ± 0.151	-8.43	ns
Cu	1.27 ± 0.046	1.43 ± 0.011	+13.16	0.004
Cr	2.10 ± 0.140	1.55 ± 0.150	-26.03	0.010
Cd	0.19 ± 0.007	0.20 ± 0.011	+5.67	ns

ns: non-significant ($P>0.1$)

4.7.4 Effect on fatty acid composition

Fatty acid composition and the changes occurred with elevated CO₂ in the composition are listed in Table. 4.25. Major fatty acid in carrot root is linoleic acid followed by linolenic and palmitic acids. These major fatty acids in the present study were the same as mentioned by Dutta and Appelqvist in their study with a difference that palmitic acid is the second abundant fatty acid in the carrot root in their results followed by linolenic acid [170]. Other fatty acids are present in minor amount in carrot roots. It has been observed that elevated CO₂ decreased the major fatty acids and most of the minor fatty acids

present in carrot fats. The decrease was very much pronounced for major fatty acids like linoleic acid, linolenic acid and palmitic acids. Among the rest of fatty acids, myristic acid, pentadecanoic acid, margaric acid, oleic acid, arachidic acid and behenic acid were also reduced while elaidic acid was increased. However due to the absence of triplicate analysis, statistical analysis of the data could not be conducted. Stearic acid, tricosanoic acid and tetracosanoic acid were not affected by enhanced CO₂. The decrease in fatty acid composition of carrot roots is in agreement with reduced fat content of carrot under elevated CO₂. The effect of enhanced CO₂ on fatty acid composition of carrot fats is not studied earlier. However a decrease has been observed for potato tubers and rape oil seed [74]. Decrease in essential fatty acids like linoleic acid and linolenic acid will greatly reduce the quality of carrot roots.

Table 4.25. Fatty acid composition of carrot root grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C14:0; Myristic acid	0.002	0.001
C15:0; Pentadecanoic acid	0.004	0.002
C16:0; Palmitic acid	0.064	0.043
C17:0; Margaric acid	0.002	0.001
C18:0; Stearic acid	0.007	0.007
C18:1c; Oleic acid	0.005	0.003
C18:1n9t; Elaidic acid	0.002	0.004
C18:2c; Linoleic acid	0.183	0.111
C18:3n3; Linolenic acid	0.099	0.036
C20:0; Arachidic acid	0.007	0.005
C22:0; Behenic acid	0.009	0.007
C23:0; Tricosanoic acid	0.001	0.001
C24:0; Tetracosanoic acid	0.003	0.003

4.8 Effect of Enhanced CO₂ on Pea (*Pisum sativum*) Seeds

Pea (*Pisum sativum*) is a seed vegetable which is used fresh, frozen, dried and even canned in many parts of the world. Fresh peas are used in salads, pies and sometimes mixed with butter. They are also cooked in a number of ways, i.e. boiled, steamed and cooked with other vegetables and even rice. Dried peas are used as pulse. Dried, roasted and salted peas are used as snacks. Peas contain high amount of protein, dietary fibre, carbohydrates and minerals and are important in human nutrition [171]. They also contain a high quantity of vitamin C [172]. Antioxidant and anti-inflammatory components of pea legumes help to maintain the blood sugar level and reduce the risk of cardiovascular problems and polyphenol components reduce the risk of gastric cancer [173, 174].

In the present study the response of peas to elevated CO₂ was studied in terms of yield, nutritional, elemental and fatty acid composition. Although the yield was increased but nutritional quality was altered with reduced proteins, vitamin C, fats and increased sugars and fibers. Elemental and fatty acid composition of peas was also disturbed by elevated CO₂.

4.8.1 Effect on yield

Yield response of pea plants to elevated CO₂ was studied in terms of number of pea pods per plant as well as fresh weight of pea seeds per plant. It was observed that elevated CO₂ increased the number of pods as well as fresh weight of seeds per plant (Table 4.26). Increase in number of pods was 74.36% while fresh weight increased by 94.21%. The increases were significant at 99% confidence level ($P \leq 0.01$). Increase in plant fresh and dry weight, yield and leaf fresh weight of peas have been observed in many previous studies [126, 175]. Xu, *et al.*, observed a 48% increase in plant dry weight while Paez, *et al.*, observed no change in plant dry weight at elevated CO₂ [176, 177]. Increase in yield with elevated CO₂ is due to increase in photosynthetic rate and as a result increased plant biomass under the said condition.

4.8.2 Effect on proximate composition

Proximate composition and effect of enhanced CO₂ on proximate composition of pea is given in Table 4.26. Protein content of pea seed in the present study was $12.81 \pm 0.137\%$ which was lower than the protein value of pea reported by de Almeida Costa, *et al.*, [178] and Nikolopoulou, *et al.*, [179] but higher than that reported by Bhatti and Christison [180]. The differences in protein content arises due to the fact that nutritional quality of pea depends upon the variety, cultivation area and soil composition [179]. Elevated CO₂ decreased the protein content of pea in the present study by 13.42%. The decrease was significant at 99% confidence level ($P \leq 0.01$). The decrease in protein content of pea with elevated CO₂ is not reported earlier. The reduction in protein content is mainly thought to be the result of nutritional dilution caused by increased non-structural carbohydrates and increased biomass. Another reason may be the down-regulation of Rubisco, a major protein containing enzyme of green tissues, which lead to reduce protein content of these tissues. The reduction in nitrogen uptake by plant root due to reduced rate of transpiration at elevated CO₂ is also a possible reason for reduced protein content. Such reduction in protein content will lower the nutritional quality of pea in future atmosphere enriched with CO₂.

Total sugar contents of pea seeds in the present experiment were $30.85 \pm 0.454\%$, reducing sugars were $20.68 \pm 0.516\%$ and non-reducing sugars were $9.69 \pm 0.186\%$ (Table 4.26). The concentration of total sugars in peas in the present study was higher than those of Bhatti and Christison [180]. The ratio of reducing and non-reducing sugars was almost similar to McCance and Widdowson's [111] but different than USDA [110]. Elevated CO₂ increased the sugar content of pea (Table 4.26). Total sugars increased by 13.14%, reducing by 14.18% and non-reducing by 11.04%. The increase in total, reducing and non-reducing sugars was significant at 99% confidence level ($P \leq 0.01$).

Fiber content of pea in the present study was $5.27 \pm 0.164\%$. It was higher than the fiber content of pea observed by Bhatti and Christison [180] and lower than that of de Almeida Costa, *et al.*, [178]. Fiber content increased by 9.61% under enhanced CO₂ conditions and the increase was significant at 95% confidence level ($P \leq 0.05$) (Table

4.26). No such increase in fiber content of pea is reported earlier with elevated CO₂. Increase in sugar and fiber of pea with elevated CO₂ is mainly due to increase in photosynthesis, which increases the amount of biomass, i.e. structural and non-structural carbohydrates. This is in accordance with increase in yield and biomass of pea under the same conditions. Increase in carbohydrates with elevated CO₂ is not reported for pea, however has been observed for other plants [15].

Vitamin C content of pea in present study was 43.17 ± 1.004 mg/100g fresh weight. It was higher than ascorbic acid content reported by Ismail, *et al.*, [173] in peas however it was comparable to the standard reference value of USDA [110]. Elevated CO₂ decreased the vitamin C content by 13.95% (Table 4.26). The decrease was significant at 99% confidence level ($P \leq 0.01$). The nutritional quality of pea in terms of ascorbic acid is reduced by elevated CO₂. Total titratable acidity of pea in present study was $0.222 \pm 0.032\%$ and it was decreased by 9.52% under CO₂ enriched conditions. However the decrease was non-significant statistically ($P > 0.1$). Decrease in titratable acidity of pea is not reported earlier.

Fat content of pea seed in the present study was $5.27 \pm 0.164\%$. It was higher than the lipid content of pea reported by de Almeida Costa, *et al.*, [178] and Nikolopoulou, *et al.*, [179]. However it was lower than the standard reference value of fat content reported in McCance and Widdowson's [111]. Fat content decreased by 11.60% under elevated CO₂ conditions and the decrease was significant at 95% confidence level ($P \leq 0.05$). The decrease in fat content is also due to dilution of nutrients caused by increased biomass at elevated CO₂.

Ash content of pea in present experiment was $4.03 \pm 0.306\%$. The ash content in present study was higher than observed by de Almeida Costa, *et al.*, [178] but comparable to one variety of pea studied by Nikolopoulou, *et al.*, [179]. Ash content decreased by 3.48% under elevated CO₂ conditions however the decrease was non-significant statistically ($P > 0.1$) (Table 4.26).

Table 4.26. Yield and proximate composition of pea seeds grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (<i>P</i>)
	400 (ambient)	1000		
No. of pods/plant	13.00 ± 2.646	22.67 ± 2.517	+74.36	0.010
Fresh Weight (g/plant)	46.95 ± 4.100	91.17 ± 5.555	+94.21	<0.001
Protein (%)	12.81 ± 0.137	11.09 ± 0.094	-13.42	<0.001
Total Sugars (%)	30.85 ± 0.454	34.91 ± 0.581	+13.14	0.001
Reducing Sugars (%)	20.68 ± 0.516	23.62 ± 0.437	+14.18	0.002
Non-reducing Sugars (%)	9.69 ± 0.186	10.72 ± 0.164	+11.04	0.002
Fiber (%)	5.27 ± 0.164	5.78 ± 0.145	+9.61	0.016
Vitamin C (mg of AA/100 gram)	43.17 ± 1.004	37.15 ± 2.008	-13.95	0.010
Total Titratable Acidity (%)	0.22 ± 0.032	0.20 ± 0.018	-9.52	ns
Fat (%)	2.07 ± 0.102	1.83 ± 0.036	-11.60	0.019
Ash (%)	4.03 ± 0.306	3.87 ± 0.264	-3.48	ns

ns: non-significant (*P*>0.1)

4.8.3 Effect on elemental composition

Elemental composition and impact of enhanced CO₂ on the elemental composition of pea seed is given in Table 4.27. On comparing the elemental concentration of peas to the standard reference values reported in USDA [110] and McCance and Widdowson's [111], it was observed that N was lower than McCance and Widdowson's, Zn was higher than McCance and Widdowson's but lower than USDA, Mn was comparable to McCance and Widdowson's but lower than USDA, Ca and Mg were higher, K was comparable while Fe and Cu were lower than the two.

Elemental composition of pea was disturbed by elevated CO₂ with increased C, H and Mn, decreased N, Ca, Mg, K and Fe. Increase in C was 14.31%, H was 16.84% and in

Mn it was 8.83%. Increase in these three elements was significant at 99% confidence level ($P \leq 0.01$). Nitrogen reduced by 13.42%, Ca by 8.24%, Mg by 6.83%, K by 6.16% and Fe by 8.01%. Again the reduction in these elements was significant at 99% confidence level ($P \leq 0.01$). Zn and Pb tended to decrease by 8.93% and 6.21% respectively ($0.05 < P \leq 0.1$). A non-significant decrease was also observed for S and Cr while Ni and Cu increased non-significantly ($P > 0.1$). Increase in C and decrease in N increased the C:N ratio of pea by 32.02%. The effect of elevated CO₂ on elemental composition of pea is not studied earlier. It is said that elevated CO₂ should decrease all the elements in plant tissue except those utilized in photosynthesis and converted to photosynthate i.e. C and H [37]. Increase in C and H in the present study is in agreement with this statement. Decrease in N, Ca, Mg, K, Fe, Zn and Pb is also in accordance with the same statement. As stated earlier reduction in the elements in plant tissues at elevated CO₂ is due to nutritional dilution which is caused by increased biomass of plants. Increase in Mn is difficult to explain, however it may be due to increased uptake by increased size root under elevated CO₂ as reported by Taub and Wang for nitrogen [106]. Although Mg was increased and Pb was decreased but many other important elements like N, Ca, Mg, K and Fe were decreased and even Zn also followed a decreasing trend under enhanced CO₂ which is very unwanted from nutrition point of view.

Table 4.27. Elemental composition of pea seeds grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Macro-elements (%)				
C	44.58 ± 0.060	50.96 ±0.070	+14.31	<0.001
N	2.05 ± 0.022	1.77 ± 0.015	-13.42	<0.001
H	5.80 ± 0.04	6.78 ± 0.021	+16.84	<0.001
S	0.61 ± 0.058	0.53 ± 0.043	-13.77	ns
Ca	0.46 ± 0.005	0.42 ± 0.006	-8.24	0.001
Mg	0.26 ± 0.004	0.24 ± 0.003	-6.83	0.004
K	1.53 ± 0.005	1.44 ± 0.013	-6.16	<0.001
C/N	21.76 ± 0.263	28.73 ± 0.203	+32.02	<0.001
Micro-elements (μg g ⁻¹)				
Zn	51.53 ±2.139	46.93 ± 2.230	-8.93	(0.061)
Mn	26.12 ± 0.164	28.43 ± 0.136	+8.83	<0.001
Fe	42.77 ± 0.140	39.34 ±0.131	-8.01	<0.001
Pb	0.31 ± 0.006	0.29 ± 0.001	-6.21	(0.079)
Ni	1.67 ±0.162	1.73 ± 0.172	+3.19	ns
Cu	3.13 ± 0.170	3.41 ± 0.230	+9.17	ns
Cr	0.85 ± 0.205	0.78 ± 0.178	-7.87	ns

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.8.4 Effect on fatty acid composition

Fatty acid composition and impact of atmospheric CO₂ on fatty acid composition of pea seed is given in Table 4.28. The major fatty acid in pea in our experiment was linoleic acid followed by linolenic acid, palmitic acid, oleic acid, stearic acid and arachidic acid. Other fatty acids were present in smaller amounts. Linoleic acid was also reported to be the major fatty acid of pea lipid by Grela and Gunter [181], however in their studies palmitic acid was the second most abundant fatty acid followed by oleic acid, stearic acid and then linolenic acid. Elevated CO₂ decreased linoleic acid, linolenic acid and oleic acid in pea lipids. Palmitic acid was not altered while stearic acid was slightly increased. Among the minor fatty acids, myristic acid and margaric acid were slightly reduced;

lauric acid, pentadecanoic acid, heneicosanoic acid and behenic acid were not affected while elaidic acid, octadecadienoic acid, arachidic acid, tricosanoic acid and tetracosanoic acid increased slightly. However due to absence of any statistical analysis, nothing can be said about the significance of the results. The decrease in major fatty acids is in accordance with the reduction of fat content of pea at elevated CO₂. The effect of elevated CO₂ on fatty acid composition of pea is not studied earlier. It is observed that fatty acid composition of pea has been disturbed, although some of the minor fatty acids are increased but the essential fatty acids, i.e. linoleic acid and linolenic acid has been decreased to a larger extent which is an issue of major concern for the future CO₂ enriched atmosphere.

Table 4.28. Fatty acid composition of pea seeds grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C12:0; Lauric acid	0.001	0.001
C14:0; Myristic acid	0.005	0.004
C15:0; Pentadecanoic acid	0.004	0.004
C16:0; Palmitic acid	0.088	0.088
C17:0; Margaric acid	0.005	0.004
C18:0; Stearic acid	0.040	0.043
C18:1c; Oleic acid	0.082	0.075
C18:1n9t; Elaidic acid	0.007	0.015
C18:2c; Linoleic acid	0.366	0.232
C18:2t; Octadecadienoic acid	0.004	0.007
C18:3n3; Linolenic acid	0.167	0.138
C20:0; Arachidic acid	0.016	0.018
C21:0; Heneicosanoic acid	0.001	0.001
C22:0; Behenic acid	0.008	0.008
C23:0; Tricosanoic acid	0.002	0.003
C24:0; Tetracosanoic acid	0.008	0.010

4.9 Effect of Enhanced CO₂ on Cauliflower (*Brassica oleracea*) Flower

Cauliflower (*Brassica oleracea*) is a flower vegetable which is consumed throughout the world either raw or cooked. It is eaten as salad, fried, boiled, steamed, microwaved and sometimes roasted. Cauliflower contains high amount of dietary fibre, vitamin C, folate, manganese, potassium and low fat content. Due to lack of starch, it's a good substitute of potato for people on low carbohydrate diet [182]. Due to its high antioxidant and anti-inflammatory components, it is very important from medicinal point of view as it helps to prevent many types of cancer including breast, bladder, colon, ovarian and prostate cancer [183].

In the present study the impact of elevated CO₂ was studied on the nutritional, elemental and fatty acid composition of cauliflower. It was observed that although elevated CO₂ increased the yield of cauliflower, it disturbed the nutritional balance of vegetable with reduced protein, ascorbic acid and fats and increased fiber content, altered elemental and fatty acid composition.

4.9.1 Effect on yield

Effect of enhanced CO₂ on the yield of cauliflower is given in Table 4.29. As there is only one flower per plant in cauliflower, the yield was observed by increase in fresh weight of flower per plant. It can be seen that the fresh weight of cauliflower increased by 61.60% when grown under CO₂ enriched conditions. The increase was significant at 99% confidence level ($P \leq 0.01$). An increase of 34% in total dry weight of cauliflower was observed by Wheeler, *et al.*, when grown under elevated CO₂ [184]. Abe, *et al.*, observed increase in total dry weight of cauliflower but did not report any change in dry weight of roots and shoots at elevated CO₂ [185]. Increased photosynthesis in cauliflower in elevated CO₂ was reported by Kanechi, *et al.* [186], which led to increase in the fresh weight of cauliflower under such conditions.

4.9.2 Effect on proximate composition

Proximate composition and impact of elevated CO₂ on proximate composition of cauliflower are given in Table 4.29. Protein content of cauliflower in the present study

was $15.24 \pm 0.106\%$, slightly lower than the protein content reported by Hanif, *et al.* [182]. Elevated CO₂ decreased the protein content of cauliflower by 15.55%. The decrease was significant at 99% confidence level ($P \leq 0.01$). The effect of elevated CO₂ on the protein content of cauliflower is not studied earlier. The decrease in protein content of cauliflower under elevated CO₂ condition is in accordance with reduction in protein content of many other grain crops as well as other vegetables studied in the present study. The decrease is mainly thought to be due to nutritional dilution of proteins with enhanced biomass of the vegetable at elevated CO₂. Another reason might be the down-regulation of protein containing enzyme, Rubisco, which is present in green leaves. Any reduction in the protein content of green leaves is transferred to the other parts of the plants. Another mechanism which is thought to reduce the protein content of plants, is the decrease in transpiration rate at elevated CO₂ which decreases the uptake of water from soil by roots and alternatively reduces the uptake of minerals from soil. Reduction in nitrogen uptake from soil also reduces the protein content of all plant material [106]. Reduction in protein content of cauliflower with increase in concentration of atmospheric CO₂ will greatly reduce the nutritional quality of cauliflower in the future greener world.

Fiber content of cauliflower in the present study was $6.49 \pm 0.179\%$. Fiber content in our cauliflower sample was less than the value of fiber content reported by Hanif, *et al.* in cauliflower [182]. Enhanced CO₂ increased the fiber content of cauliflower by 8.98%. The increase was significant at 95% confidence level ($P \leq 0.05$). Increase in fiber content with elevated CO₂ is not reported earlier for cauliflower, however it is in accordance with other vegetables in the present study. The reason for such increase is the increase in biomass which is composed of structural and non-structural carbohydrates including fiber. Increase in the biomass and yield leads to increased fiber content of cauliflower.

Vitamin C content of cauliflower in the present study was 67.28 ± 1.549 mg/100g fresh weight of sample (Table 4.29). It was higher than the USA and UK standard reference value of ascorbic acid for cauliflower [110, 111]. Ascorbic acid content of cauliflower was decreased by enhanced CO₂. The decrease was 18.59% and was significant at 99% confidence level ($P \leq 0.01$). The decrease in ascorbic acid with elevated CO₂ is not reported earlier for cauliflower. Although elevated CO₂ is reported to increase carbon

containing compounds, a reduction in ascorbic acid is due to the utilization of additional carbon to the biomass production and not to antioxidants [90]. Total titratable acidity of cauliflower in the present study was $0.222 \pm 0.032\%$. Elevated CO₂ decreased the total titratable acidity by 9.52%. However the decrease was non-significant statistically ($P>0.1$). Effect of elevated CO₂ on total titratable acidity of cauliflower is not studied earlier.

Fat content of cauliflower in the present study was $1.75 \pm 0.155\%$. It was slightly lower than the fat content of cauliflower reported by Hanif, *et al.* [182]. Elevated CO₂ decreased the fat content of cauliflower by 16.79%. The decrease was significant at 95% confidence level ($P\leq 0.05$). Decrease in fat content of cauliflower is mainly due to nutritional dilution caused by increased biomass at elevated CO₂.

Ash content of cauliflower in the present study was $12.68 \pm 0.488\%$. It was higher than the ash content reported by Hanif, *et al.* in cauliflower [182]. Ash content of cauliflower decreased by 6.89% under enhanced CO₂ conditions, but the decrease was non-significant statistically ($P>0.1$).

Table 4.29. Yield and proximate composition of cauliflower grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		CO ₂ effect (%)	Probability level (<i>P</i>)
	400 (ambient)	1000		
Fresh Weight (g/plant)	175.8 ± 6.069	284.09 ± 8.481	+61.60	<0.001
Protein (%)	15.24 ± 0.106	12.87 ± 0.100	-15.55	<0.001
Fiber (%)	6.49 ± 0.179	7.08 ± 0.182	+8.98	0.017
Vitamin C (mg of AA/100 gram)	67.28 ± 1.549	54.77 ± 1.014	-18.59	<0.001
Total Titratable Acidity (%)	0.22 ± 0.032	0.20 ± 0.018	-9.52	ns
Fat (%)	1.75 ± 0.155	1.45 ± 0.094	-16.79	0.049
Ash (%)	12.68 ± 0.488	11.80 ± 0.605	-6.89	ns

ns: non-significant ($P>0.1$)

4.9.3 Effect on elemental composition

Elemental composition and impact of enhanced CO₂ on elemental composition of cauliflower is given in Table 4.30. It was observed that most of the elements in the cauliflower in the present study were much lower than the standard reference values of the corresponding elements in USDA [110] and McCance and Widdowson's [111]. Elements like N, Ca, K, Zn and Fe were lower than both US and UK standard reference values. Mg was higher than USDA and lower than McCance and Widdowson's while Cu was higher than McCance and Widdowson's and lower than USDA. Among all the elements, only Mn was higher than both USDA and McCance and Widdowson's standard reference value.

Elemental composition of cauliflower was disturbed by elevated CO₂. Concentration of C and H increased, most of the elements including N, S, Ca, Zn, Mn, Fe, Cu and Cr were decreased while K, Pb and Ni were not affected by elevated CO₂. Mg tended to decrease while Cd tended to increase. Carbon was increased by 28.50% and H by 6.31% and the increase in both the elements was significant at 99% confidence level ($P \leq 0.01$). Nitrogen was decreased by 15.54%, S by 18.60%, Ca by 3.31%, Zn by 13.24%, Mn by 2.37%, Fe by 13.94%, Cu by 10.71%, and Cr by 21.62%. The decrease in all these elements was significant at 99% confidence level ($P \leq 0.01$) except the Cr which was significant by 95% confidence level ($P \leq 0.05$). Mg tended to decrease by 1.07% while Cd tended to increase by 5.48% ($0.05 < P \leq 0.1$). Elements like K and Ni were decreased while Pb was increased, however the concentration change in these elements was non-significant statistically ($P > 0.1$). Increase in C and decrease in N increased the C:N ratio of the cauliflower by 52.16% under elevated CO₂ conditions. Increase in C and H is expected, as it is seen that enhanced CO₂ increased the biomass of cauliflower. And increase in the biomass automatically increases the concentration of C and H in the plant tissues. Decrease in the elements is mainly due to dilution of these elements due to increased biomass. The increase in uptake of these elements does not increase at the same rate at which the biomass does, the result is the dilution of these elements. The decreased transpiration rate at enhanced CO₂ also reduces the uptake of elements from the soil by the plant root, decreasing the elemental concentration of these elements. Increase in C and H and

decrease in majority of the elements is in accordance with the findings of Loladze [37]. The decrease in important nutrients like N, Ca, Zn, Mn, Fe, Cu and Cr will lower the nutritional quality of cauliflower and will result in hidden hunger in the future cauliflowers grown in CO₂ rich atmosphere.

Table 4.30. Elemental composition of cauliflower grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Macro-elements (%)				
C	29.96 ± 0.020	38.50 ± 0.070	+28.50	<0.001
N	2.44 ± 0.017	2.06 ± 0.016	-15.54	<0.001
H	3.73 ± 0.011	3.96 ± 0.039	+6.31	0.001
S	0.70 ± 0.008	0.57 ± 0.009	-18.60	<0.001
Ca	0.16 ± 0.000	0.15 ± 0.000	-3.31	<0.001
Mg	0.37 ± 0.002	0.36 ± 0.002	-1.07	(0.087)
K	0.46 ± 0.001	0.46 ± 0.001	-0.130	ns
C/N	12.29 ± 0.077	18.70 ± 0.179	+52.16	<0.001
Micro-elements (μg g ⁻¹)				
Zn	24.33 ± 0.090	21.11 ± 0.081	-13.24	<0.001
Mn	34.84 ± 0.151	34.01 ± 0.099	-2.37	0.001
Fe	28.64 ± 0.171	24.65 ± 0.050	-13.94	<0.001
Pb	0.34 ± 0.002	0.37 ± 0.002	+7.96	ns
Ni	0.99 ± 0.061	0.87 ± 0.103	-12.16	ns
Cu	3.30 ± 0.072	2.95 ± 0.031	-10.71	0.001
Cr	2.47 ± 0.186	1.93 ± 0.234	-21.62	0.037
Cd	0.21 ± 0.006	0.22 ± 0.006	+5.48	(0.089)

ns: non-significant ($P>0.1$), numbers in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.9.4 Effect on fatty acid composition

Fatty acid composition and impact of elevated CO₂ on fatty acid composition of cauliflower are given in Table 4.31. Major fatty acids in fats of cauliflower in the present

study were linolenic acid followed by palmitic acid and linoleic acid. The major fatty acid reported by Wright, et al., in cauliflower was also linolenic acid, however linoleic acid was the second most abundant fatty acid followed by palmitic acid in their study [187]. Enhanced CO₂ disturbed the fatty acid content of cauliflower oil. The three major fatty acids i.e. linolenic acid, palmitic acid and linoleic acid were all decreased by elevated CO₂. Elaidic acid, the fourth abundant fatty acid, was also reduced. Among the rest of the fatty acids, pentadecandienoic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid and arachidic acid were very slightly decreased, however due to absence of statistical analysis, significance of the results could not be concluded. The rest of the fatty acids including myristic acid, behenic acid, tricosanoic acid and tetracosanoic acid are not affected by elevated CO₂. Impact of elevated CO₂ upon fatty acid composition of cauliflower oil is not studied earlier. The decrease in fatty acid is in accordance with the decrease in fat content of cauliflower under enhanced CO₂ conditions. The decrease in most important fatty acids like linolenic acid and linoleic acid, which are essential fatty acids, will badly affect the nutritional quality of cauliflower under CO₂ enriched atmosphere.

Table 4.31. Fatty acid composition of cauliflower grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C14:0; Myristic acid	0.002	0.002
C15:0; Pentadecanoic acid	0.003	0.002
C16:0; Palmitic acid	0.039	0.033
C16:1c; Palmitoleic acid	0.003	0.001
C17:0; Margaric acid	0.002	0.001
C18:0; Stearic acid	0.007	0.005
C18:1c; Oleic acid	0.003	0.002
C18:1n9t; Elaidic acid	0.014	0.010
C18:2c; Linoleic acid	0.030	0.023
C18:3n3; Linolenic acid	0.440	0.329
C20:0; Arachidic acid	0.004	0.003
C22:0; Behenic acid	0.002	0.002
C23:0; Tricosanoic acid	0.001	0.001
C24:0; Tetracosanoic acid	0.003	0.003
C24:1; Tetracosenoic acid	0.003	0.002

4.10 Effect of Enhanced CO₂ on Radish (*Raphanus sativus*) Root Tuber

Radish (*Raphanus sativus*) is a root vegetable which is grown and consumed throughout the world. It is mostly eaten raw as salad but also cooked, dried and even pickled. The green leaves are also edible and eaten in some parts of the world but the use of bulb is more common than leaves. Radish contain a high amount of protein, crude fibre, dry matter, nitrate, total soluble sugars, vitamin C, carotene and minerals [188]. Radish is very important from medicinal point of view, it can be used in treatment of heart disease and cancer, vitamin C in radish reduces asthma and crude fibre reduces the risk of diabetes and constipation [189, 190].

In the present study the effect of enhanced CO₂ was studied on the yield, proximate, elemental and fatty acid composition of radish. It was observed that although yield of radish increased but nutritional quality of radish was disturbed with reduced protein, ascorbic acid and fat contents and increased sugars and fibers. Elemental and fatty acid composition of radish was also altered by elevated CO₂.

4.10.1 Effect on yield

Impact of elevated CO₂ on yield of radish root is given in Table 4.32. As there is only one tuberous root per plant, the impact on yield was studied by change in fresh weight of radish root. It was observed that fresh weight of radish root was increased by 139.34% at elevated CO₂. The increase was significant at 99% confidence level ($P \leq 0.01$). Increase in edible fresh weight of radish is in accordance with the results of Richards, *et al.* [191]. Increase in dry weight of radish grown under CO₂ atmosphere is also reported in a number of previous studies [192, 193]. Increase in fresh weight of radish under elevated CO₂ is in accordance with the other vegetables in the present study. Elevated CO₂ increases the rate of photosynthesis of green leaves, which increases the accumulation of carbohydrates in the leaves and as a result in the other plant tissues as well. Increase in radish yield under elevated CO₂ is very likely in a future world with an expected problem of food scarcity.

4.10.2 Effect on proximate composition

Proximate composition and impact of enhanced CO₂ on proximate composition of radish are given in Table 4.32. Protein content of radish in the present experiment was $8.79 \pm 0.944\%$. It was less than protein content of radish reported by Hanif, *et al.* [182], however it was in the range of protein content of different radish varieties analysed by Lu, *et al.* [188]. Such differences usually arises due to varietal differences, as nutritional composition of radish depends largely on the type of cultivar selected [188]. Protein content of radish root tuber decreased by 18.83% at elevated CO₂ and the decrease was significant at 95% confidence level ($P \leq 0.05$). In earlier experiment conducted by Chu, *et al.*, on radish, they observed a decrease in nitrogen and protein content of radish shoot with elevated CO₂ however that of the root tuber did not decrease [168]. The decrease in

protein content of radish root tuber is similar to that of carrot root tuber in the present experiment. It is mostly due to nutritional dilution caused by enhanced biomass at elevated CO₂ which reduces all the other nutrients in the radish root tubers. Another reason can be the decreased nitrogen and protein content of green leaves due to down-regulation of Rubisco, and the transfer of that decrease to the radish root, as it is the storage organ of the plant. The decrease in protein content of radish under enhanced CO₂ will greatly reduce its quality.

Sugar contents of radish root are given in Table 4.32. Total sugars in radish root tuber in the present experiment were $19.64 \pm 0.309\%$, of which $15.75 \pm 0.389\%$ were reducing sugars while $3.70 \pm 0.085\%$ were non-reducing sugars. The amount of Total sugars in the present study was comparable to some varieties of Lu, *et al.*, however it was much different from some other varieties in the same experiment [188]. Sugar content of radish increased with elevated CO₂. The increase was 12.99% for total sugars, 12.51% for reducing sugars and 14.91% for non-reducing sugars. The increase in total, reducing and non-reducing sugars was significant at 99% confidence level ($P \leq 0.01$). The effect of enhanced CO₂ is not studied earlier on the sugar content of radish.

Fiber content of radish root tuber in the present study was $23.75 \pm 0.336\%$. It was higher than fiber content of radish reported by Hanif, *et al.* [182], however it was comparable to some varieties of Lu, *et al.* [188]. Fiber content of radish root tuber increased by 12.04% with elevated CO₂ and the increase was significant at 99% confidence level ($P \leq 0.01$).

The increase in sugars and fiber contents of radish root tuber is mainly due to increase in total biomass. Elevated CO₂ increases the rate of photosynthesis, which increases the amount of biomass. The biomass is mostly composed of structural and non-structural carbohydrates including sugars and fibers, which are also increased in the plant tissues. Although such changes take place in photosynthetic organs of plants, i.e. leaves, however the result is also seen in the storage organ of the plant, i.e. root tuber in case of radish. An increase in the carbohydrate content of radish root and shoot with elevated CO₂ was also reported by Chu, *et al.* [168].

Vitamin C content of radish root tuber was 38.82 ± 1.534 mg/100g fresh weight of sample. It was comparable to ascorbic acid content of few radish varieties studied by Lu, *et al.* [188]. Vitamin C content of radish decreased with elevated CO₂. The decrease was 12.93% and was significant at 95% confidence level ($P \leq 0.05$). Although the amount of carbon based compounds is expected to increase, the decrease may be a result of additional carbon used up in building of carbohydrates rather than ascorbic acid in plant tissues [90]. Decrease in ascorbic acid content of radish root tuber under CO₂ enriched environment will badly affect its nutritional quality.

Total titratable acidity of radish in the present study was $0.592 \pm 0.037\%$. It was not affected by elevated CO₂. Although a decrease of 7.14% was observed, however this decrease was non-significant statistically ($P > 0.1$). Impact of elevated CO₂ on the total titratable acidity of radish root has not been investigated previously, however our results are similar to decrease in organic acid content of tomato and citric acid content of potato [54, 68].

Fat content of radish in the present experiment was $1.43 \pm 0.036\%$. It was comparable to the fat content of radish reported by Hanif, *et al.* [182]. Fat content of radish was decreased by elevated CO₂. The decrease was 15.69% and was significant at 99% confidence level ($P \leq 0.01$). The decrease in fat content of radish was similar to decrease in fat content of other vegetables and to carrot root tubers and is mainly due to dilution of fats caused by increased carbohydrates.

Ash content of radish root tuber in the present experiment was $23.42 \pm 0.631\%$. It was higher than the ash content reported by Hanif, *et al.* in their study [182]. However the difference may be due to varietal difference in nutritional composition of radish [188]. Ash content of radish was not affected by elevated CO₂. Although a decrease of 4.11% was observed in ash content, however it was non-significant statistically ($P > 0.1$). The effect of elevated CO₂ is not reported earlier on ash content of radish.

Table 4.32. Yield and proximate composition of radish root grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Fresh Weight (g/plant)	22.82 ± 4.313	54.62 ± 4.399	+139.34	0.001
Protein (%)	8.79 ± 0.944	6.96 ± 0.517	-18.83	0.043
Total Sugars (%)	19.64 ± 0.309	22.19 ± 0.390	+12.99	0.001
Reducing Sugars (%)	15.75 ± 0.389	17.72 ± 0.492	+12.51	0.006
Non-reducing Sugars (%)	3.70 ± 0.085	4.25 ± 0.107	+14.91	0.002
Fiber (%)	23.75 ± 0.336	26.61 ± 0.325	+12.04	<0.001
Vitamin C (mg of AA/100 gram)	38.82 ± 1.534	33.80 ± 2.319	-12.93	0.035
Total Titratable Acidity (%)	0.59 ± 0.037	0.55 ± 0.0366	-7.14	ns
Fat (%)	1.43 ± 0.036	1.21 ± 0.063	-15.69	0.006
Ash (%)	23.42 ± 0.631	22.46 ± 0.638	-4.11	ns

ns: non-significant ($P>0.1$)

4.10.3 Effect on elemental composition

Elemental composition and impact of enhanced CO₂ on the elemental composition of radish are given in Table 4.33. It was observed that, unlike cauliflower, the concentration of many elements in the present study was higher than the standard reference values of corresponding elements reported in USDA [110] and McCance and Widdowson's [111]. For example, Ca, Mg and Mn were higher than the two standard references while K, Zn and Cu were higher than McCance and Widdowson's and lower than USDA. Only the concentration of Fe was lower than the two standard reference values.

Elemental composition of radish root tuber was disturbed by elevated CO₂ (Table 4.33). Most of the elements analysed i.e. Ca, Mg, Zn and Cu were decreased. Only Mn and Fe were increased while K, Pb, Ni and Cr were not significantly affected. Calcium was

decreased by 5.07%, Mg by 7.63%, Zn by 12.32% and Cu by 8.33%. Reduction in Ca and Cu was significant at 95% confidence level ($P \leq 0.05$) while reduction in Mg and Zn was significant at 99% confidence level ($P \leq 0.01$). Increase in Mn was 6.77% and in Fe it was 2.98%. Increase in both these elements was significant at 99% confidence level ($P \leq 0.01$). A non-significant ($P > 0.1$) decrease of 1.38% and 4.58% has been observed for K and Pb respectively. A non-significant ($P > 0.1$) increase of 2.84% and 10.56% was also observed for Ni and Cr respectively. Decrease in elemental concentration with elevated CO₂ is mainly due to nutritional dilution, i.e. a result of increase in biomass rather than a decrease in the uptake of these elements from the soil. Another main reason which is supposed to reduce the elemental concentration of plants, is the increased water use efficiency of plants, which decreases the uptake of water from soil and as a result decreases uptake of elements which are taken up from soil with water. Increase in elements is supposed to be due to increased uptake of elements by increased size of root and larger surface of root in contact with soil [106]. The decrease in many important elements with elevated CO₂ will reduce the quality of radish and increase the chances of hidden hunger in the future.

Table 4.33. Elemental composition of radish root grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Macro-elements (%)				
Ca	0.87 ± 0.007	0.82 ± 0.017	-5.07	0.014
Mg	0.69 ± 0.003	0.64 ± 0.005	-7.63	<0.001
K	3.51 ± 0.055	3.47 ± 0.048	-1.38	ns
Micro-elements (μg g ⁻¹)				
Zn	50.87 ± 1.362	44.60 ± 1.113	-12.32	0.004
Mn	37.90 ±0.120	40.47 ± 0.110	+6.77	<0.001
Fe	37.41 ± 0.297	38.52 ± 0.223	+2.98	0.007
Pb	0.23 ± 0.002	0.22 ± 0.005	-4.58	ns
Ni	0.94 ± 0.164	0.97 ± 0.220	+2.84	ns
Cu	3.36 ± 0.111	3.08 ± 0.125	-8.33	0.044
Cr	1.07 ± 0.114	1.19 ± 0.225	+10.56	ns

ns: non-significant ($P>0.1$)

4.10.4 Effect on fatty acid composition

Fatty acid composition and impact of elevated CO₂ on the fatty acid content of radish are given in Table 4.34. Major fatty acids in radish in the present study were palmitic acid, linolenic acid and linoleic acid. Besides, stearic acid, oleic acid, elaidic and tetracosanoic acid were also present in considerable amount in radish. Almost the same pattern of fatty acids was also observed by Mandal, *et al.*, in radish fats [194]. Elevated CO₂ decreased most of the fatty acids of radish in the present experiment. Amongst the major fatty acids, palmitic acid was slightly increased while linolenic acid and linoleic acid were reduced. Among the rest of the fatty acids, myristic acid, oleic acid and elaidic acid were reduced while lauric acid was not affected. Pentadecanoic acid, margaric acid, stearic acid, arachidic acid, behenic acid and tetracosanoic acid were slightly increased. However due to the absence of statistical analysis, the significance of results could not be determined. Impact of enhanced CO₂ on fatty acid composition of radish has not been reported earlier.

Although some of the minor fatty acids were increased, however the most important fatty acid, i.e. linoleic acid and linolenic acid (essential fatty acids) were reduced. The reduction in these essential fatty acids will reduce the nutritional quality of radish at elevated CO₂.

Table 4.34. Fatty acid composition of radish root grown at two levels of atmospheric CO₂ (determined as corresponding methyl esters)

Fatty acids (%)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C12:0; Lauric acid	0.001	0.001
C14:0; Myristic acid	0.004	0.003
C15:0; Pentadecanoic acid	0.004	0.005
C16:0; Palmitic acid	0.092	0.097
C17:0; Margaric acid	0.003	0.004
C18:0; Stearic acid	0.019	0.021
C18:1c; Oleic acid	0.013	0.005
C18:1n9t; Elaidic acid	0.011	0.001
C18:2c; Linoleic acid	0.032	0.014
C18:3n3; Linolenic acid	0.070	0.055
C20:0; Arachidic acid	0.008	0.010
C22:0; Behenic acid	0.009	0.011
C24:0; Tetracosanoic acid	0.012	0.013

4.11 Effect of Enhanced CO₂ on Turnip (*Brassica rapa*) Root Tuber

Turnip (*Brassica rapa*) is a root vegetable which is grown and consumed throughout the temperate regions of the world. It is consumed in many different ways depending on the culture of the region. It is consumed raw as salad and cooked by boiling and steaming. It is mixed with other vegetables and meat. It is also pickled in some regions. Turnip leaves are also eaten in some countries however their use is comparatively less common compared to root bulbs. Turnips contain a good amount of vitamin C, proteins, fibre,

thiamine, riboflavin and minerals Ca, Mg, Fe and Zn [195]. Turnips are found effective in reducing the risk of obesity, hypertension and diabetes and in the control and prevention of many forms of cancer prevalent on lungs, stomach, pancreas, breast and bladder [183].

In the present study the response of turnip root tuber to enhanced atmospheric CO₂ was studied in terms of yield, proximate, elemental and fatty acid composition. It was observed that although yield of turnip tuber increased, its nutritional quality was disturbed with reduced protein, vitamin C and fats and increased sugars and fibers. Elemental and fatty acid composition of turnip root tuber was also altered by enhanced CO₂.

4.11.1 Effect on yield

Impact of enhanced CO₂ on yield of turnip root tuber is given in Table 4.35. As like carrot and radish, turnip also have only one root tuber per plant so the yield was determined only as fresh weight of turnip root tuber. Fresh weight of turnip root was increased by elevated CO₂. The increase was 72.57% and was significant at 99% confidence level ($P \leq 0.01$). Increase in yield was similar to other vegetables, particularly those of root vegetables. Bunce, *et al.*, observed increase in the dry mass of turnip roots when grown under elevated CO₂ [175]. Increased concentration of CO₂ in the atmosphere increases the rate of photosynthesis and as a result increases the amount of plant biomass. Increase in plant biomass was observed for other vegetables as well as for the root vegetables including radish and carrot in the present experiment.

4.11.2 Effect on proximate composition

Proximate composition and effect of enhanced CO₂ on proximate composition of turnip root tuber are given in Table 4.35. Protein content of turnip root tuber in the present experiment was $5.54 \pm 0.088\%$. It was lower than the protein content of tuber analysed by Mosha and Gaga [196]. Protein content of turnip root tuber was decreased by elevated CO₂. The decrease was 18.17% and was significant at 99% confidence level ($P \leq 0.01$). Effect of elevated CO₂ has not been studied earlier for turnip. The decrease in protein content was similar to other root vegetables investigated in the present study. The

decrease is probably due to nutritional dilution caused by increased biomass of turnip root tuber as is seen by increased turnip yield. Another reason is thought to be increased water use efficiency which decreases the uptake of water from soil and also the uptake of any other nutrients from soil. Down-regulation of photosynthetic enzyme, Rubisco, also decreases the protein content of green leaves [106]. The reduction in protein content of leaves reduces the protein content of storage organs, i.e. root in case of turnip. Decrease in protein content of turnip at elevated CO₂ will reduce the quality of vegetable in the future CO₂ rich environment.

Total sugars in the present experiment were $25.73 \pm 1.038\%$. Amount of reducing sugars was $20.95 \pm 1.212\%$ and non-reducing sugars were $4.54 \pm 0.303\%$. Although the amount of total sugars was less than that observed by Shattuck, *et al.*, however the ratio of reducing and non-reducing sugars was the same [197]. Sugar content of turnip was increased by elevated CO₂. Increase in total sugars was 19.64%, reducing sugar was 22.68% and non-reducing sugar was 6.31%. Increase in total sugars and reducing sugars was significant at 99% confidence level ($P \leq 0.01$) while increase in non-reducing sugars was non-significant statistically ($P > 0.1$).

Fiber content of turnip in the present experiment was $14.68 \pm 0.329\%$. It was lower than the fiber content observed by Mosha and Gaga in turnip with elevated CO₂ [196]. Fiber content of turnip was increased by 6.15% under elevated CO₂. The increase was significant at 95% confidence level ($P \leq 0.05$).

Although increase in carbohydrates in turnip root tuber with elevated CO₂ is not studied earlier, the increase was similar to that observed for other vegetables in the present study. Increase in sugars and fiber is due to increased photosynthetic rate at elevated CO₂ which results in greater accumulation of photosynthate i.e. carbohydrates in the green leaves. This increase is shifted to the other non-photosynthetic organs of plants. In case of turnip the main storage organ is root tuber, so the change is also reflected by root.

Vitamin C content of turnip root tuber in the present study was 26.77 ± 1.449 mg/100 gram of fresh sample. It was slightly lower than vitamin C content of tuber observed by Termine, *et al.* [198]. Vitamin C content was decreased by 21.87% with elevated CO₂.

The decrease was significant at 99% confidence level ($P \leq 0.01$). The decrease in vitamin C content of turnip root tuber has not been studied earlier. Although Carbon based compounds are expected to increase with elevated CO₂, the decrease in vitamin C may be due to the utility of additional carbon into the manufacture of biomass and not the antioxidant compounds like vitamin C [90].

Total titratable acidity of turnip root tuber in the present experiment was $0.233 \pm 0.037\%$. Elevated CO₂ did not affect the total titratable acidity of turnip roots. Although a decrease of 4.54% was observed, it was non-significant statistically ($P > 0.1$). The effect of elevated CO₂ on total titratable acidity of turnip root tuber is not studied earlier. In earlier experiments on potato, a similar non-significant decrease in citric acid with elevated CO₂ was observed by Högy and Fangmeier [54].

Fat content of turnip root tuber in the present experiment was $1.56 \pm 0.021\%$. It was lower than the fat content of turnip reported by Mosha and Gaga [196]. Elevated CO₂ decreased the fat content of turnip by 9.85%. The decrease was significant at 99% confidence level ($P \leq 0.01$). Decrease in fat content of turnip root tuber with elevated CO₂ is not reported earlier, however it is similar to decrease in fat content of other root and non-root vegetables in the present study. Main reason for such decreases is thought to be nutritional dilution caused by increased biomass and carbohydrates in the plant tissues.

Ash content of turnip root tuber in the present experiment was $14.62 \pm 0.894\%$. It was higher than the ash content reported in turnip by Mosha and Gaga [196]. Elevated CO₂ did not affect the ash content of turnip root tuber. Although a decrease of 8.57% was observed in the ash content of turnip with elevated CO₂, the decrease was non-significant statistically ($P > 0.1$).

Table 4.35. Yield and proximate composition of turnip root grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Fresh Weight (g/plant)	44.59 ± 5.088	76.95 ± 6.107	+72.57	0.002
Protein (%)	5.54 ± 0.088	4.53 ± 0.100	-18.17	<0.001
Total Sugars (%)	25.73 ± 1.038	30.79 ± 1.347	+19.64	0.007
Reducing Sugars (%)	20.95 ± 1.212	25.70 ± 1.058	+22.68	0.007
Non-reducing Sugars (%)	4.54 ± 0.303	4.83 ± 0.484	+6.31	ns
Fiber (%)	14.68 ± 0.329	15.58 ± 0.223	+6.15	0.017
Vitamin C (mg of AA/100 gram)	26.77 ± 1.449	20.92 ± 1.449	-21.87	0.008
Total Titratable Acidity (%)	0.23 ± 0.037	0.22 ± 0.032	-4.54	ns
Fat (%)	1.56 ± 0.021	1.41 ± 0.023	-9.85	0.001
Ash (%)	14.62 ± 0.894	13.36 ± 0.938	-8.57	ns

ns: non-significant ($P>0.1$)

4.11.3 Effect on elemental composition

Elemental composition and impact of elevated CO₂ on elemental composition of turnip root tuber is summarized in Table 4.36. When elemental composition of turnip root tuber in the present experiment was compared to the standard reference values of USDA [110] and McCance and Widdowson's [111], it was observed that concentration of corresponding elements differ from the standard values. Many of the elements like Ca, Mg, K and Mn were lower than the values of McCance and Widdowson's and higher than USDA, while Zn and Cu were lower than USDA and higher than McCance and Widdowson's. Nitrogen was lower than McCance and Widdowson's. Fe was higher than McCance and Widdowson's but it was almost comparable to USDA.

Elevated CO₂ disturbed the elemental composition of turnip root tuber with increased Ca, K, Mn and Cu and reduced N, Ca, Mg, Zn, Fe and Ni (Table 4.36). Carbon increased by

20.82%, K by 2.45%, Mn by 10.18% and Cu increased by 15.92% under elevated CO₂. Increase in these four elements was significant under 99% confidence level ($P \leq 0.01$). Decrease was 18.17% for N, 9.50% for Ca, 3.74% for Mg, 24.57% for Zn, 18.67% for Fe and 4.11% for Ni. The decrease in these elements with elevated CO₂ was also significant at 99% confidence level ($P \leq 0.01$). Sulphur tended to increase by 25.33% and Pb tended to increase by 5.20% ($0.05 < P \leq 0.1$). No significant trend was observed in H, Cr and Cd, although H was decreased and Cr and Cd was increased slightly, but it was non-significant ($P > 0.1$). Increase in C and decrease in N increased C:N ratio of the plants by 47.66%. Increase in C is also in accordance with increase in sugars and fiber content of the turnip root tubers. Decrease in N is in accordance with reduction in protein content of the tuber. And this is similar to other vegetables studied in the present experiment. Although the impact of elevated CO₂ has not been studied earlier on elemental composition of turnip root, the decrease in some elements can be due to increased uptake of those elements by larger sized root tuber under such conditions. Decrease in elements is mainly due to nutritional dilution of these elements and decreased uptake of these elements due to reduced transpiration [106]. Although many important nutrients like K and Mn were increased, however it was done at the cost of other important nutrients like Ca, Mg, Zn and Fe. This will lower the nutritional quality of turnip root tuber and will increase the chances of hidden hunger. Another matter of important concern is that heavy metal like Pb tended to increase in turnip root tuber which will further lower the nutritional quality of turnip.

Table 4.36. Elemental composition of turnip root grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Macro-elements (%)				
C	31.99 ± 0.135	38.65 ± 0.080	+20.82	<0.001
N	0.89 ± 0.014	0.73 ± 0.016	-18.17	<0.001
H	5.55 ± 0.220	5.22 ± 0.170	-5.88	ns
S	0.76 ± 0.114	0.95 ± 0.101	+25.33	(0.094)
Ca	0.49 ± 0.002	0.44 ± 0.002	-9.50	<0.001
Mg	0.28 ± 0.001	0.27 ± 0.001	-3.74	0.001
K	0.45 ± 0.001	0.46 ± 0.000	+2.45	0.001
C/N	36.11 ± 0.723	53.33 ± 1.067	+47.66	<0.001
Micro-elements (μg g ⁻¹)				
Zn	19.98 ± 0.285	15.07 ± 0.050	-24.57	<0.001
Mn	32.34 ± 0.031	35.63 ± 0.030	+10.18	<0.001
Fe	39.69 ± 0.227	32.28 ± 0.193	-18.67	<0.001
Pb	0.32 ± 0.010	0.33 ± 0.007	+5.20	(0.075)
Ni	5.31 ± 0.031	5.09 ± 0.070	-4.11	0.008
Cu	2.60 ± 0.062	3.02 ± 0.053	+15.92	0.001
Cr	1.06 ± 0.132	1.23 ± 0.083	+15.46	ns
Cd	0.22 ± 0.010	0.23 ± 0.006	+3.42	ns

ns: non-significant ($P>0.1$), number in parenthesis indicate trend ($0.05<P\leq 0.1$)

4.11.4 Effect on fatty acid composition

Fatty acid composition and impact of elevated CO₂ on fatty acid composition of turnip root tuber is given in Table 4.37. Major fatty acids in the turnip root tuber in the present study are linolenic acid followed by palmitic acid, linolenic acid and oleic acid. Elaidic acid, stearic acid and tetracosanoic acid were also present in considerable amount in the turnip root. Other fatty acids were present in minor amounts. The pattern was mostly similar to the fatty acid profile of other brassica species studied by Chen and Heneen [199].

The fatty acid composition of turnip root tuber was altered by elevated CO₂. Almost all of the fatty acids were reduced by elevated CO₂. The major fatty acids linolenic acid, palmitic acid, linoleic acid and oleic acid were reduced by a larger amount. Stearic, elaidic and tetracosanoic acids were also reduced. Among the minor fatty acids only lauric acid increased slightly while rest of the fatty acids decreased to a smaller extent. Due to absence of statistical analysis, significance of results could not be concluded, however the decrease in major fatty acids is quite notable. The impact of elevated CO₂ on fatty acid composition of turnip root tuber is not studied earlier. The decrease in fatty acid contents is in agreement with reduction in fat content of turnip root tuber at enhanced CO₂. The reduction in fatty acids especially the essential fatty acids, linolenic and linoleic acid will greatly affect the nutritional quality of turnip root tuber.

Table 4.37. Fatty acid composition of turnip root grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (mg/g)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C12:0; Lauric acid	0.001	0.002
C14:0; Myristic acid	0.004	0.003
C15:0; Pentadecanoic acid	0.003	0.002
C16:0; Palmitic acid	0.059	0.035
C16:1c; Palmitoleic acid	0.006	0.002
C17:0; Margaric acid	0.004	0.002
C18:0; Stearic acid	0.016	0.011
C18:1c; Oleic acid	0.022	0.008
C18:1n9t; Elaidic acid	0.017	0.006
C18:2c; Linoleic acid	0.031	0.009
C18:3n3; Linolenic acid	0.120	0.041
C20:0; Arachidic acid	0.006	0.003
C22:0; Behenic acid	0.006	0.002
C24:0; Tetracosanoic acid	0.010	0.004

4.12 Effect of Enhanced CO₂ on Eggplant (*Solanum melongena*) Fruit

Eggplant (*Solanum melongena*), a fruit vegetable, is grown and consumed worldwide. The fleshy fruits are cooked in a number of ways depending on the culture of the region. The fruit is sliced, fried and presented with yogurt. The skin of the roasted fruit is removed, the inner fruit is meshed and mixed with other vegetables. It is also stuffed with rice or meat and baked. It is also pickled in some countries. Eggplants contain a high amount of antioxidants including vitamin C and phenolics, and considerable quantities of protein, dry matter and minerals like Ca, K, Mg and P [200, 201]. It is very important from medicinal point of view as it contains nasunin which helps to improve brain functioning [202], antioxidants make them good anti-cancer and phytonutrients help to reduce the blood cholesterol [203].

In the present study the impact of enhanced CO₂ was studied on the proximate, elemental and fatty acid composition of eggplant fruits. Proximate composition was altered with reduced protein, vitamin C and fat content while increased fiber content of the fruit. Elemental and fatty acid composition of eggplant fruit were also disturbed by enhanced CO₂.

4.12.1 Effect on proximate composition

Proximate composition and impact of elevated CO₂ on proximate composition of eggplant fruit are given in Table 4.38. Protein content of eggplant in the present experiment was $12.76 \pm 0.069\%$. It was comparable to the protein content of eggplant reported by Flick, *et al.* [204]. Protein content of eggplant decreased with enhanced CO₂. The decrease was 11.36% and was significant at 99% confidence level ($P \leq 0.01$). The impact of elevated CO₂ is not studied earlier for eggplant. The decrease in protein content of eggplant with enhanced CO₂ is similar to other vegetables in the present study. The result is thought mainly to be due to increased biomass and carbohydrates of the plant tissues which results in the dilution of non-carbohydrate components including protein. Another reason might be the down-regulation of photosynthetic enzyme, Rubisco, which is the major protein containing enzyme of green leaves. Decrease in protein content of leaves will be automatically shifted to other plant organs, including

fruit in case of eggplant. Decreased uptake of nitrates and nitrites from soil due to reduced transpiration and reduced water uptake from soil is also thought to be a reason for reduced protein content [106]. The decrease in protein content of eggplant at enhanced CO₂ will lower its nutritional quality.

Fiber content of eggplant in the present experiment was $14.58 \pm 0.205\%$. It was slightly higher than the fiber content of one variety but lower than the fiber content of another eggplant variety analysed by Flick, *et al.* [204]. Fiber content of eggplant was increased by elevated CO₂. Increase was 9.67% and was significant at 99% confidence level ($P \leq 0.01$). Elevated CO₂ increases the photosynthetic rate and hence any structural and non-structural carbohydrates in plant tissues. The increase in fiber content with elevated CO₂ is also reflected by other vegetables of the present study.

Vitamin C content of eggplant fruit in the present experiment was 3.35 ± 0.203 mg/100 gram of fresh weight. This was almost identical to the ascorbic acid content of eggplant studied by Esteban, *et al.* [205]. Enhanced CO₂ decreased the vitamin C content of eggplant. The decrease was 15.96% and was significant at 95% confidence level ($P \leq 0.05$). Decrease in vitamin C content of eggplant with elevated CO₂ is not studied earlier. The decrease is similar to other vegetables of the present study. The decrease is because of the fact that additional carbon is channelized to manufacture of carbohydrates and not to the antioxidant compounds [90].

Total titratable acidity of eggplant fruit in the present experiment was $0.285 \pm 0.055\%$. It was slightly higher than the total titratable acidity of eggplant fruits analysed by Esteban, *et al.* [205]. Enhanced CO₂ did not alter the total titratable acidity of the eggplant fruit. A decrease of 7.41% was observed at elevated CO₂, but it was non-significant ($P > 0.1$). This non-significant reduction in total titratable acidity of eggplant fruit is similar to other vegetables of the present study.

Fat content of eggplant in the present study was $1.30 \pm 0.072\%$. It was slightly higher than the fat content of eggplant fruits reported by Flick, *et al.* [204]. Fat content of eggplant fruit was decreased by elevated CO₂. The decrease was 14.36% and was significant at 95% confidence level ($P \leq 0.05$). Decrease in fat content of eggplant with

elevated CO₂ is similar to other vegetables in the present study and is thought to be due to increased carbohydrates leading to nutritional dilution of other components including fats.

Ash content of the eggplant fruit in the present experiment was $11.60 \pm 0.410\%$. It was higher than the ash content of eggplant varieties analysed by Flick, *et al.* [204]. Ash content of eggplant fruit was not affected by elevated CO₂. Although a decrease 5.37% was observed, however it was non-significant statistically ($P>0.1$).

Table 4.38. Proximate composition of eggplant fruits grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		CO ₂ effect (%)	Probability level (<i>P</i>)
	400 (ambient)	1000		
Protein (%)	12.76 ± 0.069	11.31 ± 0.169	-11.36	<0.001
Fiber (%)	14.58 ± 0.205	15.99 ± 0.125	+9.67	0.001
Vitamin C (mg of AA/100 gram)	3.35 ± 0.203	2.81 ± 0.065	-15.96	0.012
Total Titratable Acidity (%FW)	0.285 ± 0.055	0.264 ± 0.048	-7.41	ns
Fat (%)	1.30 ± 0.072	1.11 ± 0.083	-14.36	0.043
Ash (%)	11.60 ± 0.410	10.98 ± 0.476	-5.37	ns

ns: non-significant ($P>0.1$)

4.12.2 Effect on elemental composition

Elemental composition and impact of elevated CO₂ on elemental composition of eggplant fruit is given in Table 4.39. By comparing the concentration of different elements of eggplant fruit in the present experiment with the standard reference values of the corresponding elements reported in USDA [110] and McCance and Widdowson's [111] many differences were observed. Nitrogen content was comparable to McCance and Widdowson's. Calcium and potassium were lower than two standard reference values. Mg was lower than USDA and higher than McCance and Widdowson's. Zn and Mn were comparable to McCance and Widdowson's however Zn was lower while Mn was higher

than McCance and Widdowson's. Fe and Cu were higher than USDA and lower than McCance and Widdowson's.

Elemental composition of eggplant fruit was altered by enhanced CO₂. Elements like C, H and Ca were increased while N, Mg, K, Zn, Mn and Fe were decreased. Elevated CO₂ increased C by 27.35%, H by 16.01% and Ca by 9.96%. Increase was significant at 99% confidence level ($P \leq 0.01$). Nitrogen was decreased by 11.36%, Mg by 10.86%, K by 5.10%, Zn by 10.95%, Mn by 5.88% and Fe by 1.49%. Decrease in N, Mg, K and Mn was significant at 99% confidence level ($P \leq 0.01$) while decrease in Zn and Fe was significant at 95% confidence level ($P \leq 0.05$). Increase in C and decrease in N increased the C:N ratio eggplant fruit by 43.69%. A non-significant ($P > 0.1$) decrease in S, Pb and Cr while a non-significant increase in Ni and Cu was also observed under elevated CO₂ conditions. Increase in C is reflected by increased fiber content while decrease in N is reflected by reduced protein content of eggplant fruit. Increase in C and H and decrease in majority of the elements is in agreement with the prediction of Loladze, that all the other elements except C and H will decrease in different plant tissue under enhanced CO₂ conditions [37]. Increase in C and H is due to increased carbohydrates which are mostly composed up of these two elements. Decrease in other elements is mainly due to dilution of these elements caused by increased carbohydrates. Increase in Ca might be due to increased uptake of this element by enlarged root size of the eggplants under enhanced CO₂ conditions. Although Ca is increased but many important elements like N, Mg, K, Zn, Mn and Fe are decreased which will greatly lower the nutritional quality of eggplant fruits.

Table 4.39. Elemental composition of eggplant fruits grown at two levels of atmospheric CO₂

Parameter	CO ₂ concentration (μmol mol ⁻¹)		CO ₂ effect (%)	Probability level (P)
	400 (ambient)	1000		
Macro-elements (%)				
C	35.76 ± 0.030	45.54 ± 0.040	+27.35	<0.001
N	2.04 ± 0.011	1.81 ± 0.027	-11.36	<0.001
H	5.34 ± 0.015	6.19 ±0.012	+16.01	<0.001
S	0.534 ± 0.013	0.528 ± 0.011	-1.12	ns
Ca	0.108 ± 0.002	0.118 ± 0.001	+9.96	0.001
Mg	0.168 ± 0.005	0.150 ± 0.001	-10.86	<0.001
K	1.97 ± 0.003	1.87 ± 0.010	-5.10	0.002
C/N	17.51 ± 0.079	25.16 ± 0.398	+43.69	<0.001
Micro-elements (μg g ⁻¹)				
Zn	22.28 ± 0.614	19.84 ± 1.228	-10.95	0.037
Mn	34.31 ± 0.213	32.29 ± 0.264	-5.88	0.001
Fe	35.49 ± 0.179	34.97 ± 0.171	-1.49	0.021
Pb	0.47 ± 0.002	0.45 ± 0.002	-6.31	ns
Ni	1.88 ± 0.219	1.96 ± 0.232	+4.33	ns
Cu	5.98 ± 0.315	6.20 ± 0.186	+3.67	ns
Cr	1.61 ±0.217	1.55 ± 0.258	-3.53	ns

ns: non-significant ($P>0.1$)

4.12.3 Effect on fatty acid composition

Fatty acid composition and effect of enhanced CO₂ on fatty acid composition of eggplant fruit is given in Table 4.40. Major fatty acids in eggplant fruit in the present study were linoleic acid followed by linolenic acid, palmitic acid and stearic acid. Oleic acid and eicosadienoic acid were also present in considerable amounts. Other fatty acids were present in minor amount in eggplant fruits in the present experiment. The fatty acid profile was similar to that of Kenrick and Bishop, where the dominant fatty acids were palmitic, linolenic and linoleic acids [206].

Fatty acid composition of eggplant fruit was altered by elevated CO₂. Most of the fatty acids were reduced including linoleic acid, linolenic acid, palmitic acid, stearic acid and oleic acid. Minor fatty acids were also reduced except myristic acid and margaric acid, which were not affected by elevated CO₂. Minor fatty acids were also slightly reduced under enhanced CO₂ conditions. The decrease in fatty acid content was also reflected by decreased fat content of eggplant fruits. Impact of enhanced CO₂ is not reported earlier on the eggplant fatty acids, however reduction in almost all fatty acid fractions will lower the nutritional quality of eggplant fruits.

Table 4.40. Fatty acid composition of eggplant fruits grown at two levels of atmospheric CO₂ (determined as corresponding methyl ester)

Fatty acids (%)	CO ₂ concentration (μmol mol ⁻¹)	
	400 (ambient)	1000
C14:0; Myristic acid	0.001	0.001
C16:0; Palmitic acid	0.034	0.029
C17:0; Margaric acid	0.001	0.001
C18:0; Stearic acid	0.021	0.014
C18:1c; Oleic acid	0.016	0.014
C18:1n9t; Elaidic acid	0.003	0.002
C18:2c; Linoleic acid	0.146	0.136
C18:2t; Octadecadienoic acid	0.003	0.002
C18:3n3; Linolenic acid	0.070	0.065
C20:0; Arachidic acid	0.007	0.005
C20:2; Eicosadienoic acid	0.012	-
C22:0; Behenic acid	0.003	0.004
C23:0; Tricosanoic acid	0.002	0.001
C24:0; Tetracosanoic acid	0.004	0.003

CONCLUSIONS

An increase in the concentration of atmospheric CO₂ is expected to increase the edible biomass of agricultural products by increasing growth and yield of plants and vegetables. Many chemical changes can also be expected to occur in plants as a result of elevated levels of CO₂. Available studies have mostly concentrated on the growth and yield of the food crops, whereas its possible impact on the nutritional balance and elemental composition has been ignored. Studies conducted are predominantly on crops like wheat, maize, rice and potato, very little information is available on vegetables. To the best of our knowledge, no comprehensive study has ever been conducted on our local dietary vegetables that have been investigated in this work. Study of the impact of enhanced atmospheric CO₂ on the nutritional, elemental and fatty acid composition in the present work has led to the following conclusions:

- Nutritional composition of tomato has been altered by enhanced CO₂ with reduced protein, vitamin C and ash content and increased sugars and fiber content.
- Enhanced CO₂ disturbed the elemental and fatty acid composition of tomato. The amount of C, H, Ca, Fe and Cu were increased and N, Mg, Zn, Mn, Pb, Ni, Cr and Cd were reduced by elevated CO₂. Most of the fatty acids were also reduced by elevated CO₂.
- Elevated CO₂ affected the proximate composition of chili fruits by decreasing protein, fat, ash and vitamin C and increasing sugar and fat contents for all five varieties including green stages of the fruit.
- Elemental composition of chili varieties was disturbed with elevated CO₂. Elements like C, H, Fe and Mn increased while Ca, Mg, N, Zn and Pb decreased. Among fatty acids, palmitic and linoleic acids were reduced while linolenic, elaidic acid and other minor fatty acids of the two varieties behaved differently to elevated CO₂.
- Elevated CO₂ affected the nutritional quality of onion bulbs by altering the proximate composition with reduced protein, fats and vitamin C and increased sugars and fiber contents in all four varieties examined in this work.

- Among the elements, C and Zn were increased and N, Mn, Fe, Pb and Cr were decreased under elevated CO₂. Most of the fatty acids followed the same trend for all varieties with major fatty acids mostly decreasing.
- Proximate composition of okra was negatively affected by elevated CO₂ with reduced proteins, vitamin C and fats and increased sugars and fiber in the two varieties studied here.
- Elemental composition of okra was disturbed by elevated CO₂ with increased C, Ca and Fe and decreased N, Mg, Zn, Mn and Pb. Fatty acids of okra were mostly decreased by enhanced CO₂.
- Proximate composition of cucumber was affected by elevated CO₂ with reduced protein, fats and vitamin C and increased sugars and fiber contents.
- Elemental and fatty acid composition of cucumber was disturbed by elevated CO₂. Elements like C, H, Ca and Mg increased and N, Zn, Mn and Fe were decreased. Fatty acid content of cucumber followed a decreasing trend under enhanced CO₂.
- Enhanced CO₂ decreased protein, vitamin C and fat content of spinach leaves however fiber content was increased in this given leafy vegetable.
- Elemental and fatty acid composition of spinach was disturbed by elevated CO₂. Elements like C, H and Ca were increased and N, Zn, Mn, Fe, Pb, Ni, Cu and Cr were decreased. Elevated CO₂ decreased almost all of the fatty acids in spinach leaf lipids.
- Protein, vitamin C and fat content of carrot root tuber were decreased while fiber content was increased by enhanced atmospheric CO₂.
- Elemental and fatty acid concentration of carrot root was disturbed by elevated CO₂ with increased C, H, Ca, Mn and Cu, decreased N, Zn, Fe and Cr and reduced fatty acids.
- Nutritional quality of pea seeds was altered by elevated CO₂ with reduced proteins, vitamin C and fats while increased sugars and fiber.
- Elemental composition of pea seeds was disturbed by elevated CO₂ with increased C, H and Mn and reduced N, Ca, Mg, K and Fe. Most of the fatty acids were also reduced by elevated CO₂.

- Enhanced CO₂ increased the yield of cauliflower but disturbed the nutritional balance of the vegetable with reduced protein, vitamin C and fats and increased fiber content,
- Elemental and fatty acid composition of cauliflower was disturbed by elevated CO₂. Concentration of C and H increased, whereas most of the elements including N, S, Ca, Zn, Mn, Fe, Cu and Cr were decreased. Major fatty acids were also reduced by elevated CO₂.
- Nutritional quality of radish was negatively affected with reduced protein, vitamin C and fat contents and increased sugars and fiber.
- Elemental and fatty acid composition of radish root tuber was disturbed by elevated CO₂. Most of the elements analysed i.e. Ca, Mg, Zn and Cu were decreased. Only Mn and Fe were increased. The major fatty acids of radish root tuber were decreased and most of the minor fatty acids were increased very slightly.
- Protein, fats and vitamin C contents of turnip root tuber were decreased with enhanced CO₂, badly affecting its nutritional quality.
- Elemental and fatty acid composition of turnip root tuber was altered under enhanced CO₂ conditions with increased C, K, Mn and Cu and reduced N, Ca, Mg, Zn, Fe and Ni. Almost all of the fatty acids present in turnip root lipids were reduced by elevated CO₂.
- In eggplant fruits, enhanced CO₂ increased the fiber content however it lowered the nutritional quality by decreasing proteins, fats and vitamin C contents of the vegetable.
- Elemental and fatty acid composition of eggplant fruit was altered by enhanced CO₂. Elements like C, H and Ca were increased while N, Mg, K, Zn, Mn and Fe were decreased. Most of the fatty acids were reduced by elevated CO₂.

Suggestions for further study:

- Further studies are needed in order to investigate the impact of enhanced atmospheric CO₂ on more vegetables and more varieties of these vegetables to find the least affected varieties.

- The least affected varieties should be crossed together to get a set of varieties for each vegetable which are more resistant to changes in concentration of atmospheric CO₂.
- Studies should be conducted to observe any change in genetics, i.e. DNA and RNA, of the plants under CO₂ enriched atmosphere.
- Similar studies should be conducted with use of different kinds of fertilizers in order to find those one which can improve the nutritional quality of plants and reduce the negative impact of enhanced CO₂.
- Response of the plants to other gases should be studied, like methane, sulphur dioxide and ozone, which are increasing in the atmosphere.
- Combined effect of all these gases as well as temperature changes on the nutritional status of plants should be investigated to get a clear picture of future food quality.

REFERENCES

1. IPCC (2007). Climate Change. The Physical Science Basis. contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Intergovernmental Panel on Climate Change, Cambridge Univ. Press, UK, **2007**.
2. Keeling, C. D. Rewards and penalties of monitoring the earth. *Annu. Rev. Energ. Env.* **1998**, 23(1), 25–82.
3. Conway, T. J.; Lang, P. M.; Masarie, K. A. Atmospheric carbon dioxide dry air mole fractions from The NOAA ESRL carbon cycle cooperative global air sampling network, 1968–2007 Version: 2008-07-24, **2008**, Available from: [<ftp://ftp.cmdl.noaa.gov/ccg/co2/flask/event/>](ftp://ftp.cmdl.noaa.gov/ccg/co2/flask/event/).
4. Keeling, C. D.; Whorf, T. P. Atmospheric CO₂ records from sites in the SIO air sampling network. Trends, A compendium of data on global change. Carbon dioxide information analysis center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn., U.S.A, **2005**.
5. Fung, I. Y.; Doney, S. C.; Linday, K.; John, J. Evolution of carbon sinks in a changing climate. *Proc. Nat. Acad. Sci. (USA)* **2005**, 102(32), 11201–11206.
6. Le Quéré, C.; Rodenbeck, C.; Buitenhuis, E. T.; Conway, T. J.; Langenfelds, R.; Gomez, A.; Labuschagne, C.; Ramonet, M.; Nakazawa, T.; Metzl, N.; Gillett, N.; Heimann, M. Saturation of the southern ocean CO₂ sink due to recent climate change. *Science* **2007**, 316(5832), 1735–1738.
7. Etheridge, D. M.; Steele, L. P.; Langenfelds, R. L.; Francey, R. J.; Barnola, J. M.; Morgan, V. I. Natural and anthropogenic changes in atmospheric CO₂ over the last 1000 years from air in Antarctic ice and firn. *J. Geophys. Res.* **1996**, 101(D2), 4115–4128.
8. Kerr, R. A. The next oil crisis looms large – and perhaps close. *Science* **1998**, 281(5380), 1128–1131.
9. Hofmann, D. J.; Butler, J. H.; Tans, P. P. A new look at atmospheric carbon dioxide. *Atmos. Environ.* **2009**, 43(12), 2084–2086.

10. IPCC (2001). Climate Change. The scientific basis. contribution of working group I to the third assessment report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Cambridge Univ. Press, UK, **2001**.
11. Le Quéré, C.; Raupach, M. R.; G., C. J.; Marland, G.; Bopp, L.; Ciais, P.; Conway, T. J.; Doney, S. C.; Feely, R. A.; Foster, P.; Friedlingstein, P.; Gurney, K.; Houghton, R. A.; House, J. I.; Huntingford, C.; Levy, P. E.; Lomas, M. R.; Majkut, J.; Metzl, N.; Ometto, J. P.; Peters, G. P.; Prentice, I. C.; Randerson, J. T.; Running, S. W.; Sarmiento, J. L.; Schuster, U.; Sitch, S.; Takahashi, T.; Viovy, N.; van der Werf, G. R.; Woodward, F. I. Trends in the sources and sinks of carbon dioxide. *Nat. Geosci.* **2009**, 2, 831 - 836.
12. Mitchell, J. F. B. "The "Greenhouse" effect and climate change". *Rev. Geophys.* **1989**, 27(1), 115–139.
13. Bengtsson, M.; Shen, Y.; Oki, T. A SRES-based gridded global population dataset for 1990–2100. *Popul. Environ.* **2006**, 28(2), 113–131.
14. Ingram, J. S. I.; Gregory, P. J.; Izac, A.-M. The role of agronomic research in climate change and food security policy. *Agr. Ecosyst. Environ.* **2008**, 126(1-2), 4–12.
15. Long, S. P.; Ainsworth, E. A.; Rogers, A.; Ort, D. R. Rising atmospheric carbon dioxide: plants FACE the future. *Annu. Rev. Plant Biol.* **2004**, 55(591–628).
16. Norby, R. J.; Luo, Y. Q. Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytol.* **2004**, 162(2), 281–293.
17. Pearson, P. N.; Palmer, M. R. Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature* **2000**, 406, 695-699.
18. Stitt, M. Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant Cell Environ.* **1991**, 14(8), 741–762.
19. Long, S. P.; Ainsworth, E. A.; Leakey, A. D. B.; Ort, D. R. Food for thought: Lower-than-expected crop yield stimulation with rising CO₂ conditions. *Science* **2006**, 312(5782), 1918–1921.

20. DaMatta, F. M.; Grandis, A.; Arenque, B. C.; Buckeridge, M. S. Impacts of climate changes on crop physiology and food quality. *Food Res. Int.* **2010**, *43*(7), 1814–1823.
21. von Caemmerer, S.; Furbank, R. T. The C₄ pathway: An efficient CO₂ pump. *Photosynth. Res.* **2003**, *77*(2-3), 191–207.
22. Leakey, A. D. B.; Ainsworth, E. A.; Bernacchi, C. J.; Rogers, A.; Long, S. P.; Ort, D. R. Elevated CO₂ effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. *J. Exp. Bot.* **2009**, *60*(10), 2859–2876.
23. Ainsworth, E. A.; Rogers, A. The response of photosynthesis and stomatal conductance to rising [CO₂]: Mechanisms and environmental interactions. *Plant Cell Environ.* **2007**, *30*(3), 258–270.
24. Ainsworth, E. A.; Davey, P. A.; Bernacchi, C. J.; Dermody, O. C.; Heaton, E. A.; Moore, D. J.; Morgan, P. B.; Naidu, S. L.; Yoo, H.; Zu, X.; Curtis, P. S.; Long, S. P. A metaanalysis of elevated [CO₂] effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biol.* **2002**, *8*(8), 695–709.
25. Ziska, L. H.; Manalo, P. A.; Ordonez, R. Intraspecific variation in the response of rice (*Oryza sativa* L.) to increased CO₂: Evaluation of 17 cultivars. *J. Exp. Bot.* **1996**, *47*(302), 1353–1359.
26. Jablonski, L. M.; Xianzhong, W.; Curtis, P. S. Plant reproduction under elevated CO₂ conditions: A meta-analysis of reports on 79 crop and wild species. *New Phytol.* **2002**, *156*(1), 9–26.
27. Yang, L.; Wang, H.; Liu, Y.; Zhu, J.; Huang, J.; Liu, G.; Dong, G.; Wang, Y. Yield formation of CO₂-enriched inter-subspecific hybrid rice cultivar Liangyoupeijiu under fully open-air field condition in a warm sub-tropical climate. *Agr. Ecosyst. Environ.* **2009**, *129*(1-3), 193–200.
28. Miglietta, F.; Magliulo, V.; Bindi, M.; Cerio, L.; Vaccari, F. P.; Loduca, V.; Peressotti, A. Free air CO₂ enrichment of potato (*Solanum tuberosum* L.): Development, growth and yield. *Global Change Biol.* **1998**, *4*(2), 163–172.
29. De Souza, A. P.; Gaspar, M.; da Silva, E. A.; Ulian, E. C.; Waclawovsky, A. J.; Nishiyama, M. Y. J.; dos Santos, R. V.; Teixeira, M. M.; Souza, G. M.; Buckeridge, M. S. Elevated CO₂ increases photosynthesis, biomass and

- productivity, and modifies gene expression in sugarcane. *Plant Cell Environ.* **2008**, *31*(8), 1116–1127.
30. Kimball, B. A.; Idso, S. B.; Johnson, S.; Rillig, M. T. Seventeen years of carbon dioxide enrichment of sour orange trees: final results. *Global Change Biol.* **2007**, *13*(10), 2171–2183.
 31. Gifford, R. M. Plant respiration in productivity models: Conceptualisation, representation and issues for global terrestrial carbon-cycle research. *Funct. Plant Biol.* **2003**, *30*(2), 171–186.
 32. Leakey, A. D. B.; Xu, F.; Gillespie, K. M.; McGrath, J. M.; Ainsworth, E. A.; Ort, D. R. The genomic basis for stimulated respiratory carbon loss to the atmosphere by plants growing under elevated [CO₂]. In: Proceedings of the National Academy of Sciences. USA. **2009**.
 33. Taiz, L.; Zeiger, E. Plant physiology. 4th ed.: Sinauer Associates, Inc. Publishers, Sunderland, MA., **2006**.
 34. Ziska, L. H.; Bunce, J. A. Plant responses to rising atmospheric carbon dioxide. Plant growth and climate change, ed. Morison, J. I. L., Morecroft, M. D.: Blackwell Publishing, Oxford, UK, **2006**. pp. 17–47.
 35. Ainsworth, E. A.; Long, S. P. What have we learned from 15 years of free air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol.* **2005**, *165*(2), 351–372.
 36. Goldman, I. L.; Kader, A. A.; Heintz, C. Influence of production, handling, and storage on phytonutrient content of foods. *Nutr. Rev.* **1999**, *57*(9), S46–S52.
 37. Loladze, I. Rising atmospheric CO₂ and human nutrition: toward globally imbalanced plant stoichiometry. *Trends Ecol. Evol.* **2002**, *17*(10), 457–461.
 38. Thompson, G. B.; Woodward, F. I. Some influences of CO₂ enrichment, nitrogen nutrition and competition on grain yield and quality in spring wheat and barley. *J. Exp. Bot.* **1994**, *45*(7), 937–942.
 39. van Vuuren, M. M. I.; Robinson, D.; Fitter, A. H.; Chaselow, S. D.; Williamson, L.; Raven, J. A. Effects of elevated atmospheric CO₂ and soil water availability

- on root biomass, root length, and N, P and K uptake by wheat. *New Phytol.* **1997**, *135*(3), 455-465.
40. Fangmeier, A.; Grüters, U.; Högy, P.; Vermehren, B.; Jäger, H. J. Effects of elevated CO₂, nitrogen supply and tropospheric ozone on spring wheat— II. nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Zn). *Environ. Pollut.* **1997**, *96*(1), 43-59.
 41. Fangmeier, A.; De Temmerman, L.; Mortensen, L.; Kemp, K.; Burke, J.; Mitchell, R.; van Oijen, M.; Weigel, H. J. Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO₂ concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat. *Eur. J. Agron.* **1999**, *10*(3-4), 215–229.
 42. Pleijel, H.; Mortensen, L.; Fuhrer, J.; Ojanperä, K.; Danielsson, H. Grain protein accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L.) grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. *Agr. Ecosyst. Environ.* **1999**, *72*(3), 265-270.
 43. Sinclair, T. R.; Pinter, P. J.; Jr, K., B. A.; Adamsen, F. J.; LaMorte, R. L.; Wall, G. W.; Hunsaker, D. J.; Adam, N.; Brooks, T. J.; Garcia, R. L.; Thompson, T.; Leavitt, S.; Matthias, A. Leaf nitrogen concentration of wheat subjected to elevated [CO₂] and either water or N deficits. *Agr. Ecosyst. Environ.* **2000**, *79*(1), 53-60.
 44. Agrawal, M.; Deepak, S. S. Physiological and biochemical responses of two cultivars of wheat to elevated levels of CO₂ and SO₂, singly and in combination. *Environ. Pollut.* **2003**, *121*(2), 189-197.
 45. Wu, D. X.; Wang, G. X.; Bai, Y. F.; Liao, J. X. Effects of elevated CO₂ concentration on growth, water use, yield and grain quality of wheat under two soil water levels. *Agr. Ecosyst. Environ.* **2004**, *104*(3), 493–507.
 46. Wieser, H.; Manderscheid, R.; Erbs, M.; Weigel, H. J. Effects of Elevated Atmospheric CO₂ Concentration on the Quantitative Protein Composition of Wheat Grain. *J. Agric. Food Chem.* **2008**, *56*(15), 6531–6535.
 47. Högy, P.; Fangmeier, A. Effects of elevated atmospheric CO₂ on grain quality of wheat. *J. Cereal Sci.* **2008**, *48*(3), 580–591.

48. Porteous, F.; Hill, J.; Ball, A. S.; Pinter, P. J.; Kimball, B. A.; Wall, G. W.; Adamsen, F. J.; Hunsaker, D. J.; LaMorte, R. L.; Leavitt, S. W.; Thompson, T. L.; Matthias, A. D.; Brooks, T. J.; Morris, C. F. Effect of Free Air Carbon dioxide Enrichment (FACE) on the chemical composition and nutritive value of wheat grain and straw. *Anim. Feed Sci. Tech.* **2009**, *149*(3-4), 322–332.
49. Högy, P.; Keck, M.; Niehaus, K.; Franzaring, J.; Fangmeier, A. Effects of atmospheric CO₂ enrichment on biomass, yield and low molecular weight metabolites in wheat grain. *J. Cereal Sci.* **2010**, *52*(2), 215-220.
50. Erbs, M.; Manderscheid, R.; Jansen, G.; Seddig, S.; Pacholski, A.; Weigel, H. J. Effects of free-air CO₂ enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley grown in a crop rotation. *Agr. Ecosyst. Environ.* **2010**, *136*(1-2), 59–68.
51. Wheeler, R. M.; Mackowiak, C. L.; Sager, J. C.; Knott, W. M.; Berry, W. L. Proximate nutritional composition of CELSS crops grown at different CO₂ partial pressures. *Adv. Space Res.* **1994**, *14*(11), 171-176.
52. Donnelly, A.; Lawson, T.; Craigon, J.; Black, C. R.; Colls, J. J.; Landon, G. Effects of elevated CO₂ and O₃ on tuber quality in potato (*Solanum tuberosum* L.). *Agric. Ecosys. Environ.* **2001**, *87*(3), 273–285.
53. Vorne, V.; Ojanpera, K.; De Temmerman, L.; Bindi, M.; Högy, P.; Jones, M. B.; Lawson, T.; Persson, K. Effects of elevated carbon dioxide and ozone on potato tuber quality in the European multiple-site experiment ‘CHIP project’. *Eur. J. Agron.* **2002**, *17*(4), 369-381.
54. Högy, P.; Fangmeier, A. Atmospheric CO₂ enrichment affects potatoes: 2. Tuber quality traits. *Eur. J. Agron.* **2009**, *30*(2), 85-94
55. Fangmeier, A.; De Temmerman, L.; Black, C.; Persson, K.; Vorne, V. Effects of elevated CO₂ and/or ozone on nutrient concentrations and nutrient uptake of potatoes. *Eur. J. Agron.* **2002**, *17*(4), 353-368.
56. Mulchi, C. L.; Slaughter, L.; Saleem, M.; Lee, E. H.; R., P.; Rowland, R. Growth and physiological characteristics of soybean in open-top chambers in response to ozone and increased atmospheric CO₂. *Agr. Ecosyst. Environ.* **1992**, *38*(1-2), 107-118.

57. Jurgonski, L. J.; Smart, D. J.; Bugbee, B.; Nielsen, S. S. Controlled environments alter nutrient content of soybeans. *Adv. Space Res.* **1997**, *20*(10), 1979-1988.
58. Deepak, S. S.; Agrawal, M. Influence of elevated CO₂ on the sensitivity of two soybean cultivars to sulphur dioxide. *Environ. Exp. Bot.* **2001**, *46*(1), 81–91.
59. Bai, Y.; Wang, C.; Wen, M. Responses of soybean to O₃, CO₂ and their combination. *Chin. J. Appl. Ecol.* **2005**, *16*(3), 545-549.
60. Dong, G.; Wang, Y.; Huang, J.; Yang, H.; Gu, H.; Peng, B.; Ju, J.; Yang, L.; Zhu, J.; Shan, Y. Response of rice grain quality traits to free-air CO₂ enrichment. *Chin. J. Appl. Ecol.* **2004**, *15*(7), 1217-1222.
61. Terao, T.; Miura, S.; Yanagihara, T.; Hirose, T.; Nagata, K.; Tabuchi, H.; Kim, H. Y.; Lieffering, M.; Okada, M.; Kobayashi, K. Influence of free-air CO₂ enrichment (FACE) on the eating quality of rice. *J. Sci. Food Agric.* **2005**, *85*(11), 1861–1868.
62. Yang, L.; Wang, Y.; Dong, G.; Gu, H.; Huang, J.; Zhu, J.; Yang, H.; Liu, G.; Han, Y. The impact of free-air CO₂ enrichment (FACE) and nitrogen supply on grain quality of rice. *Field Crop Res.* **2007**, *102*(2), 128–140.
63. Fukayama, H.; Fukuda, T.; Masumoto, C.; Taniguchi, Y.; Sakai, H.; Cheng, W.; Hasegawa, T.; Miyao, M. Rice plant response to long term CO₂ enrichment: Gene expression profiling. *Plant Sci.* **2009**, *177*(3), 203–210.
64. Idso, S. B.; Kimball, B. A.; Shawb, P. E.; Widmer, W.; Vanderslice, J. T.; Higgs, D. J.; Montanari, A.; Clark, W. D. The effect of elevated atmospheric CO₂ on the vitamin C concentration of (sour) orange juice. *Agr. Ecosyst. Environ.* **2002**, *90*(1), 1-7.
65. Idso, S. B.; Kimball, B. A.; Hendrix, D. L. Effects of atmospheric CO₂ enrichment on chlorophyll and nitrogen concentrations of sour orange tree leaves. *Environ. Exp. Bot.* **1996**, *36*(3), 323-331.
66. Keutgen, N.; Chen, K. Responses of citrus leaf photosynthesis, chlorophyll fluorescence, macronutrient and carbohydrate contents to elevated CO₂. *J. Plant Physiol.* **2001**, *158*(10), 1307–1316.

67. Wheeler, R. M.; C. L. Mackowiak, S., G. W.; Yorio, N. C.; Berry, W. L. Effect of elevated carbon dioxide on nutritional quality of tomato. *Adv. Space Res.* **1997**, 20(10), 1975-1978.
68. Islam, M. S.; Matsui, T.; Yoshida, Y. Effect of carbon dioxide enrichment on physico-chemical and enzymatic changes in tomato fruits at various stages of maturity. *Sci. Hort.* **1996**, 65(2-3), 137-149.
69. Li, J.; Zhou, J.; Duan, Z. Q.; Du, C. W.; Wang, H. Y. Effect of CO₂ enrichment on the growth and nutrient uptake of tomato seedlings. *Pedosphere* **2007**, 17(3), 343-351.
70. Jain, V.; Pal, M.; Raj, A.; Khetarpal, S. Photosynthesis and nutrient composition of spinach and fenugreek grown under elevated carbon dioxide concentration. *Biol. Plantarum* **2007**, 51(3), 559-562.
71. McKeehen, J. D.; Smart, D. J.; Mackowiak, C. L.; Wheeler, R. M.; Nielsen, S. S. Effect of CO₂ levels on nutrient content of lettuce and radish. *Adv. Space Res.* **1996**, 18(4-5), 85-92.
72. Reddy, G. V. P.; Tossavainen, P.; Nerg, A. M.; Holopainen, J. K. Elevated Atmospheric CO₂ Affects the Chemical Quality of Brassica Plants and the Growth Rate of the Specialist, *Plutella xylostella*, but Not the Generalist, *Spodoptera littoralis*. *J. Agric. Food Chem.* **2004**, 52(13), 4185–4191.
73. Himanen, S. J.; Anne Nissinen, A.; Auriola, S.; Poppy, G. M.; Stewart Jr., C. N.; Holopainen, J. K.; Nerg, A. M. Constitutive and herbivore-inducible glucosinolate concentrations in oilseed rape (*Brassica napus*) leaves are not affected by *Bt Cry1Ac* insertion but change under elevated atmospheric CO₂ and O₃. *Planta* **2008**, 227(2), 427–437.
74. Högy, P.; Franzaring, J.; Schwadorf, K.; Breuer, J.; Schütze, W.; Fangmeier, A. Effects of free-air CO₂ enrichment on energy traits and seed quality of oilseed rape. *Agr. Ecosyst. Environ.* **2010**, 139(1-2), 239–244.
75. Wullschleger, S. D.; Norby, R. J.; Hendrix, D. L. Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment. *Tree Physiol.* **1992**, 10(1), 21-31.

76. Overdieck, D. Elevated CO₂ and the mineral content of herbaceous and woody plants. *Plant Ecol.* **1993**, 104-105(1), 403-411.
77. Liu, X.; Kozovits, A. R.; Grams, T. E. E.; Blaschke, H.; Rennenberg, H.; Matyssek, R. Competition modifies effects of enhanced ozone/carbon dioxide concentrations on carbohydrate and biomass accumulation in juvenile Norway spruce and European beech. *Tree Physiol.* **2004**, 24(9), 1045–1055.
78. Pal, M.; Karthikeyapandian, V.; Jain, V.; Srivastava, A. C.; Raj, A.; Sengupta, U. K. Biomass production and nutritional levels of berseem (*Trifolium alexandrium*) grown under elevated CO₂. *Agr. Ecosyst. Environ.* **2004**, 101(1), 31–38.
79. Barbehenn, R. V.; Chen, Z.; Karowe, D. N.; Spickard, A. C₃ grasses have higher nutritional quality than C₄ grasses under ambient and elevated atmospheric CO₂. *Global Change Biol.* **2004**, 10(9), 1565–1575.
80. Hattas, D.; Stock, W. D.; Mabusela, W. T.; Green, I. R. Phytochemical changes in leaves of subtropical grasses and fynbos shrubs at elevated atmospheric CO₂ concentrations. *Global Planet. Change* **2005**, 47(2-4), 181–192.
81. Hunt, M. G.; Rasmussen, S.; Newton, P. C. D.; Parsons, A. J.; Newman, J. A. Near-term impacts of elevated CO₂, nitrogen and fungal endophyte-infection on *Lolium perenne* L. growth, chemical composition and alkaloid production. *Plant Cell Environ.* **2005**, 28(11), 1345–1354.
82. Gleadow, R. M.; Edwards, E. J.; Evans, J. R. Changes in nutritional value of cyanogenic *Trifolium repens* grown at elevated atmospheric CO₂. *J. Chem. Ecol.* **2009**, 35(4), 476–478.
83. Schenk, U.; Jäger, H. J.; Weigel, H. J. The response of perennial ryegrass/white clover mini-swards to elevated atmospheric CO₂ concentrations: effects on yield and fodder quality. *Grass Forage Sci.* **1997**, 52(3), 232–241.
84. Wu, W. H.; Lu, J. Y.; Mortley, D. G.; Loretan, P. A.; Bonsi, C. K.; Hill, W. A. Proximate composition, amino acid profile, fatty acid composition, and mineral concentration of peanut seeds hydroponically grown at elevated CO₂ levels. *J. Agric. Food. Chem.* **1997**, 45(10), 3863–3866.

85. Bindi, M.; Fibbi, L.; Miglietta, F. Free air CO₂ enrichment (FACE) of grapevine (*Vitis vinifera* L.): II. growth and quality of grape and wine in response to elevated CO₂ concentrations. *Eur. J. Agron.* **2001**, *14*(2), 145–155.
86. Srivastava, A. C.; Pal, M.; Sengupta, U. K. Changes in Nitrogen Metabolism of *Vigna Radiata* in Response to Elevated CO₂. *Biol. Plantarum* **2002**, *45*(3), 395-399.
87. Agüera, E.; Ruano, D.; Cabello, P.; de la Haba, P. Impact of atmospheric CO₂ on growth, photosynthesis and nitrogen metabolism in cucumber (*Cucumis sativus* L.) plants. *J. Plant Physiol.* **2006**, *163*(8), 809-817.
88. Marinari, S.; Calfapietra, C.; De Angelis, P.; Mugnozza, G. S.; Grego, S. Impact of elevated CO₂ and nitrogen fertilization on foliar elemental composition in a short rotation poplar plantation. *Environ. Pollut.* **2007**, *147*(3), 507-515.
89. Erice, G.; Irigoyen, J. J.; Díaz, M. S.; Avice, J. S.; Ourry, A. Effect of drought, elevated CO₂ and temperature on accumulation of N and vegetative storage proteins (VSP) in taproot of nodulated alfalfa before and after cutting. *Plant Sci.* **2007**, *172*(5), 903–912.
90. Levine, L. H.; Paré, P. W. Antioxidant capacity reduced in scallions grown under elevated CO₂ independent of assayed light intensity. *Adv. Space Res.* **2009**, *44*(8), 887–894.
91. Manderscheid, R.; Pacholski, A.; Weigel, H. J. Effect of free air carbon dioxide enrichment combined with two nitrogen levels on growth, yield and yield quality of sugar beet: Evidence for a sink limitation of beet growth under elevated CO₂. *Eur. J. Agron.* **2010**, *32*(3), 228–239.
92. Mortensen, L. M. Review: CO₂ enrichment in greenhouses. Crop responses. *Sci Hortic.* **1987**, *33*(1-2), 1-25.
93. Conroy, J. P. Influence of elevated atmospheric CO₂ concentrations on plant nutrition. *Aust. J. Bot.* **1992**, *40*(5), 445 – 456.
94. Idso, S. B.; Idso, K. E. Effects of atmospheric CO₂ enrichment on plant constituents related to animal and human health. *Environ. Exp. Bot.* **2001**, *45*(2), 179–199.

95. Erda, L.; Wei, X.; Hui, J.; Yinlong, X.; Yue, L.; Liping, B.; Liyong, X. Climate change impacts on crop yield and quality with CO₂ fertilization in China. *Phil. Trans. R. Soc. B.* **2005**, 360(1463), 2149-2154.
96. Taub, D. R.; Miller, B.; Allen, H. Effects of elevated CO₂ on the protein concentration of food crops: a meta-analysis. *Global Change Biol.* **2008**, 14(3), 565–575.
97. Moretti, C. L.; Mattos, L. M.; Calbo, A. G.; Sagent, S. A. Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: a review. *Food Res. Int.* **2010**, 43(7), 1824–1832.
98. AOAC International. Association of Official Analytical Chemists. Official methods of analysis. 17th ed.: Washington, DC., **2000**.
99. Allen, S. E.; Grimshaw, H. M.; Rowland, A. P. Chemical Analysis, In; Methods in Plant ecology, Moore, P. D., Chapman, S. B., Editors. Blackwell Scientific Publication, Oxford: London, **1986**. pp. 285-344.
100. Thybo, A. K.; Edelenbos, M.; Christensen, L. P.; Sørensen, J. N.; Thorup-Kristensen, K. Effect of organic growing systems on sensory quality and chemical composition of tomatoes. *LWT-Food Sci. Technol.* **2006**, 39, 835–843.
101. Guil-Guerrero, J. L.; Reboloso-Fuentes, M. M. Nutrient composition and antioxidant activity of eight tomato (*Lycopersicon esculentum*) varieties. *J. Food. Compos. Anal.* **2009**, 22, 123–129.
102. Özçelik, N.; Akilli, M. Effects of CO₂ enrichment on vegetative growth, yield and quality of greenhouse - grown tomatoes in soil and soilless cultures. In; ISHS Acta Horticulturae 491: International Symposium Greenhouse Management for Better Yield & Quality in Mild Winter Climates. **1999**.
103. Reinert, R. A.; Eason, G.; Barton, J. Growth and fruiting of tomato as influenced by elevated carbon dioxide and ozone. *New Phytol.* **1997**, 137, 411-420.
104. Li, F.; Chen, Y.; Wang, X.; Yue, M. Combined effects of enhanced UV-B radiation and doubled CO₂ on tomato growth and its fruit quality. *Chin. J. Appl. Ecol.* **2006**, 17(1), 71-74.

105. Bruggink, G. T. Effects of CO₂ concentration on growth and photosynthesis of young tomato and carnation plants. In; ISHS Acta Horticulturae 162: Symposium on CO₂ Enrichment. **1984**.
106. Taub, D. R.; Wang, X. Why are Nitrogen Concentrations in Plant Tissues Lower under Elevated CO₂? A Critical Examination of the Hypotheses. *J. Integr. Plant Biol.* **2008**, 50(11), 1365–1374.
107. Fangmeier, A.; Chrost, B.; Högy, P.; Krupinska, K. CO₂ enrichment enhances flag leaf senescence in barley due to greater grain nitrogen sink capacity. *Environ. Exp. Bot.* **2000**, 44, 151-164.
108. Suárez, M. H.; Rodríguez, E. M. R.; Romero, C. D. Chemical composition of tomato (*Lycopersicon esculentum*) from Tenerife, the Canary Islands. *Food Chem.* **2008**, 106, 1046-1056.
109. Dalal, K. B.; Sallunkhe, D. K.; Boe, A. A.; Olson, L. E. Certain physiological and biochemical changes in the developing tomato fruit (*Lycopersicon esculentum* Mill.). *J. Food Sci.* **1965**, 30(3), 504-508.
110. USDA, United States Department of Agriculture, **2007**. (www.fns.usda.gov/fdd).
111. McCance and Widdowson's The Composition of Foods, Sixth summary edition. Food Standards Agency.: Royal Society of Chemistry, Cambridge, **2002**.
112. Burr, G. O.; Burr, M. M.; Miller, E. On the nature and role of the fatty acids essential in nutrition. *J. Biol. Chem.* **1930**, 86, 587-589.
113. Howard, L. R.; Talcott, S. T.; Brenes, C. H.; B., V. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J. Agric. Food. Chem.* **2000**, 48, 1713-1720.
114. Donald, G.; Barceloux, M. D. Pepper and Capsaicin (*Capsicum* and *Piper* Species), In; Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Plants, and Venomous Animals, Barceloux, D. G., Editor. John Wiley & Sons, Inc.: Hoboken, New Jersey, USA, **2008**. pp. 71-76.
115. Furlan, R. A.; Rezende, F. C.; Alves, D. R. B.; Folegatti, M. V. Effect of irrigation water depth and CO₂ application on sweet pepper yield cv. Mayata in plastic greenhouse. *Hortic. bras.* **2002**, 20(4), 547-550.

116. Penuelas, J.; Biel, C.; Estiarte, M. Growth, biomass allocation, and Phenology responses of pepper to elevated CO₂ concentrations and different water and nitrogen supply. *Photosynthetica* **1995**, 31(1), 91-99.
117. Cook, J. A.; VanderJagt, D. J.; Pastuszyn, A.; Mounkaila, G.; Glew, R. S.; Millson, M.; Glew, R. H. Nutrient and chemical composition of 13 wild plant foods of Niger. *J. Food Comp. Anal.* **2000**(13), 83-92.
118. Gifford, R. M.; Barrett, D. J.; Lutze, J. L. The effects of elevated [CO₂] on the C:N and C:P mass ratios of plant tissues. *Plant. Soil* **2000**, 224(1–14).
119. Martinez, S.; Curros, A.; Bermudez, J.; Carballo, J.; Franco, I. The composition of Aronia peppers (*Capsicum annum* L.) at different stages of maturity. *Int. J. Food Sci. Nutr.* **2007**, 58(2), 150-161.
120. Simonne, A. H.; Simonne, E. H.; Eitenmiller, R. R.; Mills, H. A.; Green, N. R. Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annum* L.). *J. Food Comp. Anal.* **1997**, 10, 299–311.
121. Hägg, M.; Ylikoski, S.; Kumpulainen, J. Vitamin C and alfa- and beta-carotene contents in vegetables consumed in Finland during 1988-1989 and 1992-1993. *J. Food Comp. Anal.* **1994**, 7(252-259).
122. Villanueva, M. J.; Tenorio, M. D.; Esteban, M. A.; Mendoza, M. C. Compositional changes during ripening of two cultivars of muskmelon fruits. *Food Chem.* **2004**, 87, 179-185.
123. Ketiku, A. O. The chemical composition of nigerian onions (*Allium cepa*, linn). *Food Chem.* **1976**, 1(1), 41-47.
124. Block, E. The chemistry of garlic and onions. *Sci. Am.* **1985**, 252(3), 114-119.
125. Jasoni, R.; Kane, C.; Green, C.; Peffley, E.; Tissue, D.; Thompson, L.; Payton, P.; Paré, P. W. Altered leaf and root emissions from onion (*Allium cepa* L.) grown under elevated CO₂ conditions. *Environ. Exp. Bot.* **2004**, 51, 273-280.
126. Mortensen, L. M. Effects of elevated CO₂ concentrations on growth and yield of eight vegetable species in a cool climate. *Sci. Hort.* **1994**, 58, 177-185.
127. Mota, C. L.; Luciano, C.; Dias, A.; Barroca, M. J.; F., G. R. P. Convective drying of onion: Kinetics and nutritional evaluation. *Food Bioprod. Process.* **2010**, 88, 115-123.

128. Thompson, L.; Peffley, E.; Green, C.; Paré, P.; Tissue, D. Biomass, flavonol levels and sensory characteristics of *Allium* cultivars grown hydroponically at ambient and elevated CO₂. SAE International, **2004**.
129. Conn, J. S.; Cochran, V. L. Response of potato (*Solanum tuberosum* L.) to elevated atmospheric CO₂ in the North American Subarctic. *Agric. Ecosys. Environ.* **2006**, *112*, 49–57.
130. Jacob, J.; Greitner, C.; Drake, B. G. Acclimation of photosynthesis in relation to Rubisco and nonstructural carbohydrate contents and in situ carboxylase activity in *Scirpus olneyi* grown at elevated CO₂ in the field. *Plant Cell Environ* **1995**, *18*, 875-884.
131. Kahane, R.; Vialle-Guérin, E.; Boukema, I.; Tzanoudakis, D.; Bellamy, C.; Chamaux, C.; Kik, C. Changes in non-structural carbohydrate composition during bulbing in sweet and high-solid onions in field experiments. *Environ. Exp. Bot.* **2001**, *45*, 73-83.
132. Bibak, A.; Behrens, A.; Sturup, S.; Knudsen, L.; Gundersen, V. Concentrations of 63 major and trace elements in Danish agricultural crops measured by Inductively Coupled Plasma Mass Spectrometry. 1. Onion (*Allium cepa* Hysam). *J. Agric. Food Chem.* **1998**, *46*, 3139–3145.
133. Tsiagani, M. C.; Laskari, K.; Melissari, E. Fatty acid composition of *Allium* species lipids. *J. Food Comp. Anal.* **2006**, *19*, 620–627.
134. Tindall, H. D. Commercial Vegetable Growing. Oxford University Press, Oxford, UK, **1968**.
135. Calisir, S.; Ozcan, M.; Haciseferogullari, H.; Yidiz, M. U. A study on some physico-chemical properties of Turkey okra (*Hibiscus esculenta*, L.) seeds. *J. Food Eng.* **2005**, *68*, 73–78.
136. Mortensen, E.; Bullard, E. T. Handbook of Tropical and Subtropical Horticulture. AID Publisher, Washington DC, USA, **1984**.
137. Sionit, N.; Strain, B. R.; Beckford, H. A. Environmental Controls on the Growth and Yield of Okra. I. Effects of Temperature and of CO₂ Enrichment at Cool Temperature. *Crop Sci.* **1981**, *21*, 885-888.

138. Longe, O. G.; Fetuga, B. L.; Akenova, M. E. Changes in the composition and carbohydrate constituents of okra (*Abelmoschus esculentus*, linn.) with age. *Food. Chem.* **1982**, 8, 27-32.
139. Camciuc, M.; Deplagne, M.; Vilarem, G.; Gaset, A. Okra—*Abelmoschus esculentus* L. (Moench.) a crop with economic potential for set aside acreage in France. *Ind. Crop. Prod.* **1998**, 7, 257–264.
140. Zaidi, A. A.; Iahi, A.; Tanvir, R.; Ruby, S. Persistence of dichlorvos residues in okra and its effects on sugar content of the vegetable. *Pak. J. Agri. Sci.* **1993**, 30(2), 224-227.
141. Olaniyi, J. O.; Akanbi, W. B.; Olaniran, O. A.; Ilupeju, O. T. The effect of organo-mineral and inorganic fertilizers on the growth, fruit yield, quality and chemical compositions of okra. *J. Anim. Plant Sci.* **2010**, 9(1), 1135- 1140.
142. Babarinde, G. O.; Fabunmi, O. A. Effects of packaging materials and storage temperature on quality of fresh okra (*Abelmoschus esculentus*) fruit. *Agricultura Tropica Et Subtropica* **2009**, 42(4), 151-156.
143. Berry, S. K. The fatty acid composition and cyclopropene fatty acid content of the maturing okra (*Hibiscus esculentus* L.) fruits. *Pertanika* **1980**, 3(2), 82-86.
144. Miao, M.; Xu, X.; Chen, X.; Xue, L.; Cao, B. Cucumber carbohydrate metabolism and translocation under chilling night temperature. *J. Plant. Physiol.* **2007**, 164(5), 621-628.
145. Murray, M.; Pizzorno, J. E.; Pizzorno, L. U. The Encyclopaedia of Healing Foods. Atria Books, New York, USA, **2005**.
146. Luomala, E. M.; Särkkä, L.; Kaukoranta, T. Altered plant structure and greater yield of cucumber grown at elevated CO₂ in a semi-closed greenhouse. In; ISHS Acta Horticulturae 801: International Symposium on High Technology for Greenhouse System Management: Greensys2007. **2008**.
147. Agüera, E.; Ruano, D.; Cabello, P.; de la Hab, P. Impact of atmospheric CO₂ on growth, photosynthesis and nitrogen metabolism in cucumber (*Cucumis sativus* L.) plants. *J. Plant. Physiol.* **2006**, 163, 809-817.
148. Akilli, M.; Özmerzi, A.; Ercan, N. Effect of CO₂ enrichment on yield of some vegetables grown in greenhouses. *ISHS Acta Horticulturae 534: International*

Conference and British-Israeli Workshop on Greenhouse Techniques towards the 3rd Millennium 2000.

149. Ruiz, J. M.; Romero, L. Commercial yield and quality of fruits of cucumber plants cultivated under greenhouse conditions: Response to increases in nitrogen fertilization. *J. Agric. Food Chem.* **1998**, *46*, 4171-4173.
150. Widders, I. E.; Kwantes, M. Ontogenic changes in seed weight and carbohydrate composition as related to growth of cucumber (*Cucumis sativus* L.) fruit. *Sci Hortic.* **1995**, *63*, 155-165.
151. Du, Y. C.; Tachibana, S. Effect of supraoptimal root temperature on the growth, root respiration and sugar content of cucumber plants. *Sci Hortic.* **1994**, *58*, 289-301.
152. Fishwick, M. J.; Wright, A. J.; Galliard, T. Quantitative composition of the lipids of cucumber fruit (*Cucumis sativus*). *J. Sci. Food Agric.* **1977**, *28*, 394-398.
153. Draper, S. R. Lipid changes in senescing cucumber cotyledons. *Phytochemistry* **1969**, 1641-1647.
154. Prakash, D.; Pal, M. Nutritional and antinutritional comparison of vegetable and grain *Amaranthus* leaves. *J. Sci. Food. Agric.* **1991**, *57*, 573–585.
155. Torres-Sanchez, L.; Lopez-Carillio, L.; Lopez-Cervantes, M.; Rueda-Neria, C.; Wolff, M. S. Food sources of phytoestrogens and breast cancer risk in Mexican women. *Nutr. Cancer* **2000**, *37*(2), 134-139.
156. Castenmiller, J. J. M.; van de Poll, C. J.; West, C. E.; Brouwer, I. A.; Thomas, C. M. G.; van Dusseldorp, M. Bioavailability of folate from processed spinach in humans. *Ann. Nutr. Metab.* **2000**, *44*(4), 163-169.
157. Holbrook, G. P.; Hansen, J.; Wallick, K.; Zinnen, T. M. Starch accumulation during hydroponic growth of spinach and basil plants under carbon dioxide enrichment. *Environ. Exp. Bot.* **1993**, *33*(2), 313-321.
158. Singh, G.; Kawatra, A.; Sehgal, S. Nutritional composition of selected green leafy vegetables, herbs and carrots. *Plant Food Hum. Nutr.* **2001**, *56*, 359–364.
159. Bergquist, S. A.; Gertsson, U. E.; Olsson, M. E. Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (*Spinacia oleracea* L.). *J. Sci. Food. Agric.* **2006**, *86*, 346–355.

160. McDermid, K. J.; Stuercke, B. Nutritional composition of edible Hawaiian seaweeds. *J. Appl. Phycol.* **2003**, *15*, 513–524.
161. Constantopoulos, J.; Kenyon, C. N. Release of free fatty acids and loss of hill activity by aging spinach chloroplasts. *Plant Physiol.* **1968**, *43*, 531-536.
162. Theodosiou, M.; Laudet, V.; Schubert, M. From carrot to clinic: an overview of the retinoic acid signaling pathway. *Cell Mol. Life Sci.* **2010**, *67*(9), 1423-1445.
163. Idso, S. B.; Kimball, B. A.; Anderson, M. G.; Mauney, J. R. Effects of atmospheric CO₂ enrichment on plant growth: the interactive role of air temperature. *Agric. Ecosys. Environ.* **1987**, *20*, 1-10.
164. Idso, S. B.; Kimball, B. A.; Mauney, J. R. Effects of atmospheric CO₂ enrichment on root:shoot ratios of carrot, radish, cotton and soybean. *Agric. Ecosys. Environ.* **1988**, *21*, 293-299.
165. Wheeler, T. R.; Morison, J. I. L.; Ellis, R. H.; Hadley, P. The effects of CO₂, temperature and their interaction on the growth and yield of carrot (*Daucus carota* L.). *Plant Cell Environ.* **1994**, *17*(12), 1275–1284.
166. Bao, B.; Chang, K. C. Carrot pulp chemical composition, color, and water-holding capacity as affected by blanching. *J. Food Sci.* **1994**, *59*(6), 1159-1161.
167. Brunsgaard, G.; Kidmose, U.; Sorensen, L.; Kaack, K.; Eggum, B. O. The influence of variety and growth conditions on the nutritive value of carrots. *J. Sci. Food Agric.* **1994**, *65*, 163-170.
168. Chu, C. C.; Coleman, J. S.; Mooney, H. A. Controls of biomass partitioning between roots and shoots: Atmospheric CO₂ enrichment and the acquisition and allocation of carbon and nitrogen in wild radish. *Oecologia* **1992**, *89*, 580-587.
169. Phan, C. T.; Hsu, H. Physical and chemical changes occurring in the carrot root during growth. *Can. J. Plant Sci.* **1973**, *53*, 629-634.
170. Dutta, P. C.; Appelqvist, L. A. Lipids and fatty acid patterns in developing seed, leaf, root, and in tissue culture initiated from embryos of *Daucus carota* L. *Plant Sci.* **1991**, *75*, 177--183.
171. Deshpande, U. S.; Deshpande, S. S. Legumes, In: Production, technology, and human nutrition, Salunkhe, D. K., Deshpande, S. S., Editors. Van Nostrand Reinhold: New York, USA, **1991**. pp. 137–300.

172. Moriyama, M.; Oba, K. Comparative study on the vitamin C contents of the food legume seeds. *J. Nutr. Sci. Vitaminol (Tokyo)* **2008**, *54*(1), 1-6.
173. Ismail, A.; Tiong, N. W.; Tan, S. T.; Azlan, A. Antioxidant properties of selected non-leafy vegetables. *Nutr. Food Sci.* **2009**, *39*(2), 176-180.
174. Hernández-Ramírez, R. U.; Galván-Portillo, M. V.; Ward, M. H.; Agudo, A.; González, C. A.; Oñate-Ocaña, L. F.; Herrera-Goepfert, R.; Palma-Coca, O.; López-Carrillo, L. Dietary intake of polyphenols, nitrate and nitrite and gastric cancer risk in Mexico City. *Int. J. Cancer* **2009**, *125*(6), 1424-1430.
175. Bunce, J. A. Variation in growth stimulation by elevated carbon dioxide in seedlings of some C₃ crop and weed species. *Global Change Biol.* **1997**, *3*, 61-66.
176. Xu, D. Q.; Gifford, R. M.; Chow, W. S. Photosynthetic Acclimation in Pea and Soybean to High Atmospheric CO₂ Partial Pressure. *Plant Physiol.* **1994**, *106*, 661-671
177. Paez, A.; Hellmers, H.; Strain, B. R. CO₂ effects on apical dominance in *Pisum sativum*. *Physiol. Plantarum* **1980**, *50*(1), 43-46.
178. de Almeida Costa, G. E.; da Silva Queiroz-Monici, K.; Reis, S. M. P. M.; de Oliveira, A. C. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food. Chem.* **2006**, *94*, 327-330.
179. Nikolopoulou, D.; Grigorakis, K.; Stasini, M.; Alexis, M. N.; Iliadis, K. Differences in chemical composition of field pea (*Pisum sativum*) cultivars: Effects of cultivation area and year. *Food. Chem.* **2007**, *103*, 847-852.
180. Bhatti, R. S.; Christison, G. I. Composition and nutritional quality of pea (*Pisum sativum* L.), faba bean (*Vicia faba* L. spp. minor) and lentil (*Lens culinaris* Medik.) meals, protein concentrates and isolates. *Qual. Plant. Plant. Foods Hum. Nutr.* **1984**, *34*, 41-51.
181. Grela, E. R.; Gunter, K. D. Fatty acid composition and tocopherol content of some legume seeds. *Anim. Feed Sci. Tech.* **1995**, *52*, 325-331.
182. Hanif, R.; Iqbal, Z.; Iqbal, M.; Hanif, S.; Rasheed, M. Use of vegetables as nutritional food: Role in human health. *Journal of Agricultural and Biological Science* **2006**, *1*(1), 18-22.

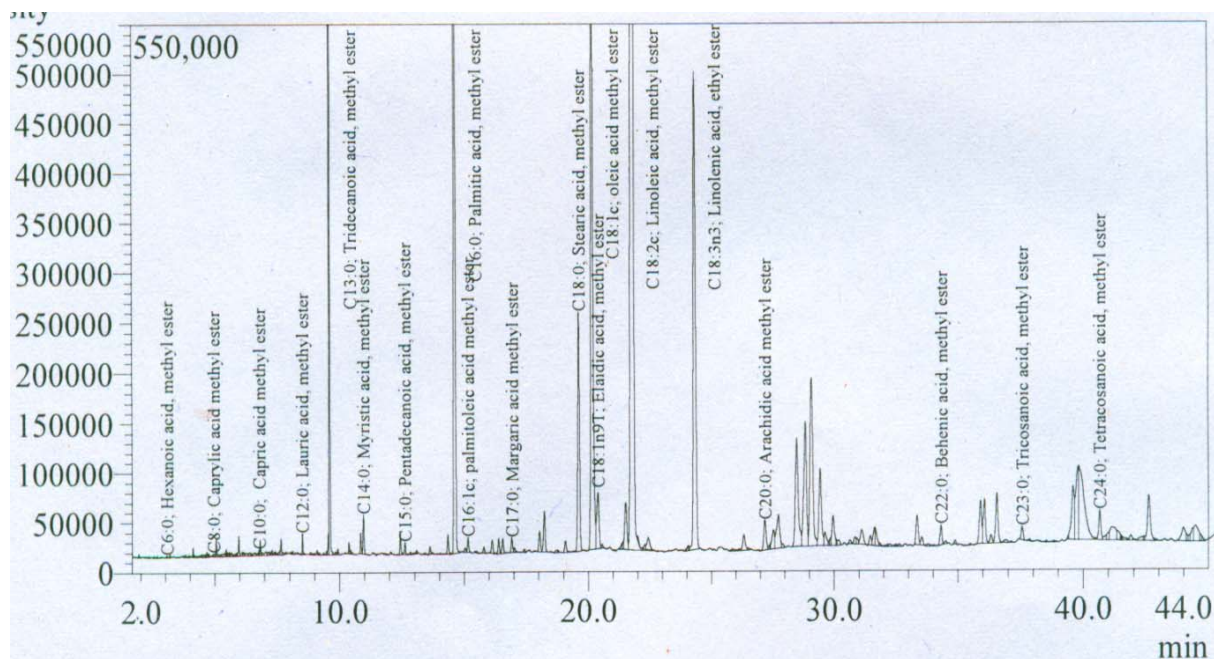
183. Ambrosone, C. B.; Tang, L. Cruciferous vegetable intake and cancer prevention: role of nutrigenetics. *Cancer Prev. Res. (Phila)* **2009**, 2(4), 298-300.
184. Wheeler, T. R.; Ellis, R. H.; Hadley, P.; Morison, J. I. L. Effects of CO₂, temperature and their interaction on the growth, development and yield of cauliflower (*Brassica oleracea* L. botrytis) *Sci. Hort.* **1995**, 60, 181-197.
185. Abe, M.; Kanechi, M.; Inagaki, N.; Taniguchi, M.; Maekawa, S. Effects of CO₂ enrichment on the growth and the photosynthetic rate of in vitro cauliflower plantlets. In; ISHS Acta Horticulturae 393: Environmental Effects and their Control in Plant Tissue Culture. **1995**.
186. Kanechi, M.; Ochi, M.; Abe, M.; Inagaki, N.; Maekawa, S. The effects of carbon dioxide enrichment, natural ventilation, and light intensity on growth, photosynthesis, and transpiration of cauliflower plantlets cultured in vitro photoautotrophically and photomixotrophically. *J. Amer. Soc. Hort. Sci.* **1998**, 123(2), 176-181.
187. Wright, L. C.; McMurchie, E. J.; Pomeroy, M. K.; Raison, J. K. Thermal behavior and lipid composition of cauliflower plasma membranes in relation to ATPase activity and chilling sensitivity. *Plant Physiol.* **1982**, 69, 1356-1360.
188. Lu, Z. L.; Liu, L. W.; Li, X. Y.; Gong, Y. Q.; Hou, X. L.; Zhu, X. W.; Yang, J. L.; Wang, L. Z. Analysis and evaluation of nutritional quality in chinese radish (*Raphanus sativus* L.). *Agricultural Sciences in China* **2008**, 7(7), 823-830
189. Curtis, I. S. The noble radish: past, present and future. *Trends Plant Sci.* **2003**, 8(7), 305-307.
190. Ramulu, P.; Rao, P. U. Total, insoluble and soluble dietary fiber contents of Indian fruits. *J. Food. Comp. Anal.* **2003**, 16(6), 677-685.
191. Richards, J. T.; Edney, S. L.; Yorio, N. C.; Stutte, G. W.; Cranston, N.; Wheeler, R. M.; Goins, G. D. Effects of Lighting Intensity and Supplemental CO₂ on Yield of Potential Salad Crops for ISS. SAE Paper# 2004-01-2296. In; 34th International Conferences on Environmental Systems, Colorado Springs. Colorado. **2004**.
192. Usuda, H. Evaluation of the effect of photosynthesis on biomass production with simultaneous analysis of growth and continuous monitoring of CO₂ exchange in

- the whole plants of radish, cv kosena under ambient and elevated CO₂. *Plant Prod. Sci.* **2004**, 7(4), 386-396.
193. Usuda, H. Effects of elevated CO₂ on the capacity for photosynthesis of a single leaf and a whole plant, and on growth in a radish. *Plant Cell Physiol.* **2006**, 47(2), 262-269.
 194. Mandal, S.; Yadav, S.; Singh, R.; Begum, G.; Suneja, P.; M. Singh, M. Correlation studies on oil content and fatty acid profile of some Cruciferous species. *Genet. Resour. Crop Ev.* **2002**, 49, 551–556.
 195. Lyimo, M. H.; Nyagwegwe, S.; Mnkeni, A. P. Investigations on the effect of traditional food processing, preservation and storage methods on vegetable nutrients: a case study in Tanzania. *Plant Food Hum. Nutr.* **1991**, 41(1), 53-57.
 196. Mosha, T. C.; Gaga, H. E. Nutritive value and effect of blanching on the trypsin and chymotrypsin inhibitor activities of selected leafy vegetables. *Plant Food Hum. Nutr.* **1999**, 54, 271–283.
 197. Shattuck, V. I.; Kakuda, Y.; Shelp, B. J.; Kakuda, N. Chemical composition of turnip roots stored or intermittently grown at low temperature. *J. Amer. Soc. Hort. Sci.* **1991**, 116(5), 818-822.
 198. Termine, E.; Lairon, D.; Letage, B. T.; Gautier, S.; Lafont, R.; Lafont, H. Yield and content in nitrates, minerals and ascorbic acid of leeks and turnips grown under mineral or organic nitrogen fertilizations. *Plant Food Hum. Nutr.* **1987**, 37, 321-332.
 199. Chen, B. Y.; Heneen, W. K. Fatty acid composition of resynthesized *Brassica napus* L., *B. campestris* L. and *B. alboglabra* Bailey with special reference to the inheritance of erucic acid content. *Heredity* **1989**, 63, 309-314.
 200. Flick, G. J.; Burnette, F. S.; Aung, L. H.; Ory, R. L.; Angelo, A. S. Chemical composition and biochemical properties of mirlitons (*Sechium edule*) and purple, green, and white eggplants (*Solanum melongena*). *J. Agric. Food Chem.* **1978**, 26, 1000–1005.
 201. Raigón, M. D.; Prohens, J.; Muñoz-Falcón, J. E.; Nuez, F. Comparison of eggplant landraces and commercial varieties for fruit content of phenolics, minerals, dry matter and protein. *J. Food. Comp. Anal.* **2008**, 21(5), 370-376

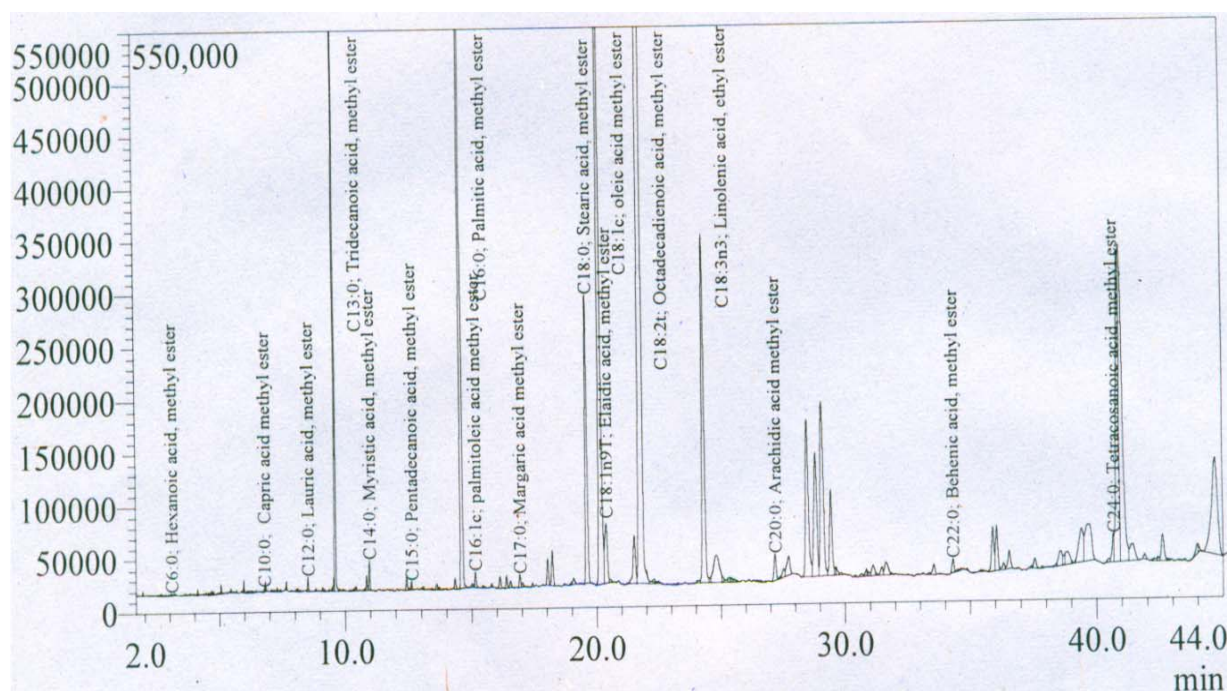
- 202. Noda, Y.; Kneyuki, T.; Igarashi, K.; Mori, A.; Packer, L. Antioxidant activity of nasunin, an anthocyanin in eggplant peels. *Toxicology* **2000**, *148*(2-3), 119-123.
- 203. Jorge, P. A.; Neyra, L. C.; Osaki, R. M.; de Almeida, E.; Bragagnolo, N. Effect of eggplant on plasma lipid levels, lipidic peroxidation and reversion of endothelial dysfunction in experimental hypercholesterolemia. *Arq. Bras. Cardiol.* **1998**, *70*(2), 87-91.
- 204. Flick, G. J.; Burnette, F. S.; Aung, L. H.; Ory, R. L.; St. Angelo, A. J. Chemical composition and biochemical properties of mirlitons (*Sechium edule*) and purple, green, and white eggplants (*Solanum melongena*). *J. Agric. Food Chem.* **1978**, *26*(5), 1000-1005.
- 205. Esteban, R. M.; Molla, E.; Villarroya, M. B.; Lopex-Andreu, F. J. Changes in the chemical composition of eggplant fruits during storage. *Sci. Hort.* **1989**, *41*, 19-25.
- 206. Kenrick, J. R.; Bishop, D. G. The fatty acid composition of phosphatidylglycerol and sulfoquinovosyldiacylglycerol of higher plants in relation to chilling sensitivity. *Plant Physiol.* **1986**, *81*, 946-949.

Appendix

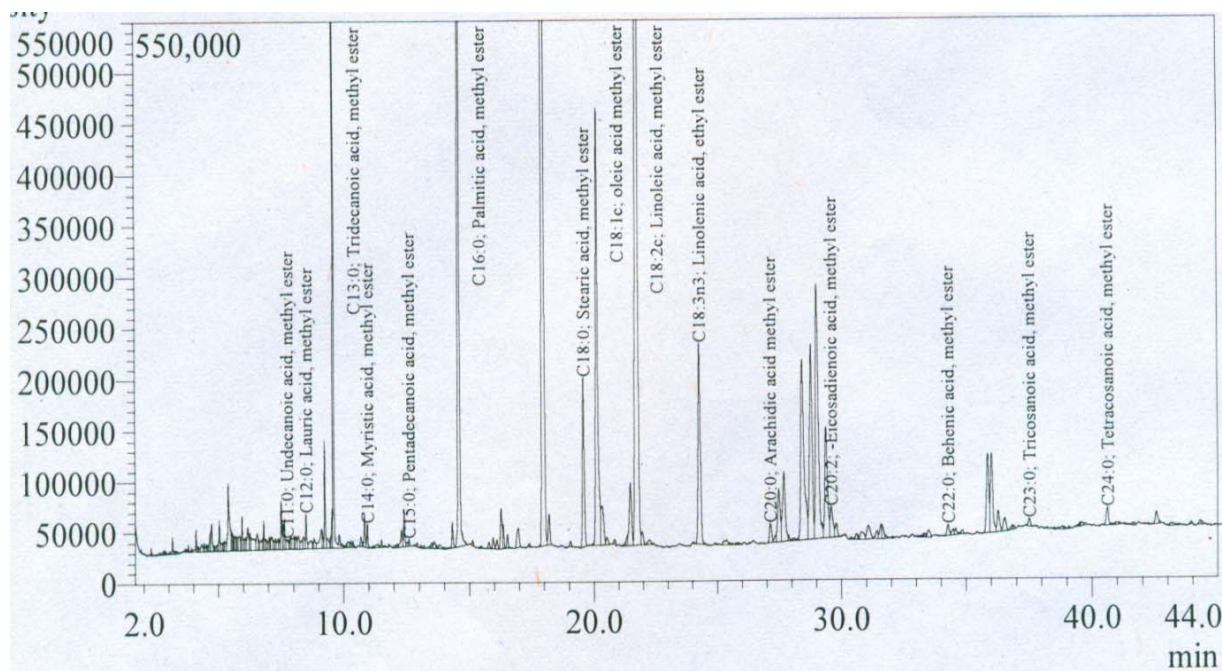
Appendix 1.1. Fatty acid profile of tomato (Eureka) grown at ambient level of CO₂



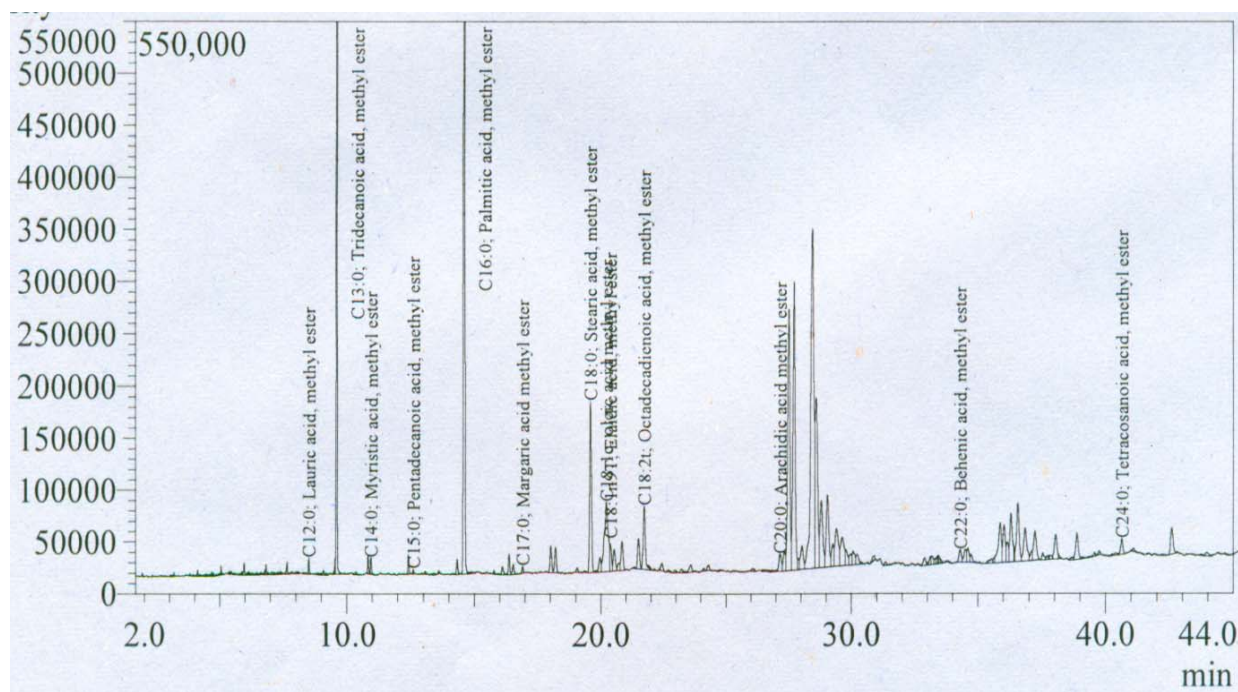
Appendix 1.2. Fatty acid profile of tomato (Eureka) grown at elevated level of CO₂



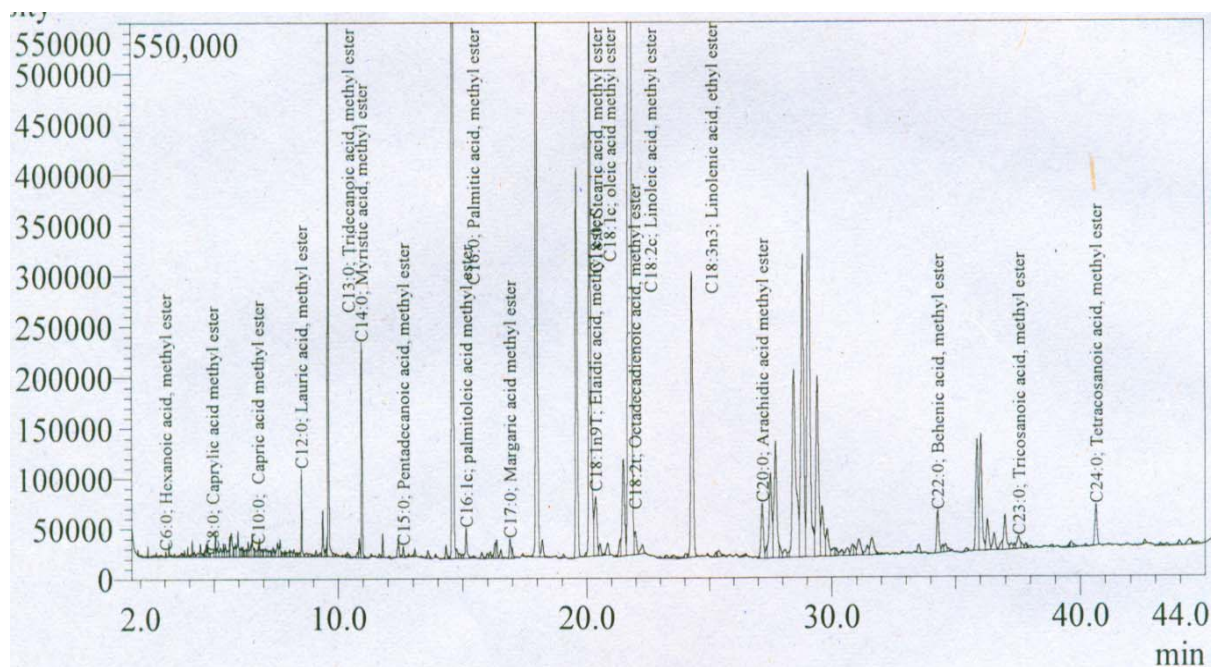
Appendix 1.3. Fatty acid profile of tomato (Astra) grown at ambient level of CO₂



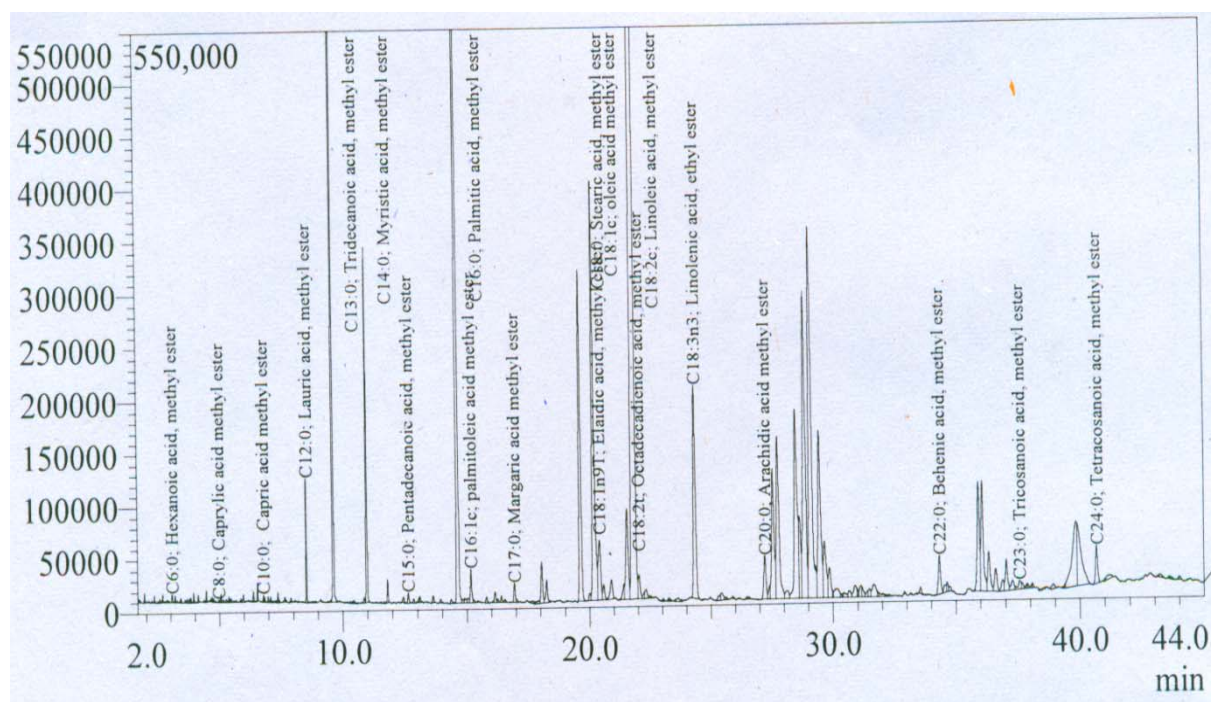
Appendix 1.4. Fatty acid profile of tomato (Astra) grown at elevated level of CO₂



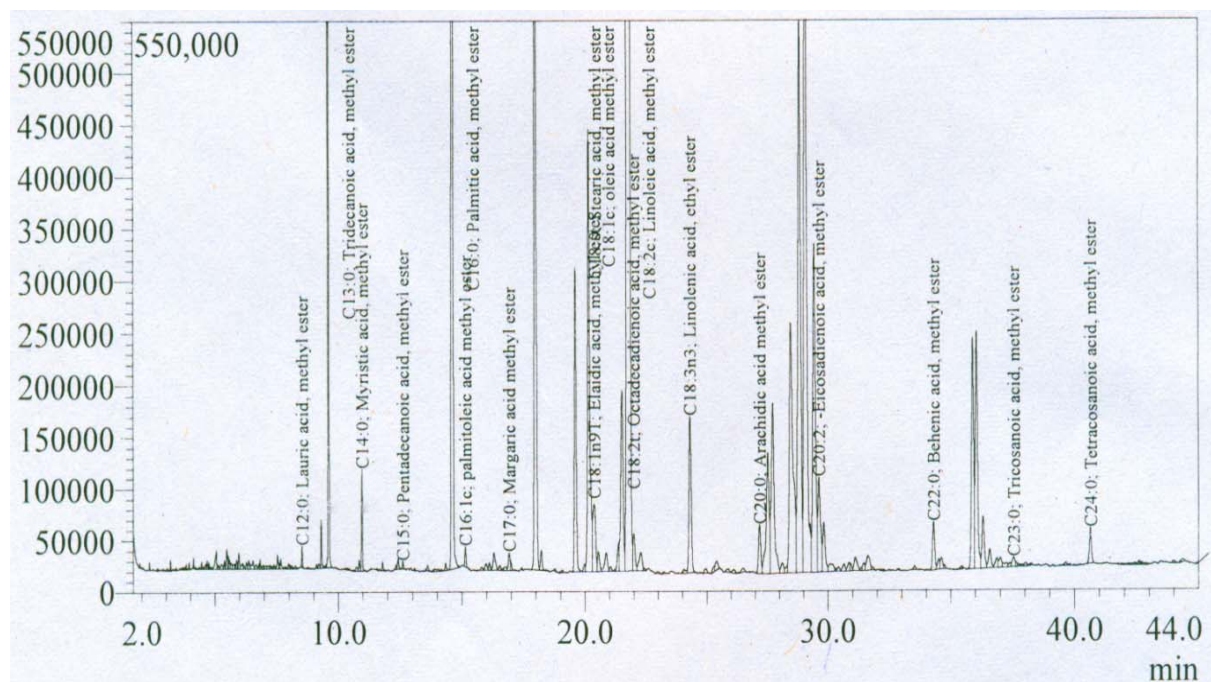
Appendix 1.5. Fatty acid profile of chili (99PE-1689) grown at ambient level of CO₂



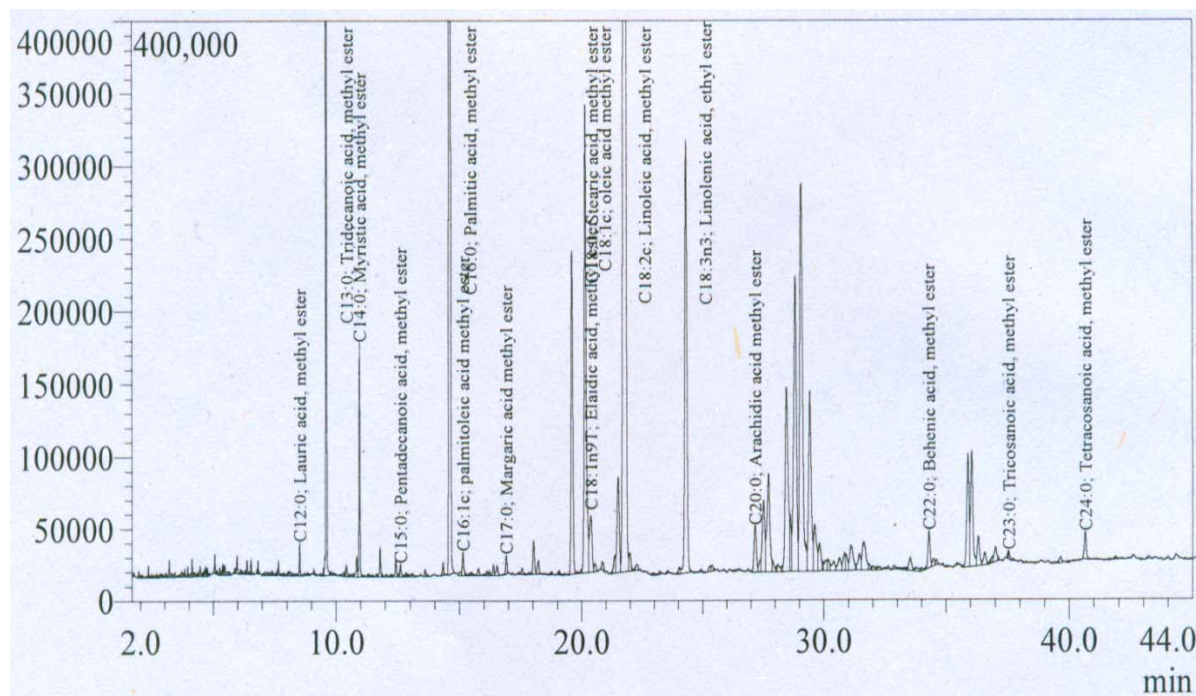
Appendix 1.6. Fatty acid profile of chili (99PE-1689) grown at elevated level of CO₂



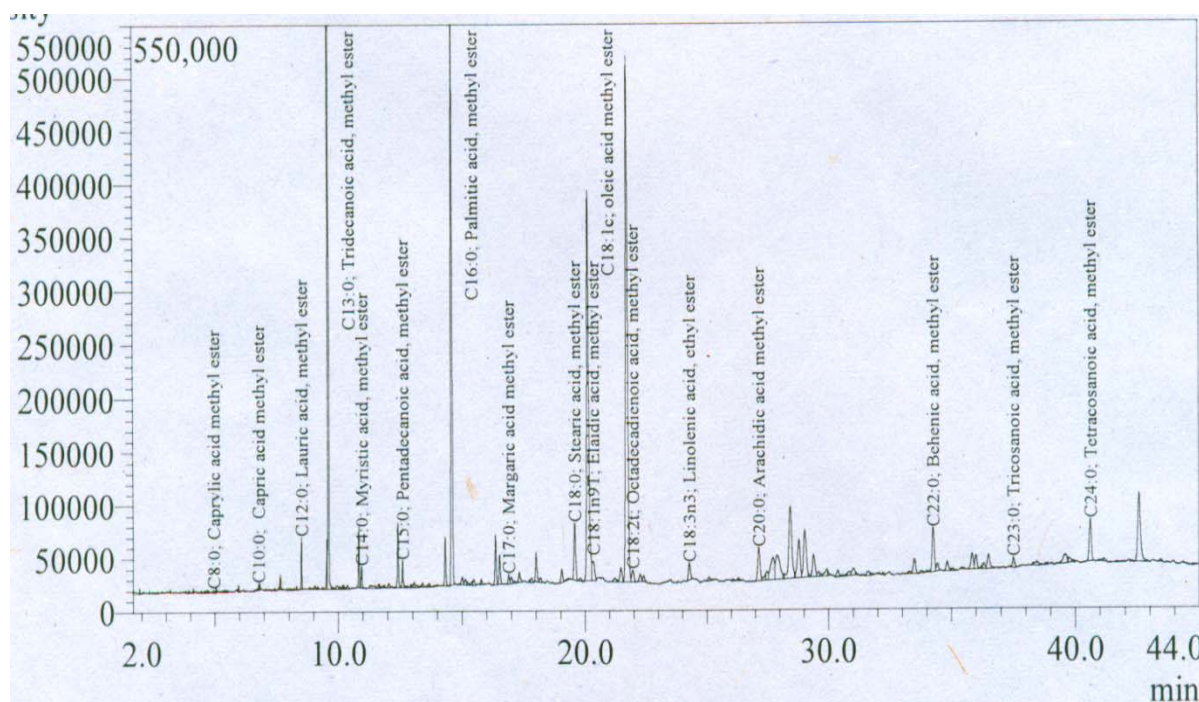
Appendix 1.7. Fatty acid profile of chili (Best Choice) grown at ambient level of CO₂



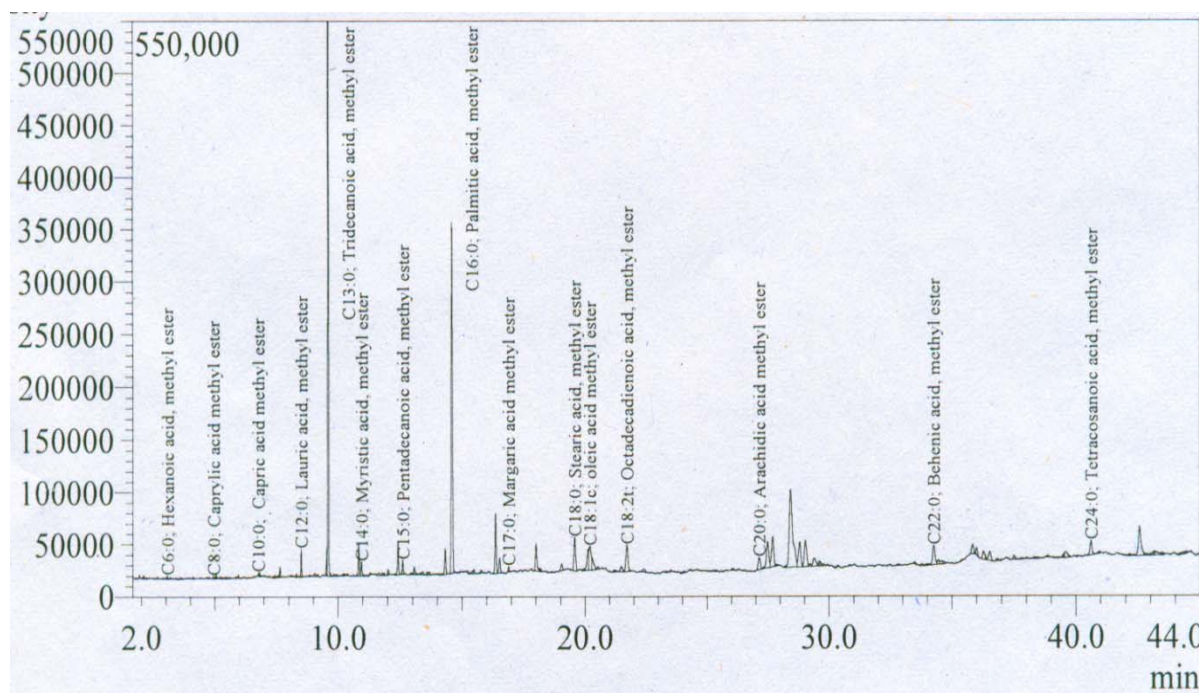
Appendix 1.8. Fatty acid profile of chili (Best Choice) grown at elevated level of CO₂



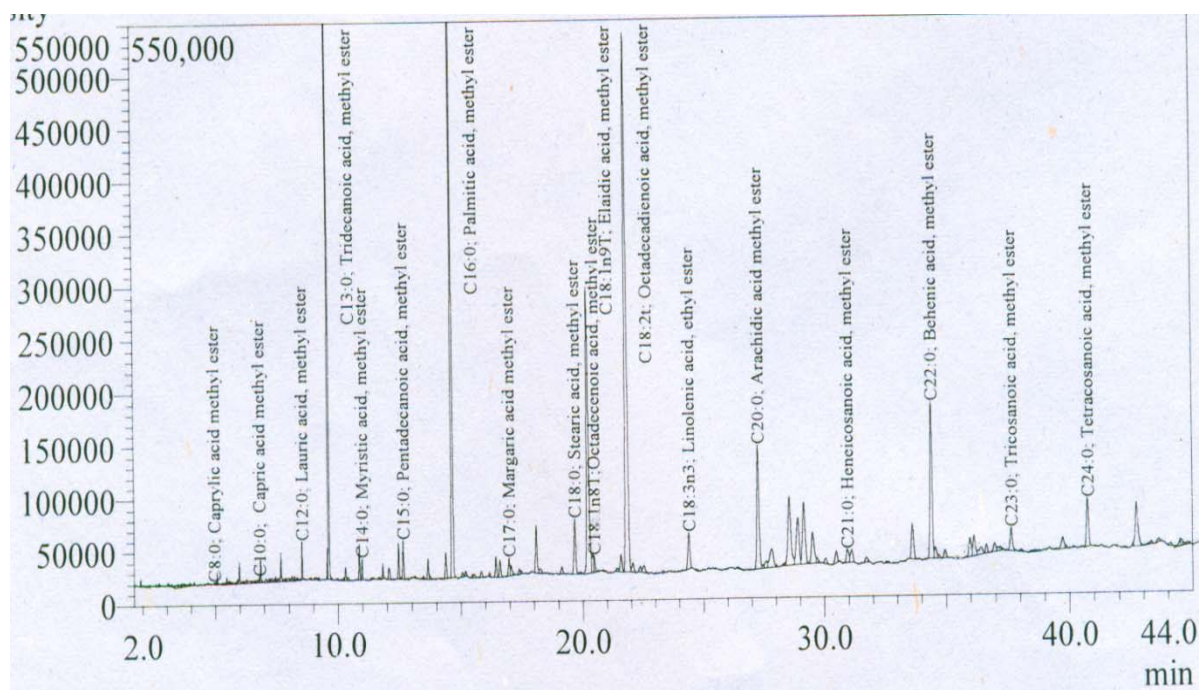
Appendix 1.9. Fatty acid profile of Onion (Rosita) grown at ambient level of CO₂



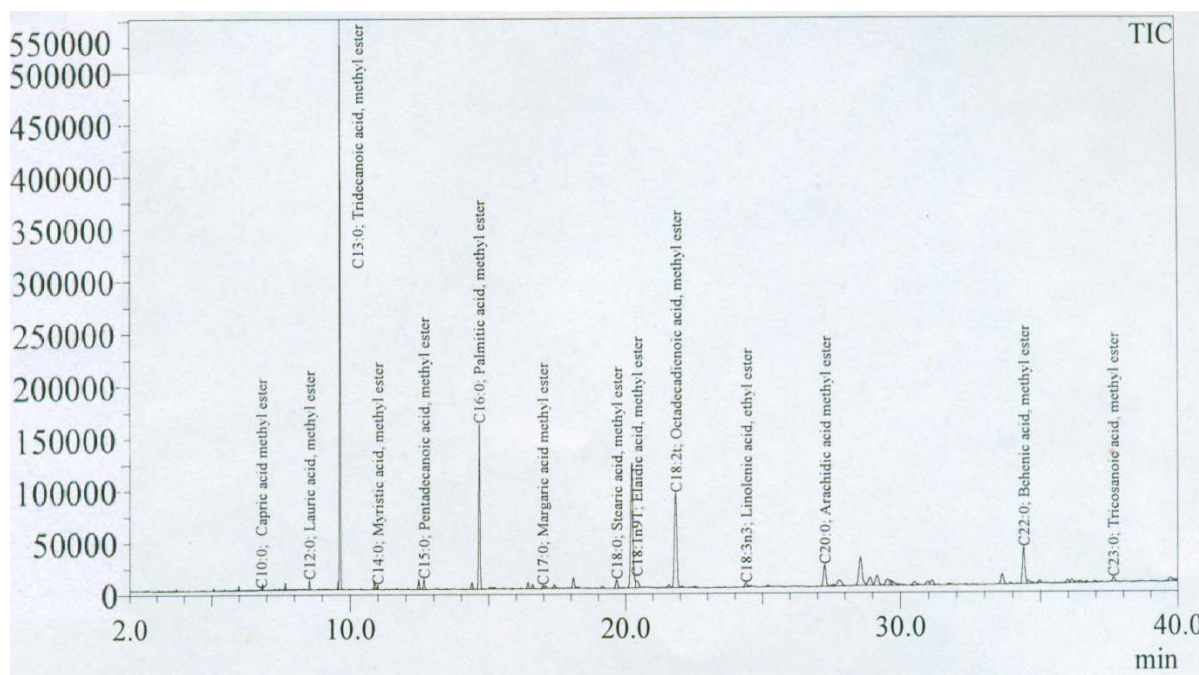
Appendix 1.10. Fatty acid profile of Onion (Rosita) grown at elevated level of CO₂



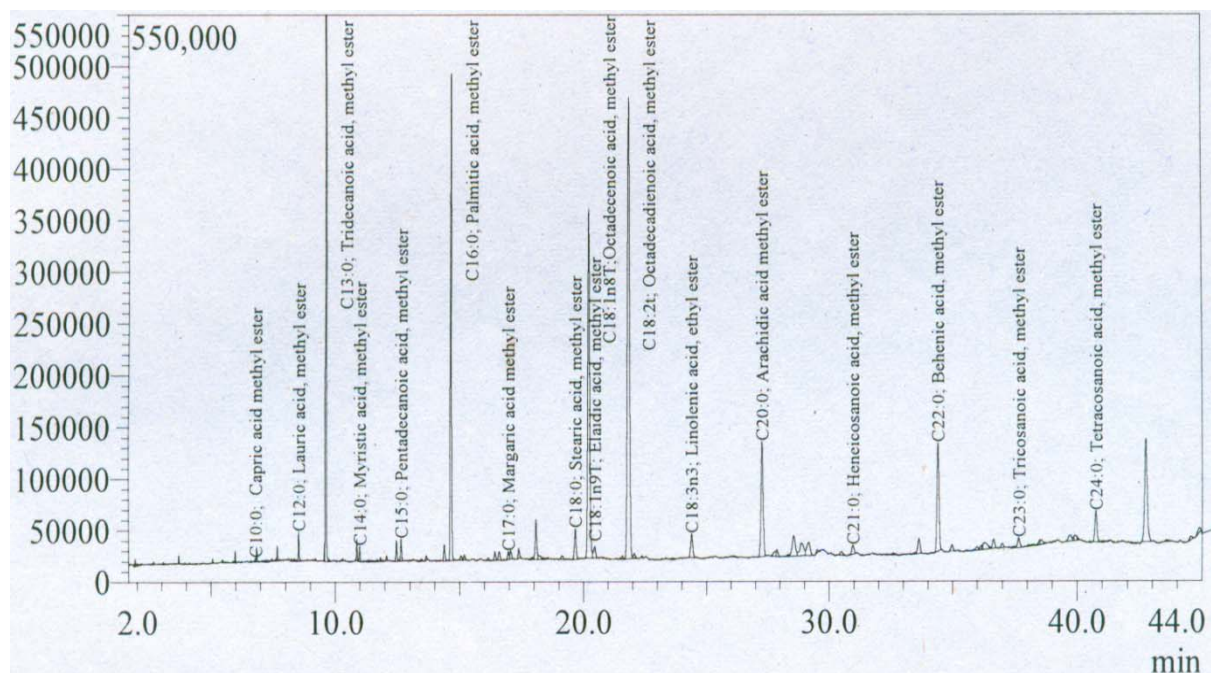
Appendix 1.11. Fatty acid profile of Onion (Red Brown) grown at ambient level of CO₂



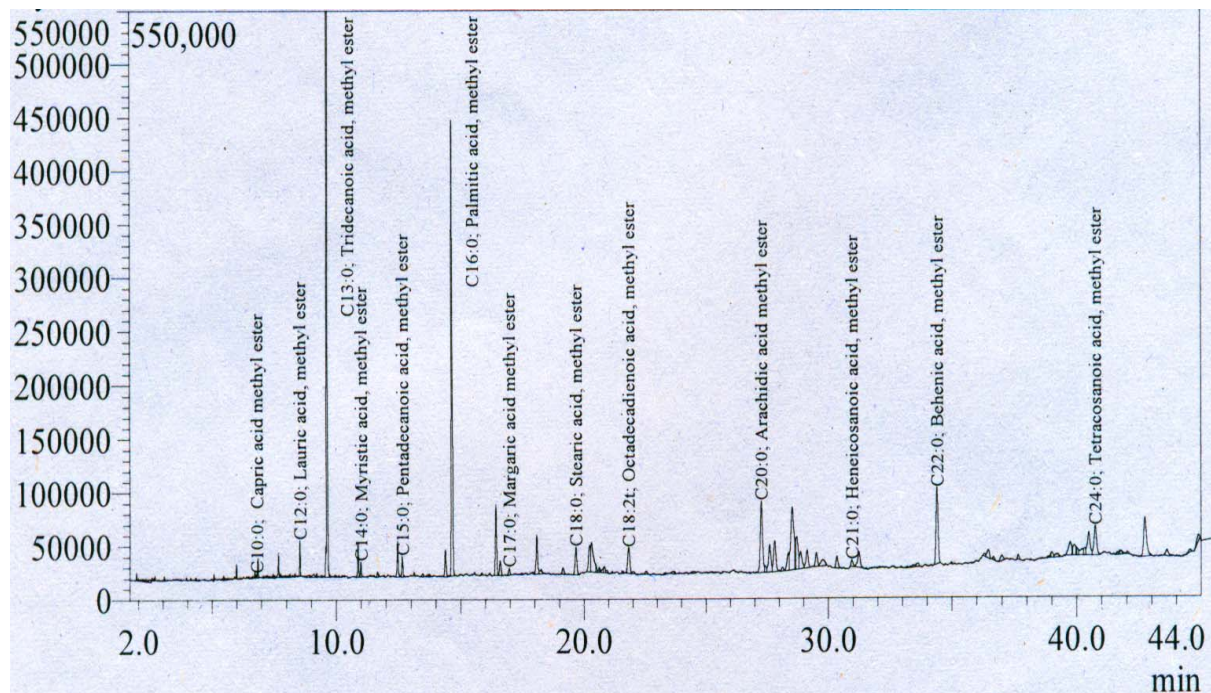
Appendix 1.12. Fatty acid profile of Onion (Red Brown) grown at elevated level of CO₂



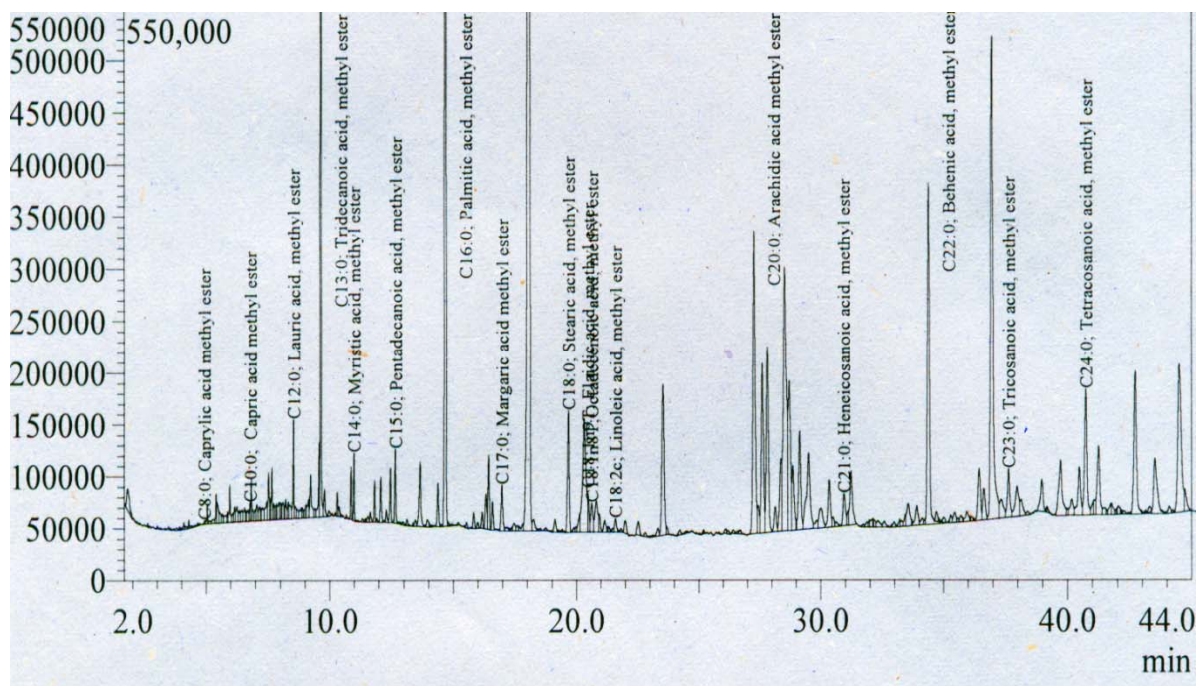
Appendix 1.13. Fatty acid profile of Onion (Dark Red) grown at ambient level of CO₂



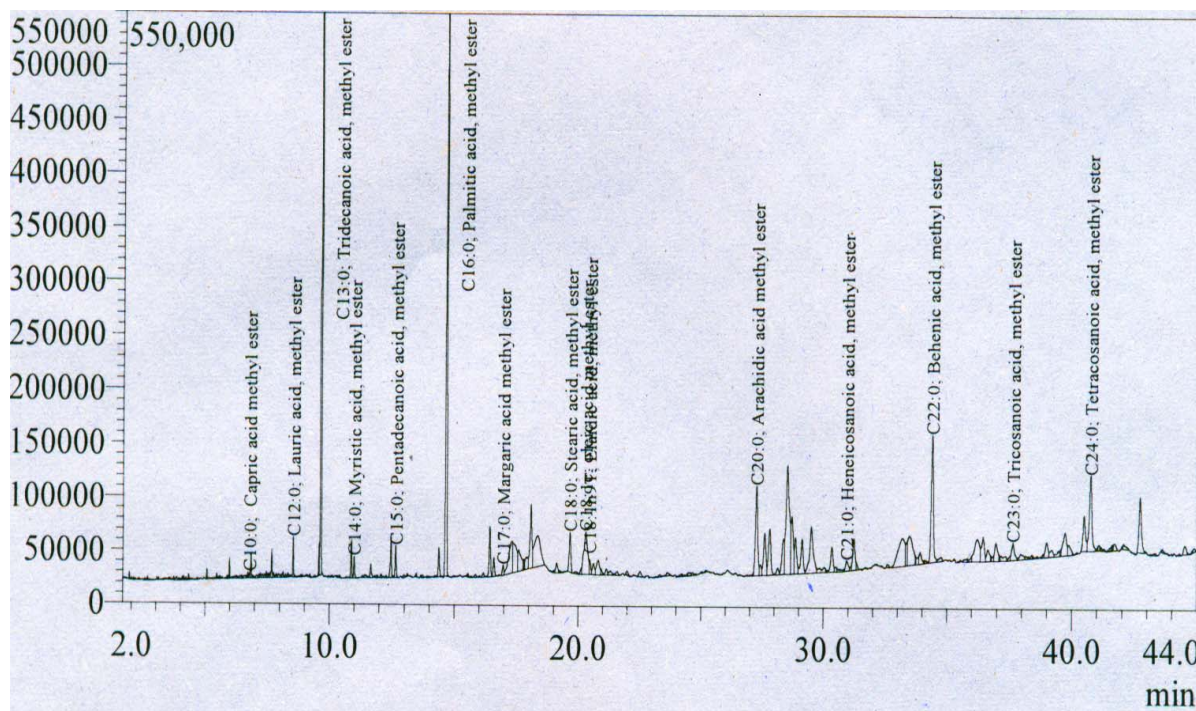
Appendix 1.14. Fatty acid profile of Onion (Dark Red) grown at elevated level of CO₂



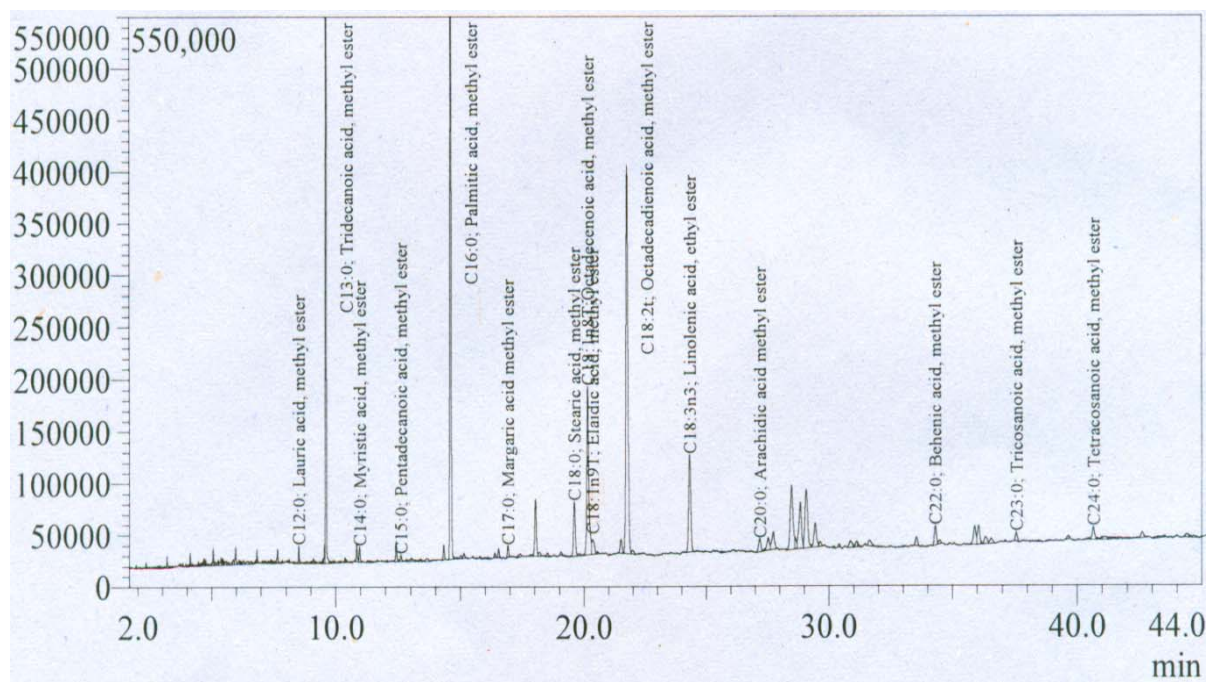
Appendix 1.15. Fatty acid profile of Onion (T1-172) grown at ambient level of CO₂



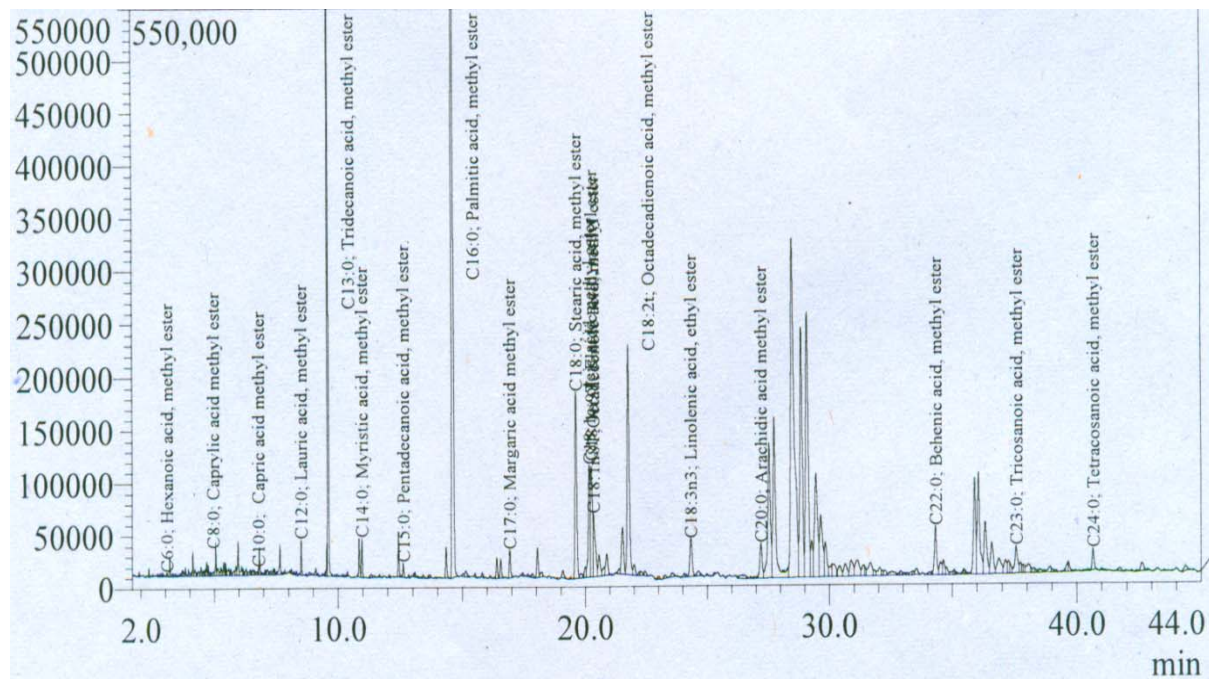
Appendix 1.16. Fatty acid profile of Onion (T1-172) grown at elevated level of CO₂



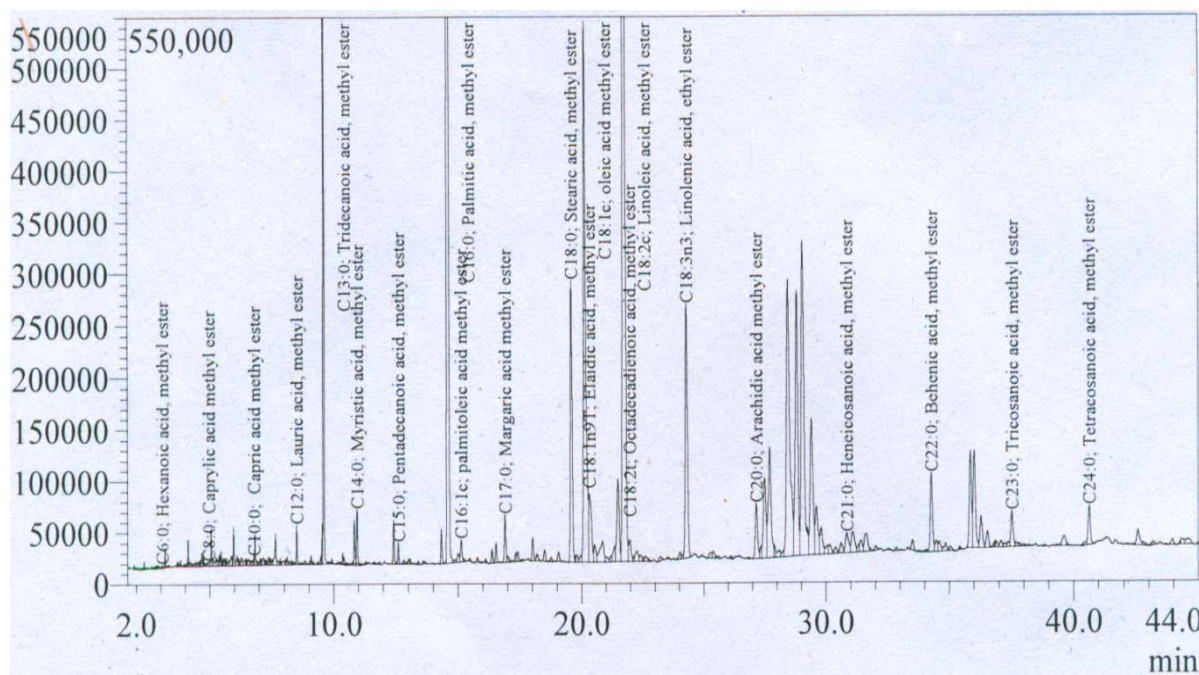
Appendix 1.17. Fatty acid profile of Okra (Arkanamika) grown at ambient level of CO₂



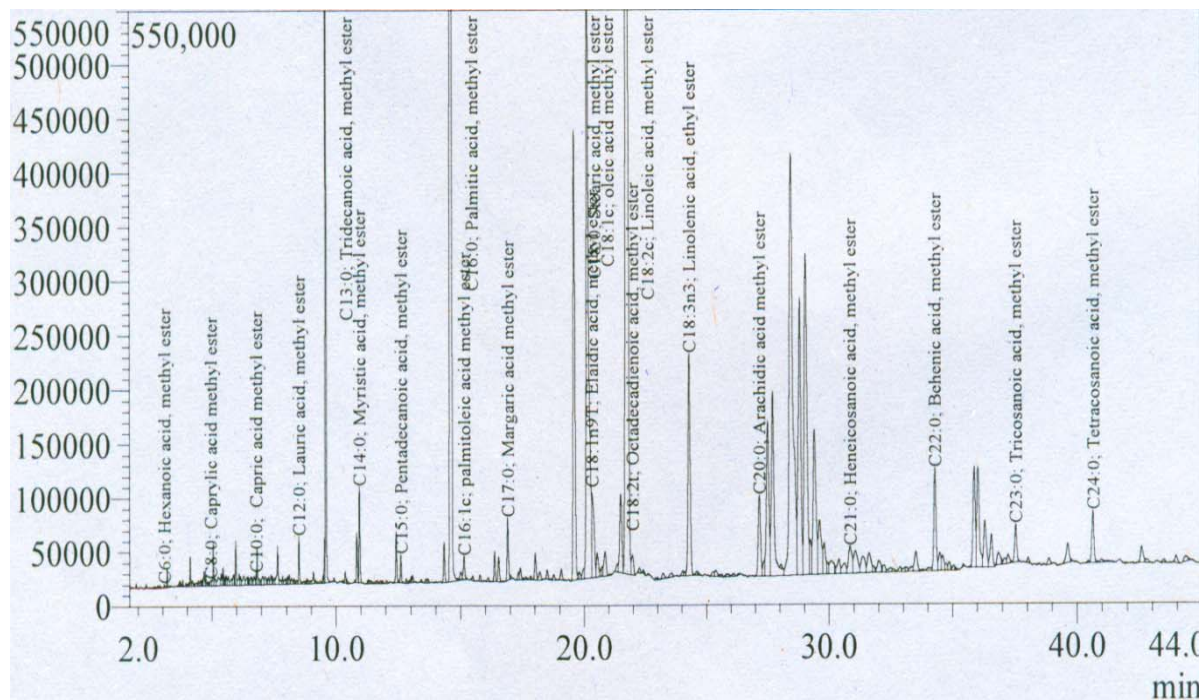
Appendix 1.18. Fatty acid profile of Okra (Arkanamika) grown at elevated level of CO₂



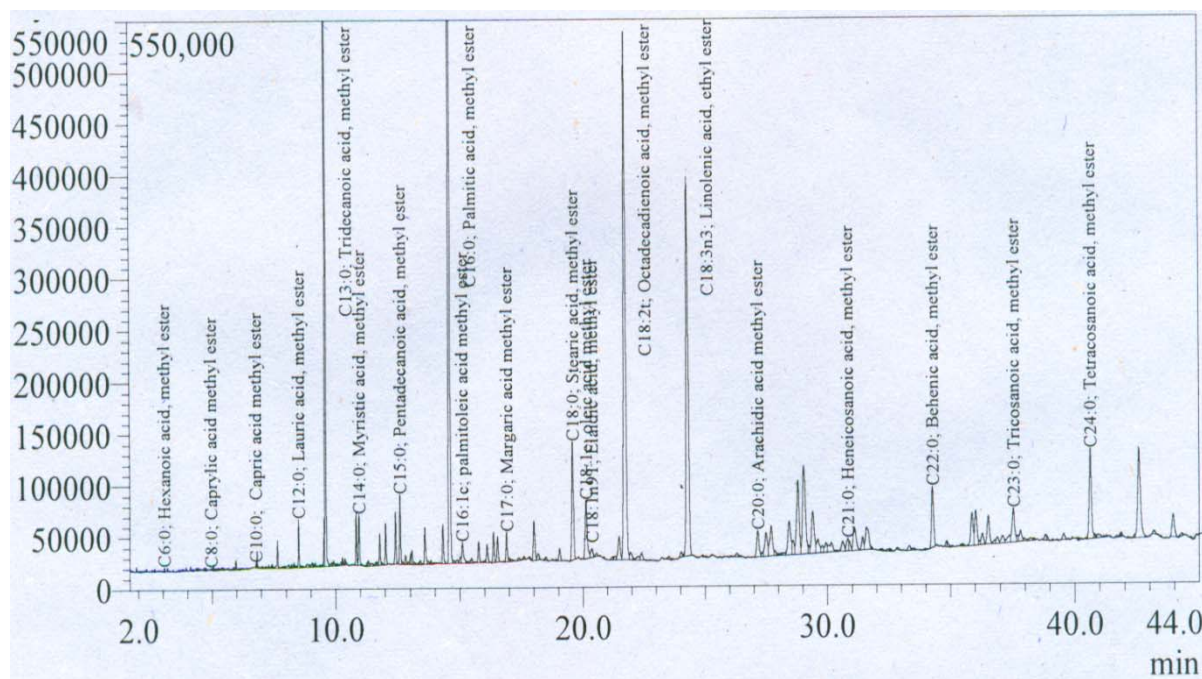
Appendix 1.19. Fatty acid profile of Okra (Sabz Pari) grown at ambient level of CO₂



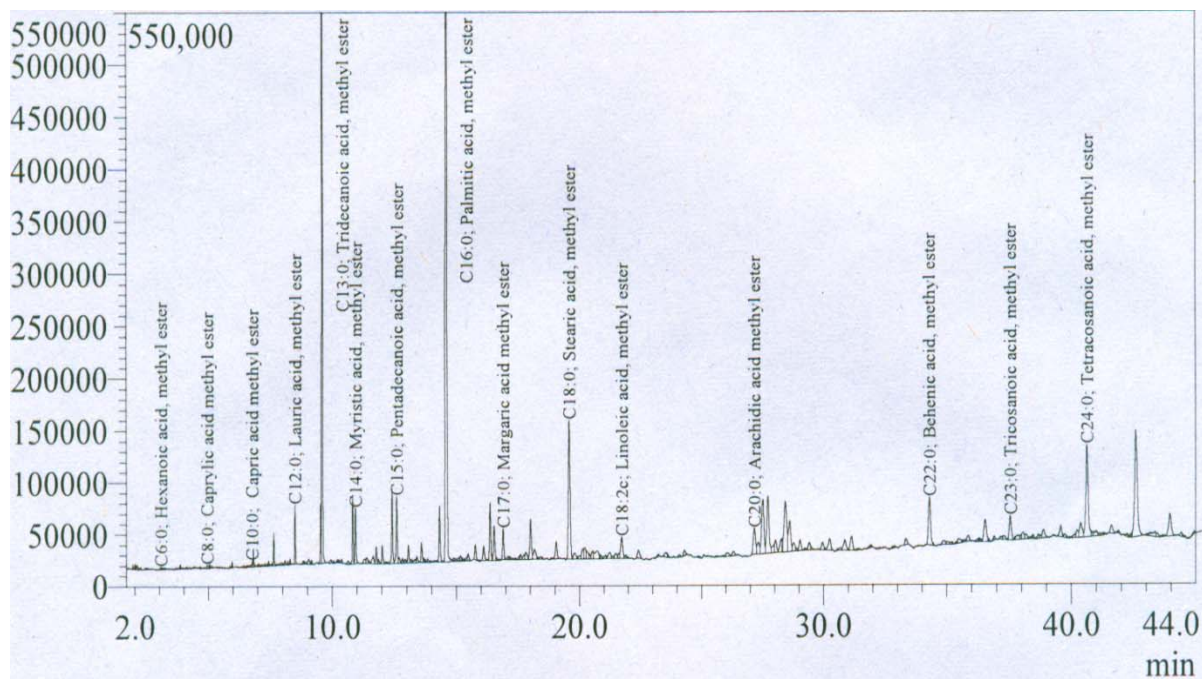
Appendix 1.20. Fatty acid profile of Okra (Sabz Pari) grown at elevated level of CO₂



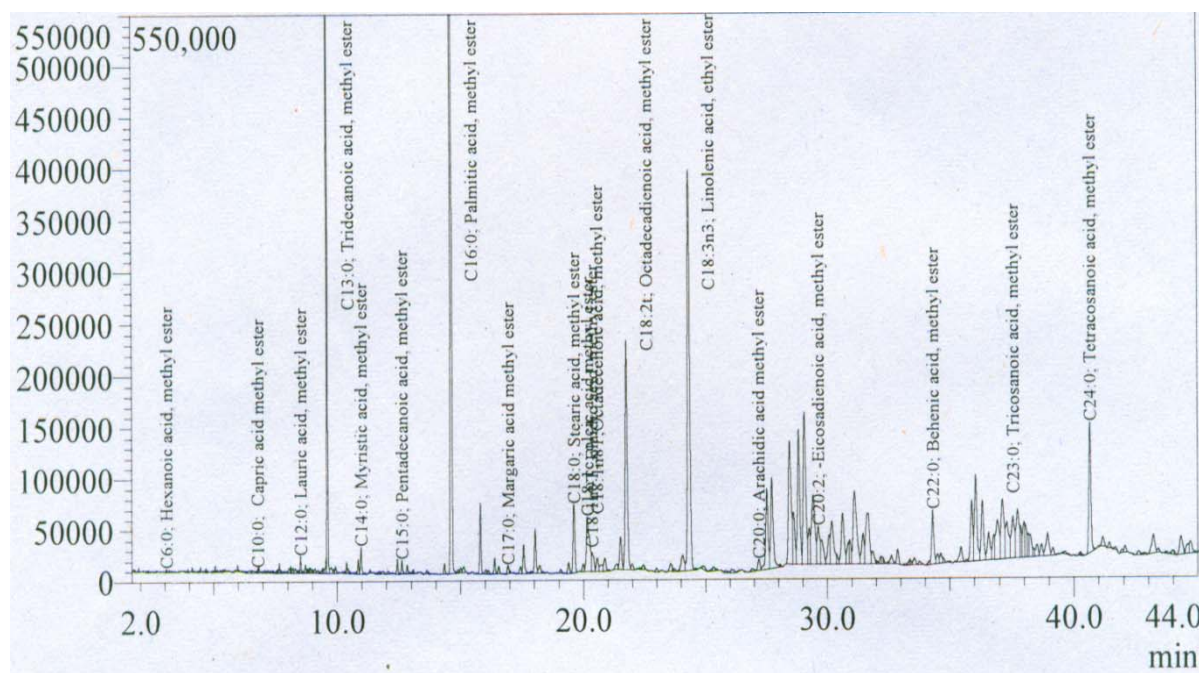
Appendix 1.21. Fatty acid profile of cucumber (Dvnasty) grown at ambient level of CO₂



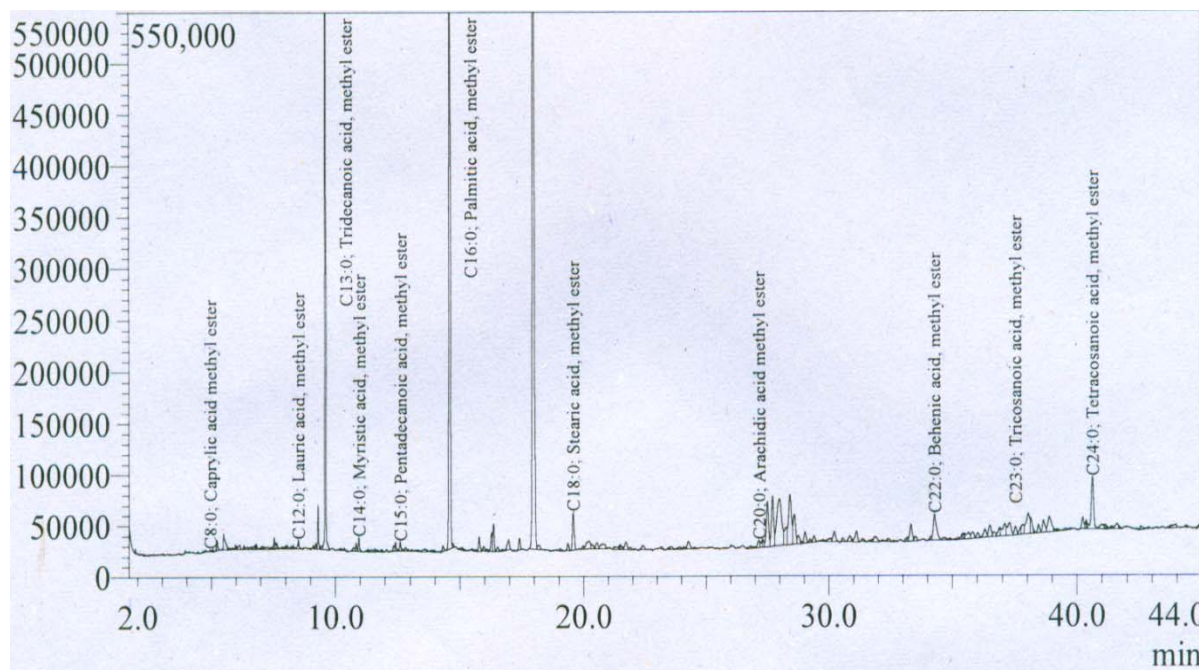
Appendix 1.22. Fatty acid profile of cucumber (Dvnasty) grown at elevated level of CO₂



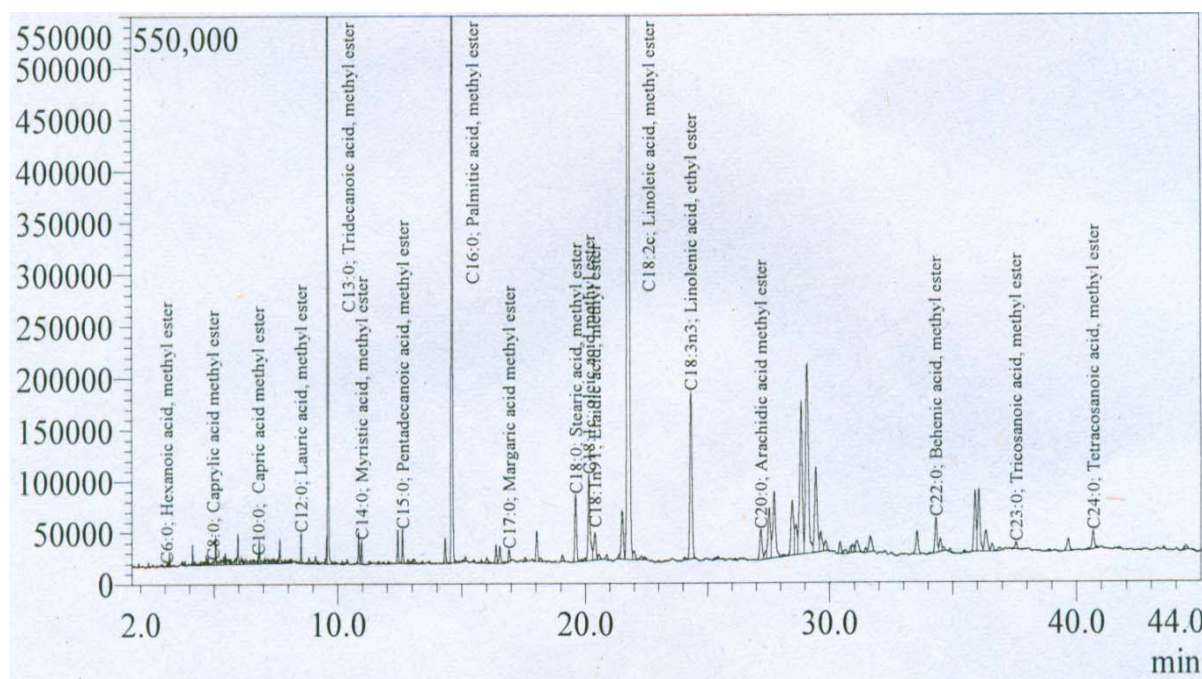
Appendix 1.23. Fatty acid profile of spinach (Local) grown at ambient level of CO₂



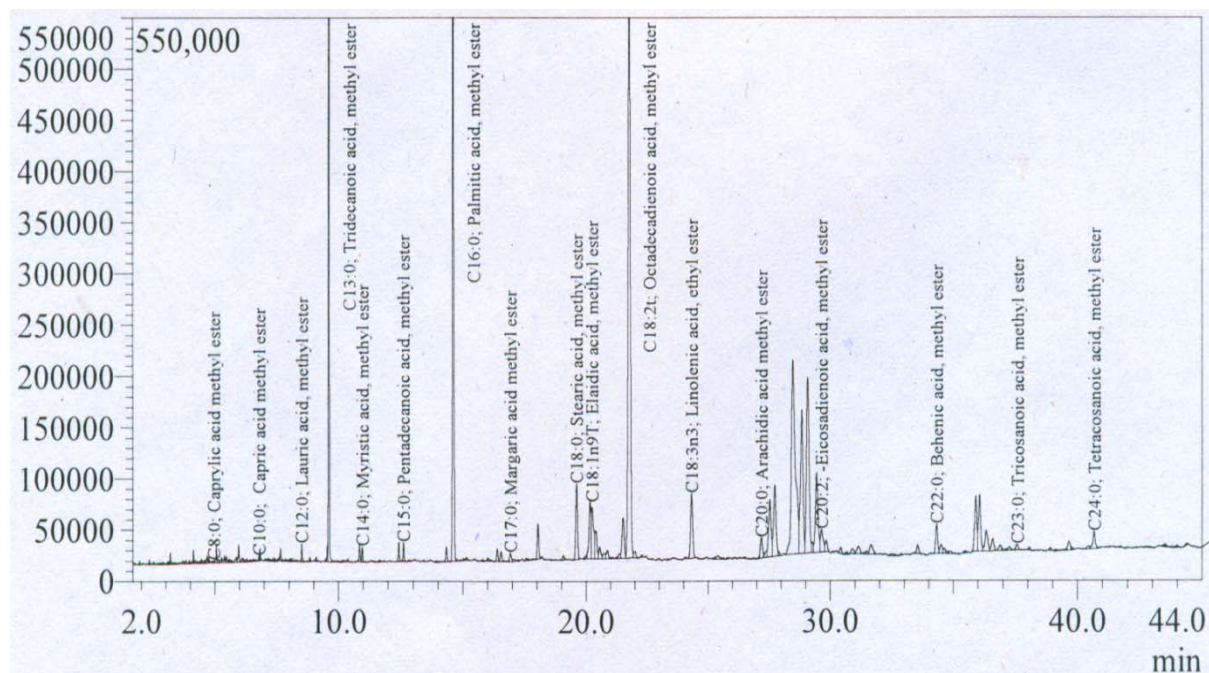
Appendix 1.24. Fatty acid profile of spinach (Local) grown at elevated level of CO₂



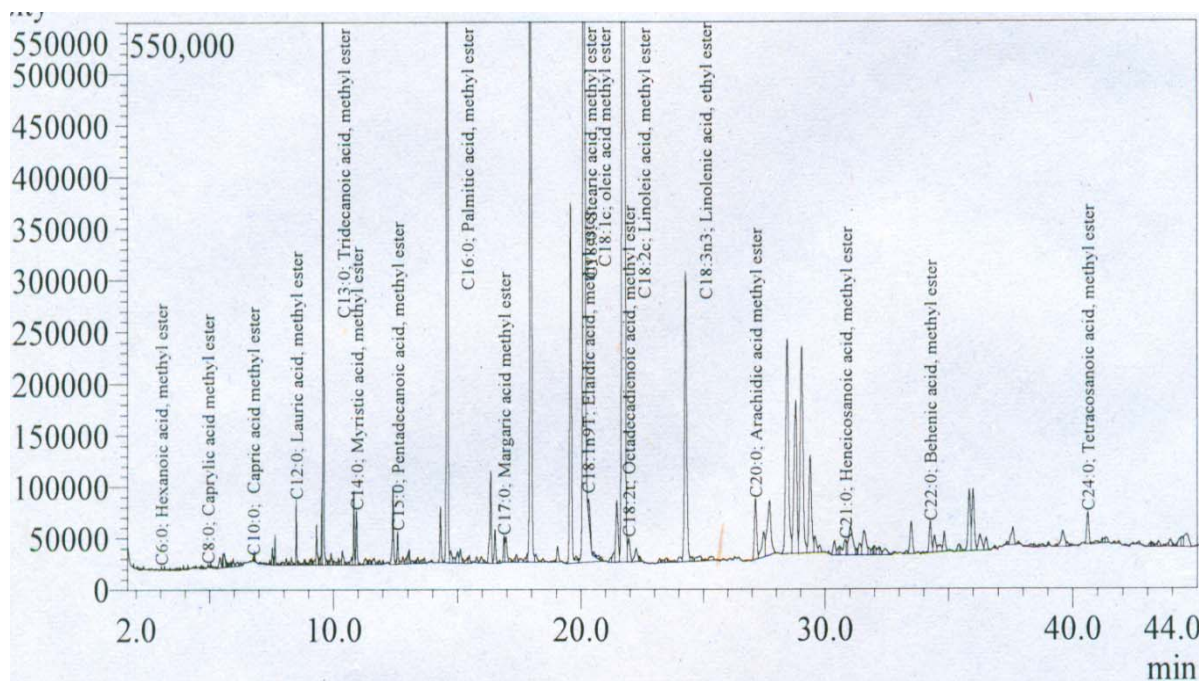
Appendix 1.25. Fatty acid profile of carrot (T-1-111) grown at ambient level of CO₂



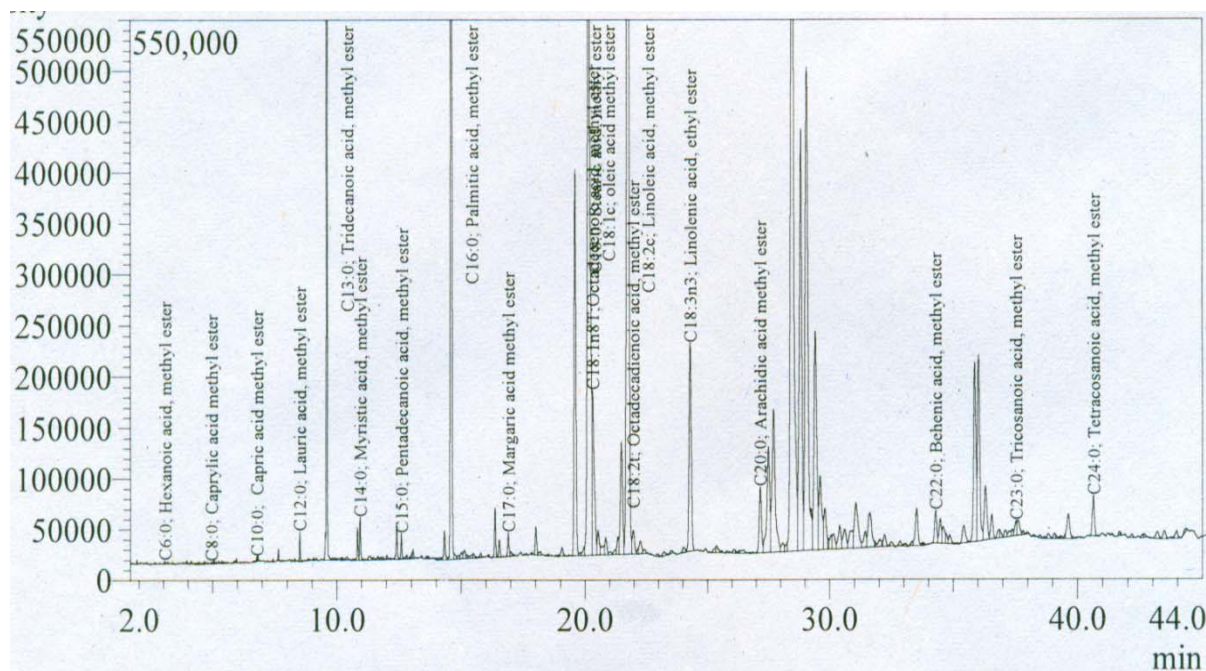
Appendix 1.26. Fatty acid profile of carrot (T-1-111) grown at elevated level of CO₂



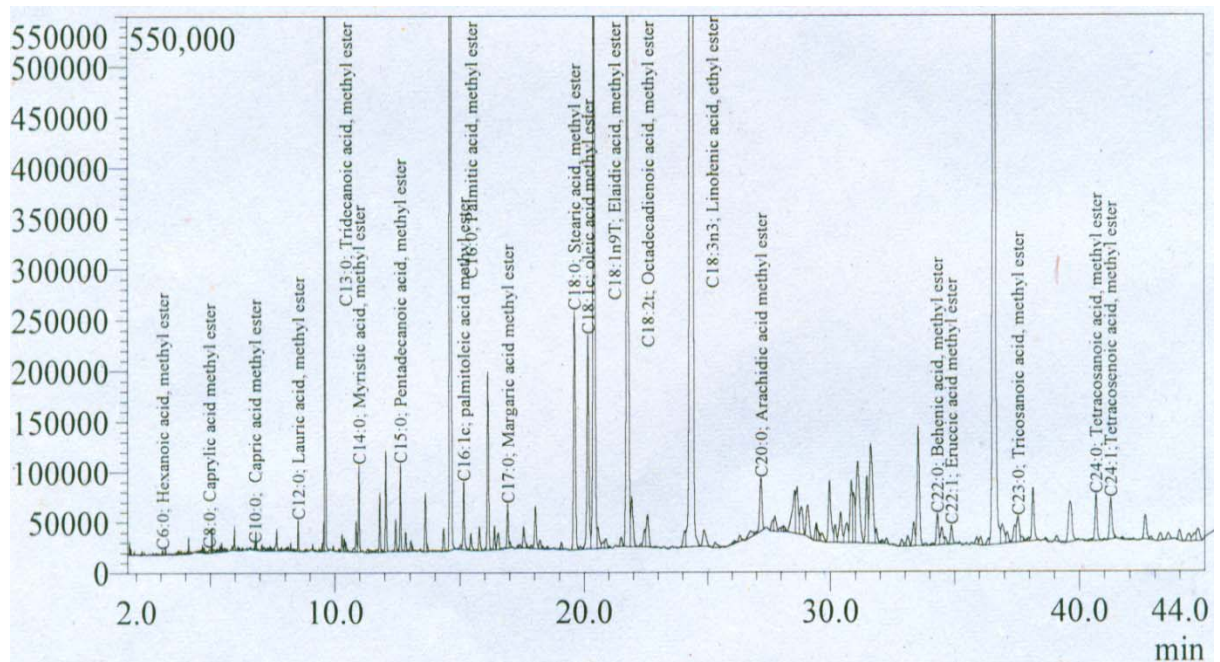
Appendix 1.27. Fatty acid profile of pea (10691) grown at ambient level of CO₂



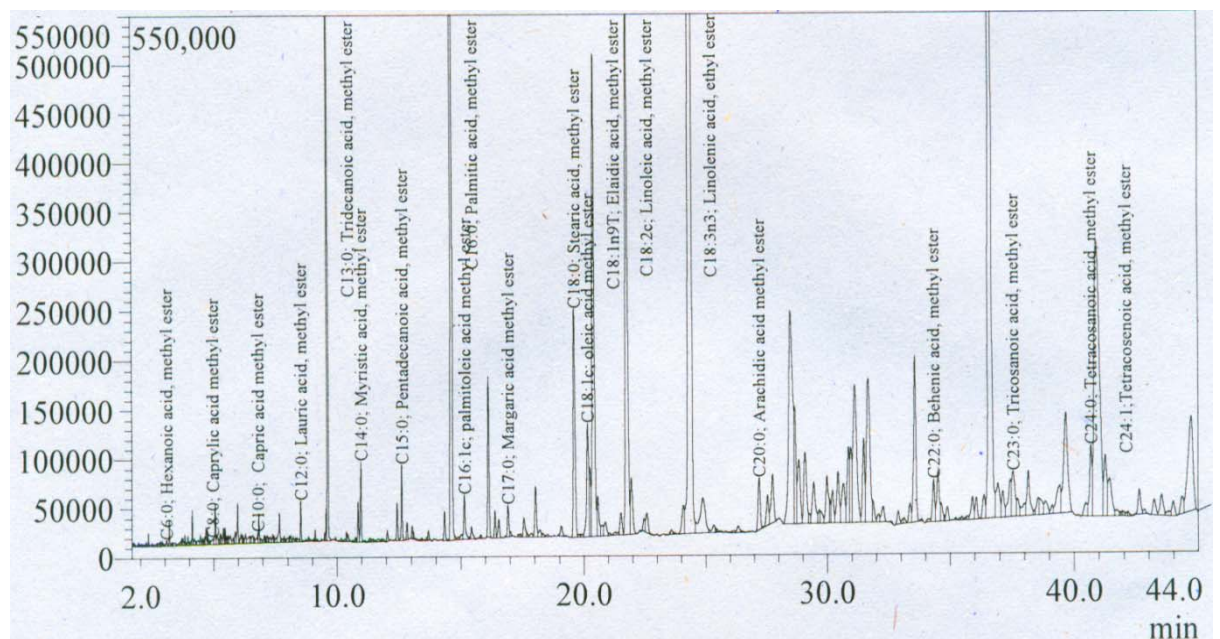
Appendix 1.28. Fatty acid profile of pea (10691) grown at elevated level of CO₂



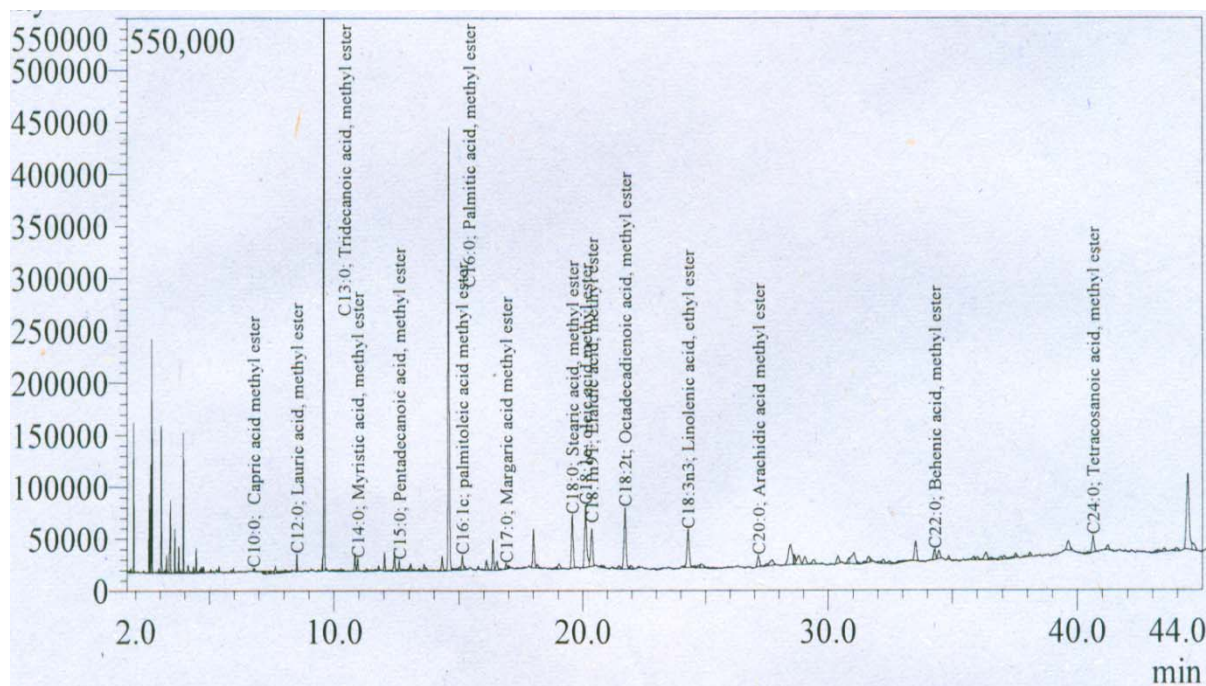
Appendix 1.29. Fatty acid profile of cauliflower (Snow Grace) grown at ambient level of CO₂



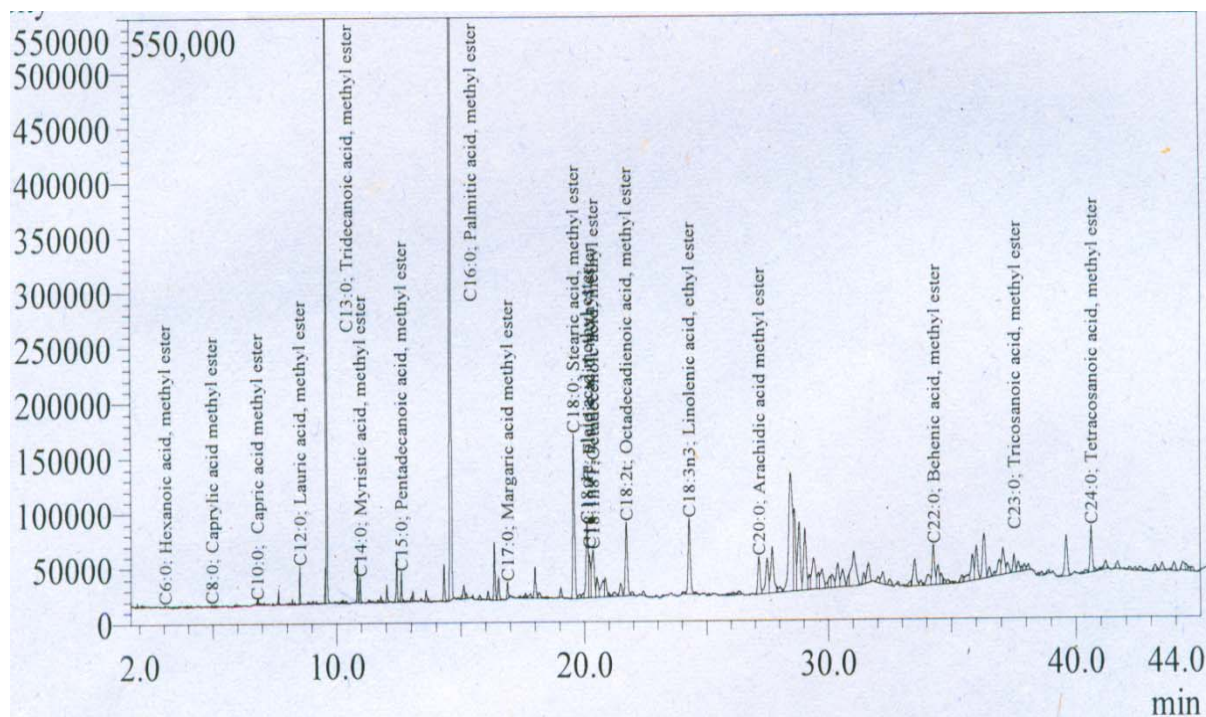
Appendix 1.30. Fatty acid profile of cauliflower (Snow Grace) grown at elevated level of CO₂



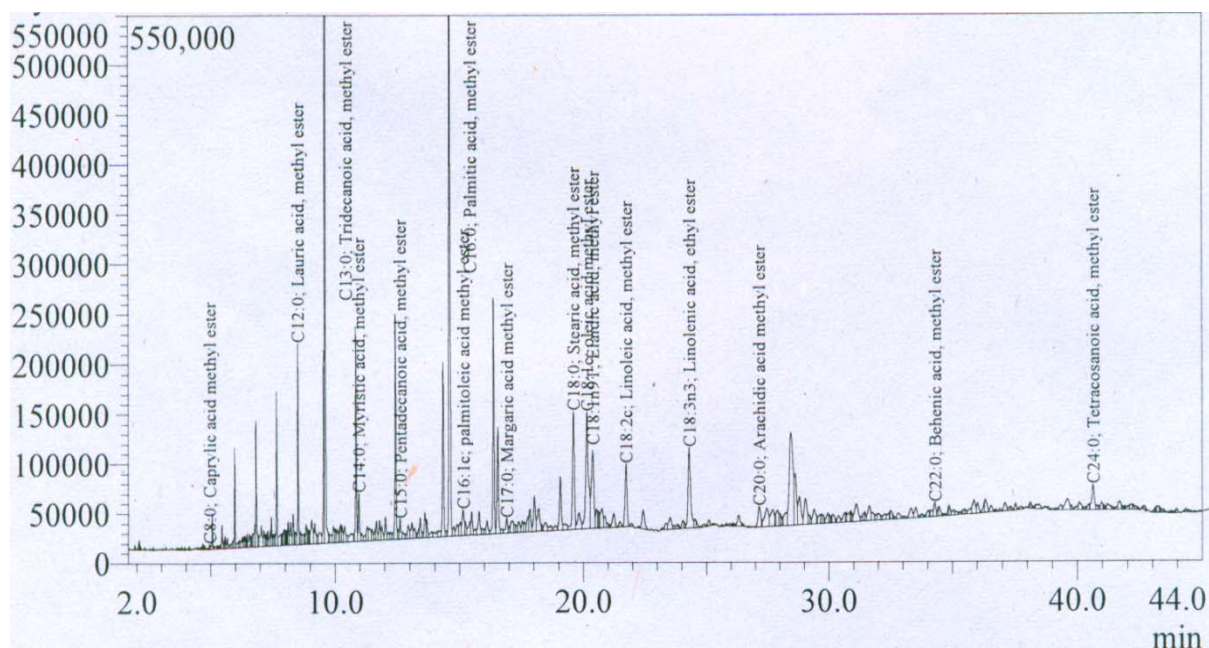
Appendix 1.31. Fatty acid profile of radish (Mino) grown at ambient level of CO₂



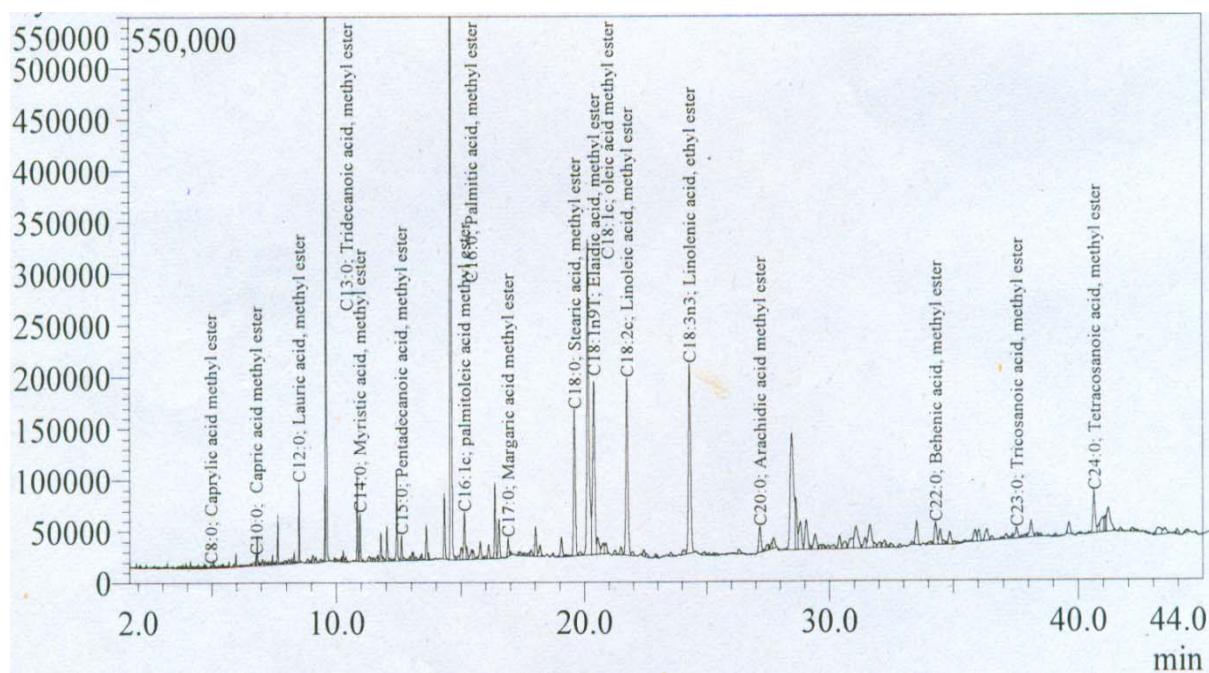
Appendix 1.32. Fatty acid profile of radish (Mino) grown at elevated level of CO₂



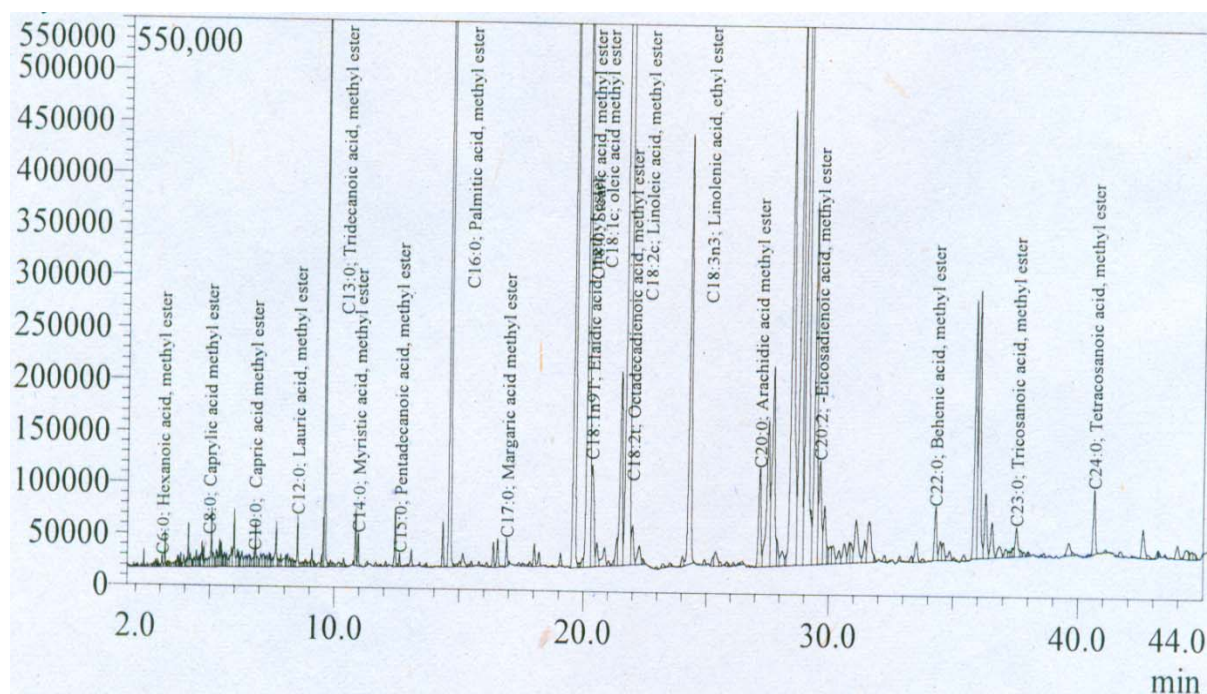
Appendix 1.33. Fatty acid profile of turnip (Grabe) grown at ambient level of CO₂



Appendix 1.34. Fatty acid profile of turnip (Grabe) grown at elevated level of CO₂



Appendix 1.35. Fatty acid profile of eggplant (Dilnashin) grown at ambient level of CO₂



Appendix 1.36. Fatty acid profile of eggplant (Dilnashin) grown at elevated level of CO₂

