



UNIVERSITI PUTRA MALAYSIA

**NUTRIENT COMPOSITION, ANTIOXIDANT AND ANTIPROLIFERATIVE
PROPERTIES OF CHILLI AND TOMATO CULTIVATED BY
FERTIGATION AND CONVENTIONAL METHODS**

NOR ASHIKEEN ABD. MOKTI @ MUKTI

FPSK(M) 2005 12

**NUTRIENT COMPOSITION, ANTIOXIDANT AND
ANTIPROLIFERATIVE PROPERTIES OF CHILLI AND TOMATO
CULTIVATED BY FERTIGATION AND CONVENTIONAL METHODS**

By

NOR ASHIKEEN ABD. MOKTI @ MUKTI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia in Fulfilment of the Requirements for the Degree of Master of
Science**

May 2005



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**NUTRIENT COMPOSITION, ANTIOXIDANT AND
ANTIPROLIFERATIVE PROPERTIES OF CHILLI AND TOMATO
CULTIVATED BY FERTIGATION AND CONVENTIONAL METHODS**

By

NOR ASHIKEEN ABD. MOKTI @ MUKTI

May 2005

Chairperson: Associate Professor Asmah Rahmat, PhD

Faculty: Medicine and Health Sciences

Chilli (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) are two fruit-type vegetables which are commonly found in multiethnic Malaysians' daily menu. They are widely consumed, easily available and edible in a variety of ways. Chillies and tomatoes which are found in the local markets are usually grown by farmers using the conventional way of cultivation, i.e. soil-based method. Fertigation is a relatively new cultivation technique in Malaysia which uses nutrient solution for the growth of the plants instead of relying on the soil as the nutrient provider.

For this research, both chilli and tomato plants were cultivated in MARDI and the green and red fruits of both plants were later analysed in UPM. Proximate analyses were carried out to determine the content of macro and micronutrients of the individual plants. Their antioxidant activities



determined by Beta-carotene bleaching and DPPH Assay, and antiproliferative properties (MTT Assay) were also determined. All the data of the individual plant was later compared between the two cultivation methods as well as between green and red fruits of the plants. Overview of results showed significant differences in the content of certain macronutrients. Significant differences were exhibited in the contents of antioxidant vitamins (A, C and E) of both plants. Generally, the contents of minerals in the samples were not significantly different when compared between cultivation techniques. On the contrary, the differences were significant between green and red fruits of the plants. Lycopene, as well as total phenolics contents were significantly different between conventional and fertigation red tomato. Chilli and tomato in both cultivation methods showed antioxidant activities but they were not significant in low concentrations. The crude ethanolic extracts of both plants did not display potent antiproliferative properties, since IC_{50} values were only obtained from one cancer cell lines, i.e. cervical cancer (HeLa) out of five tested at 5-100 $\mu\text{g}/\text{ml}$ concentration range.

Therefore, it can be generalized that green and red chilli, as well as green tomato cultivated using fertigation technique produce nutritionally higher quality of yields, whereas conventional red tomato is of higher quality



compared with its fertigation counterpart. Conclusively, fertigation produces nutritionally superior yields than that of conventional.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KOMPOSISI NUTRIEN, ANTIOKSIDAN DAN SIFAT
ANTIPROLIFERATIF CILI DAN TOMATO YANG DITANAM
DENGAN KAEDAH FERTIGASI DAN KONVENSIONAL**

Oleh

NOR ASHIKEEN ABD. MOKTI @ MUKTI

Mei 2005

Pengerusi: Profesor Madya Asmah Rahmat, PhD

Fakulti: Perubatan dan Sains Kesihatan

Cili (*Capsicum annuum*) dan tomato (*Lycopersicon esculentum*) merupakan dua sayuran jenis buah yang lazim ditemui dalam menu harian rakyat Malaysia pelbagai etnik. Penggunaan kedua-duanya rencam, mudah diperolehi di pasaran dan dijual pada harga yang mampu dibeli oleh segenap lapisan masyarakat. Cili dan tomato yang didapati di pasaran biasanya ditanam secara konvensional, iaitu menggunakan tanah. Fertigasi merupakan satu kaedah penanaman yang agak baru di Malaysia yang menggunakan larutan nutrien dan tidak lagi bergantung kepada tanah untuk membekalkan nutrient bagi pertumbuhan tanaman.

Dalam kajian ini, pokok cili dan tomato ditanam di MARDI digunakan.

Buah cili dan tomato muda dan masak ini kemudiannya dianalisis di UPM.

Analisis proksimat dijalankan untuk menentukan kandungan makro dan

mikronutrien setiap tanaman tersebut. Aktiviti antioksidasi (pelunturan beta-karoten, Asai DPPH) dan sifat antiproliferatif (Asai MTT) juga ditentukan. Data yang diperolehi daripada setiap tanaman ini dibuat perbandingan antara kedua-dua jenis kaedah penanaman serta di antara buah yang muda dengan yang masak.

Secara keseluruhannya, keputusan menunjukkan perbezaan yang signifikan bagi kandungan beberapa makronutrien tertentu. Perbezaan yang signifikan ditunjukkan dalam kandungan vitamin antioksidasi (A, C dan E) bagi kedua-dua tanaman. Amnya, kandungan mineral dalam semua sampel tidak berbeza secara signifikan apabila perbandingan dibuat antara teknik penanaman. Walau bagaimanapun, buah yang muda menunjukkan perbezaan yang signifikan apabila dibandingkan dengan buah yang masak. Kandungan likopen dan fenolik juga menunjukkan perbezaan yang signifikan. Cili dan tomato konvensional dan fertigasi mempunyai aktiviti antioksidasi, tetapi pada kepekatan yang rendah, kuasanya juga rendah. Ekstrak kasar kedua-dua tanaman ini juga tidak mempamerkan sifat antiproliferatif yang baik, memandangkan nilai IC_{50} (kepekatan yang merencat pertumbuhan sel kanser sebanyak 50%) diperolehi daripada satu sel sahaja, iaitu kanser serviks (HeLa) daripada lima jenis sel kanser yang diuji pada julat kepekatan 5-100 $\mu\text{g/ml}$.

Berdasarkan hasil kajian, umumnya cili hijau dan merah serta tomato hijau yang ditanam menggunakan teknik fertigasi memberi hasil yang lebih bermutu tinggi daripada segi zat pemakanannya, manakala tomato merah yang ditanam secara konvensional lebih bermutu daripada tomato merah fertigasi. Namun bolehlah disimpulkan bahawa, secara keseluruhannya, teknik penanaman fertigasi membuahkan hasil yang mempunyai nilai pemakanan yang lebih tinggi berbanding teknik konvensional.



ACKNOWLEDGEMENTS

In the Name of Allah, the Most Gracious, Most Merciful. Peace be to Prophet Muhammad, his Family and Companions.

One page is never enough to convey my gratitude to all individuals who have helped me through my “battle”, in the pursuit of a Masters degree. My sincere and deep respect goes to my main and co-supervisors, Associate Professor Dr. Asmah Rahmat, Associate Professor Dr. Fauziah Othman and Puan Normah Hashim. Dr. Mahamud Shahid of MARDI, the man behind fertigation in Malaysia, deserves a special mention. Your guidance and motivation were of tremendous help. Millions of thanks!

Not forgetting the laboratory personnels, namely Puan Siti Muskinah, Puan Che Maznah and Encik Simon. They are simply the professionals when it comes to lab work! They made me believe that an ounce of experience actually worth more than a tonne of knowledge! My sincere appreciation and gratitude goes out to these people.

To my dear friends, old and new, without you, I don't think I can make it this far with my sanity still intact! Your presence made my (sometimes) gloomy days in the lab looked rosier. Kak Timah, Kak Susi, Abang Herman, Siti, Dilla, Shashi, Kak Abdah, Jah, Izzah, Nida, Azah, Hafzan, Anim, Padly, Zaidi, Faizul, Emmy and Atul...thank you for being there. Dessy Arisanty, my “partner in crime” deserves my sincere appreciation. She was with me all through my lab works. Two heads are better than one, and four hands are definitely better than two! Thank you very much, Dessy.

To the most significant persons in my life, Mak and Babah, and my family members, only Allah can repay you for what you have sacrificed for me. You are the pillars of my strength!

May Allah bless all of you!

Nor Ashikeen Mukti



I certify that an Examination Committee met on 9th May 2005 to conduct the final examination of Nor Ashikeen Abd. Mokti @ Mukti on her Master of Science thesis entitled "Nutrient Composition, Antioxidant and Antiproliferative Properties of Chilli and Tomato Cultivated by Fertigation and Conventional Methods" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Mirnalini Kandiah, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Johnson Stanslas, PhD

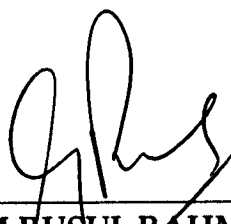
Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Rokiah Mohd Yusof, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Yasmin Anum Mohd Yusof, PhD

Associate Professor
Faculty of Medicine
Universiti Kebangsaan Malaysia
(External Examiner)



GULAM RUSUL RAHMAT ALI, PhD

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: **21 JUL 2005**



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Asmah Rahmat, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairperson)

Fauziah Othman, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Normah Hashim

Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 AUG 2005



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



NOR ASHIKEEN ABD. MOKTI @ MUKTI

Date: 11 July 2005



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiv
LIST OF FIGURES	xviii
CHAPTER	
1 INTRODUCTION	1.1
2 LITERATURE REVIEW	2.4
2.1 Chilli	2.4
2.1.1 Capsaicin	2.8
2.2 Tomato	2.10
2.2.1 Lycopene	2.14
2.3 Fertigation	2.15
2.4 Antioxidant	2.21
2.5 Carcinogenesis	2.24
2.6 Chemoprevention	2.25
3 MATERIALS AND METHOD	3.28
3.1 Samples	3.28
3.2 Cultivation Methods	
3.2.1 Conventional	3.31
3.2.2 Fertigation	3.34
3.3 Macronutrients	
3.3.1 Moisture	3.38
3.3.2 Ash	3.38
3.3.3 Protein	3.39
3.3.4 Fat	3.40
3.3.5 Carbohydrate	3.41
3.3.6 Crude Fibre	3.42
3.4 Micronutrients	
3.4.1 Vitamins A,C,E - HPLC	3.43
3.4.2 Minerals - AAS	3.46
3.4.3 Minerals - Energy Dispersive X-Ray (EDX)	3.46



3.5	Lycopene Content in Red Tomato	3.48
3.6	Total Phenolic Content	3.49
3.7	Antioxidant Activities	
3.7.1	Beta-carotene Bleaching Assay	3.50
3.7.2	DPPH Free Radical Scavenging Assay	3.51
3.8	Antiproliferative Properties (MTT Assay)	3.52
3.9	Statistical Analysis	3.55
4	RESULTS	4.56
4.1	Macronutrients	4.57
4.1.1	Moisture content	4.57
4.1.2	Ash content	4.58
4.1.3	Protein content	4.59
4.1.4	Fat content	4.60
4.1.5	Carbohydrate content	4.61
4.1.6	Crude fibre content	4.62
4.2	Micronutrients	4.63
4.2.1	Vitamin A	4.63
4.2.1	Vitamin C	4.65
4.2.3	Vitamin E	4.66
4.2.4	Minerals (AAS)	4.67
4.2.5	Energy Dispersive X-Ray	4.75
4.3	Lycopene Content in Red Tomato	4.80
4.4	Total Phenolics	4.81
4.5	Antioxidant Activities	4.82
4.5.1	Beta-carotene Bleaching Assay	4.82
4.5.2	DPPH Free Radical Scavenging Assay	4.83
4.6	Antiproliferative Properties (MTT Assay)	4.84
4.6.1	MCF-7	4.85
4.6.2	MDA-MB-231	4.85
4.6.3	HepG2	4.86
4.6.4	CaOV3	4.86
4.6.5	HeLa	4.87
5	DISCUSSION	5.88
5.1	Introduction	5.88
5.2	Macronutrients	5.88
5.3	Micronutrients	5.91
5.4	Lycopene and Total Phenolics	5.94
5.5	Antioxidant Activity	5.96
5.6	Antiproliferative Activity	5.100
6	CONCLUSION	6.103



REFERENCES/BIBLIOGRAPHY	111
APPENDICES	119
BIODATA OF THE AUTHOR	125



LIST OF TABLES

Table		Page
1	Lycopene content of common fruits and vegetables	14
2	Chilli and tomato yields of conventional and fertigation cultivation methods	17
3	Approximate cost of starting a fertigation project	18
4	Approximate cost of producing chillies and tomatoes	19
5	Yields and return of chillies and tomatoes of fertigation	20
6	List of antioxidants obtained from the diet	22
7	Major classes of phytochemicals with antioxidant activity	23
8	Conditions for HPLC separation of antioxidant vitamins	45
9	Moisture contents of chilli and tomato of conventional and fertigation methods	57
10	Ash contents of chilli and tomato of conventional and fertigation methods	58
11	Protein contents of chilli and tomato of conventional and fertigation methods	59
12	Fat contents of chilli and tomato of conventional and fertigation methods	60
13	Carbohydrate contents of chilli and tomato of conventional and fertigation methods	61
14	Crude fibre contents of chilli and tomato of conventional and fertigation methods	62



15	Vitamin A contents of chilli and tomato of conventional and fertigation methods	64
16	Vitamin C contents of chilli and tomato of conventional and fertigation methods	65
17	Vitamin E contents of chilli and tomato of conventional and fertigation methods	66
18	Comparison between contents of seven selected minerals between conventional and fertigation green chilli	67
19	Comparison between contents of seven selected minerals between conventional and fertigation red chilli	68
20	Comparison between contents of seven selected minerals between green and red conventional chilli	69
21	Comparison between contents of seven selected minerals between green and red fertigation chilli	70
22	Comparison between contents of seven selected minerals between conventional and fertigation green tomato	71
23	Comparison between contents of seven selected minerals between conventional and fertigation red tomato	72
24	Comparison between contents of seven selected minerals between green and red conventional tomato	73
25	Comparison between contents of seven selected minerals between green and red fertigation tomato	74
26	Detected elements in conventional and fertigation green chilli	75



27	Detected elements in conventional and fertigation red chilli	76
28	Detected elements in conventional green and red chilli	76
29	Detected elements in fertigation green and red chilli	77
30	Detected elements in conventional and fertigation green tomato	77
31	Detected elements in conventional and fertigation red tomato	78
32	Detected elements in conventional green and red tomato	78
33	Detected elements in fertigation green and red tomato	79
34	Lycopene content in conventional and fertigation red tomato	80
35	Total phenolics content in conventional and fertigation samples	81
36	Percentage of antioxidant activity in conventional and fertigation samples	82
37	Percentage of scavenging activity of conventional and fertigation samples on DPPH• radical at highest concentration tested	83
38	IC ₅₀ of crude extracts of chilli and tomato against MCF-7 from MTT Assay	85
39	IC ₅₀ of crude extracts of chilli and tomato against MDA-MB-231 from MTT Assay	85
40	IC ₅₀ of crude extracts of chilli and tomato against HepG2 from MTT Assay	86



41	IC ₅₀ of crude extracts of chilli and tomato against CaOV3 from MTT Assay	86
42	IC ₅₀ of crude extracts of chilli and tomato against HeLa from MTT Assay	87
43	Summary of results for green chilli	104
44	Summary of results for red chilli	106
45	Summary of results for green tomato	107
46	Summary of results for red tomato	109



LIST OF FIGURES

Figure		Page
1	Chilli plants bearing some ripe fruits	6
2	An upclose of some unripe fruits of chilli	6
3	Chemical structure of capsaicin	10
4	Yellow flowers and the green, hairy stalk of tomato	11
5	An upclose of some unripe tomato fruits	12
6	Chemical structure of lycopene	14
7	Schematic representation of multistage carcinogenesis	24
8	Classification of chemopreventive phytochemicals based on their mechanism of action	29
9	Seedlings of chilli in rockwools containing vermiculite; ready to be transplanted into polybags	30
10	Seedlings of tomato in grounded peats containing vermiculite; ready to be transplanted into polybags	27
11	Top-soil	32
12	Sand	32
13	Fertilizer	33
14	Top-soil, sand and fertilizer were combined and mixed in the mixer	34
15	Coco-peat	35
16	1 kg of dry coco-peat was weighed into each polybag	35
17	Polybag containing coco-peat; ready for transplantation	36



18	Overall view in Rainshelter House	37
19	Rainshelter House, Horticulture Unit, MARDI	37
20	Variable Pressure Scanning Electron Microscope (Model: Leo VP 1450)	47
21	Samples attached onto the stab	48



CHAPTER 1

INTRODUCTION

Chilli (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) are two fruit-type vegetables which are commonly found in multiethnic Malaysians' daily menu. They are widely consumed, easily available and affordable to all walks of life. In addition to that, they are edible in a variety of ways, either eaten fresh or cooked, and also used creatively as food garnishing.

Chillies and tomatoes which are found in the local markets are usually grown by farmers using the conventional way of cultivation, i.e. soil-based method.

This method is called "geoponic system", which literally describes the roots of the plant creeping in the soil. Even though this method seemed to satisfy the demand for these fruit-type vegetables in the market, research are ongoing to produce yields that are of high quality and more economical to produce.

Universiti Putra Malaysia (UPM) and Malaysian Agriculture Research and Development Institute (MARDI) are examples of organizations that are actively involved in the related research.

Hydroponic system is an alternative cultivation method to the existing geoponic system practiced in the country. Compared to the old system,



hydroponic uses nutrient solution for the growth of the plants instead of relying on the soil as the nutrient provider.

Fertigation is a new cultivation technique which is almost similar to the concept as well as the technique used in hydroponic. In Malaysia, it was pioneered by Dr. Mahamud Shahid, a senior researcher in MARDI. This method has been applied widely in other countries such as India (Dalvi *et al.*, 1998; Singandhupe *et al.*, 2002; Hebbar *et al.*, 2003), Canada (Xu *et al.*, 1995), Belgium (De Rijck & Schrevens, 1997) and Israel (Chapagain & Wiesman, 2003). In our country, it is widely applied in Cameron Highlands, certain parts of Johore, Terengganu, Kedah and Negeri Sembilan (Mahamud, 2002).

Importance of Study

Fertigation yields, specifically chilli and tomato which are produced in Malaysia have yet to be studied, specifically in terms of their nutrient composition. Even though the data could be obtained from Nutrient Composition of Malaysian Foods (Tee *et al.*, 1996), they are not representative of chilli and tomato in this study. Furthermore, the cultivar and the cultivation technique used for the plants in Tee *et al.* (1996) are unknown. In this study, fertigation yields will be compared to their conventional counterparts to determine which cultivation method produce superior yield from the perspective of their nutritional values.



Objective

General objective

To determine and compare the nutrient composition, antioxidant activities and antiproliferative property of chilli and tomato cultivated by fertigation and conventional methods.

Specific objectives

1. To determine and compare the nutrient composition, antioxidant activities and antiproliferative property of green and red chillies cultivated by fertigation and conventional methods.
2. To determine and compare the nutrient composition, antioxidant activities and antiproliferative property of green and red tomatoes cultivated by fertigation and conventional methods.

CHAPTER 2

LITERATURE REVIEW

Food nourishes life, and the substances in food on which life depends are the nutrients. These provide the energy and building materials for the countless substances that are essential to the growth and survival of living things (Mahan and Escott-Stump, 1996).

Plants can provide most of the nutrients required in the human diet (Tucker, 2003). Through the process called photosynthesis, green plants intercept a portion of the sunlight reaching their leaves and capture it within the chemical bonds of glucose. Proteins, fats and other carbohydrates are synthesized from this basic carbohydrate to meet the needs of the plant. Animals and humans obtain these nutrients and the energy they contain by consuming plants and the flesh of other animals (Mahan and Escott-Stump, 1996).

2.1 Chilli

Capsicum annuum L., the botanical name for chilli, belongs to the family of Solanaceae. In the Malay community, it is called "cili", "cabai" or "lada". It is reported to be originated from Central or South America (Pruthi, 1976;

