



UNIVERSITI PUTRA MALAYSIA

***BIOACTIVE COMPOUNDS AND ANTIOXIDANT ATTRIBUTE OF
CARAMBOLA (AVERRHOA CARAMBOLA L.) FRUIT AS AFFECTED BY
CULTIVARS AND RIPENING STAGES***

MOHD ASRAF BIN MOHD ZAINUDIN

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BERILMU BERBAKTI

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CULTIVARS AND RIPENING STAGES**

By

MOHD ASRAF BIN MOHD ZAINUDIN

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

October 2013

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

**BIOACTIVE COMPOUNDS AND ANTIOXIDANT ATTRIBUTE OF
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October 2013

**Chairman: Professor Azizah Abd Hamid, PhD.
Faculty: Food Science and Technology**

Study on bioactive compounds and antioxidant activity was conducted on three different carambola cultivars (B2, B17 and B10) planted in Malaysia and the best cultivars in terms of antioxidant potential was selected and evaluated at different ripening stages. Carambola fruits were analyzed for ascorbic acid (AA), total phenolic compound (TPC), total flavonoid content (TFC), total carotenoid content (TCC), beta-carotene (β -carotene), tocopherols (α , β , γ and δ) and sugars (glucose, fructose and sucrose). Free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl assay (DPPH) and β -carotene-linoleic acid model (BCLAM) assays were performed to evaluate their antioxidant activities. Results of the study showed that there is no significant difference in AA content between cv. B2 and cv. B17. Among the cultivars, cv. B17 was found to contain significantly ($P < 0.05$) higher TPC, sugars, β -carotene and β -tocopherol levels while cv. B10 only had significantly ($P < 0.05$) higher content of γ - and δ -tocopherols. However, cv. B10 and cv. B17 did not show any significant difference in TCC, TFC, β -carotene and α -tocopherol content. The study showed that each cultivar had their own advantages in terms of bioactive compounds that could contribute to antioxidant activity. As expected, cv. B10 and cv. B17 exhibited significantly ($P < 0.05$) higher antioxidant activity than that of cv. B2 for both DPPH and BCLAM assays. However, BCLAM result showed that cv. B17 had the highest antioxidant activity among the cultivars. Pearson's correlation and Partial Least Square (PLS) were used to identify the link between antioxidant activity and bioactive compounds. Carambola cv. B17 had been selected for quantifying the bioactive compounds and antioxidant activity changes at different ripening stages. Carambola cv. B17 was tagged at fruit set stage and harvested at week 9, 10, 11, 12 and 13. Ascorbic acid, TCC and sugar composition were significantly ($P < 0.05$) increased while TPC, TFC and β -carotene exerted the reverse trend during ripening. Tocopherols homologue changes were varied where α - and β -tocopherols levels were increased until week 12 before decreased in week 13. On the contrary, γ - and δ -tocopherols levels were significantly ($P < 0.05$) decreased during ripening. Fruit harvested at a later ripening stage allow for the synthesis and accumulation of bioactive compounds, thus explained the increasing trend. On the

other hand, decreased trend in the bioactive compounds are probably due to their functions as precursor compounds. Antioxidant activities of the samples were significantly ($P < 0.05$) decreased as measured by DPPH and BCLAM assays. Multivariate analysis revealed clear separations between the ripening stages caused by transitional changes in bioactive compounds and antioxidant activity during fruit ripening. In conclusion, bioactive compound and antioxidant activity were cultivar- and ripening stage-dependent. Carambola fruit cv. B17 was found to be the best cultivar in terms of antioxidant potential and should be harvested at week 9 to get its full benefit of antioxidant property.



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

**SEBATIAN BIOAKTIF DAN SIFAT ANTIOKSIDA PADA BUAH
BELIMBING (*Averrhoa carambola* L.) DIPENGARUHI OLEH KULTIVAR
DAN TAHAP KERANUMAN**

Oleh

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Kajian terhadap sebatian bioaktif dan sifat antioksidan telah dijalankan ke atas tiga kultivar buah belimbing berbeza (B2, B10, B17) yang ditanam di Malaysia dan kultivar yang terbaik dari segi potensi antioksidan akan dipilih dan dinilai pada tahap peranakan yang berbeza. Buah belimbing telah dianalisa kandungan asid askorbik (AA), sebatian fenolik (TPC), sebatian flavonoid (TFC), sebatian karotenoid (TCC), beta karotena (β -karotena), tokoferol (α , β , γ , δ) dan gula (glukosa, fruktosa dan sukrosa). Esei aktiviti penghapus sisa radikal bebas menggunakan esei 2,2-diphenyl-2-picrylhydrazyl (DPPH) dan esei model β -karotena/asid linoleik telah dilakukan untuk mengukur aktiviti antioksidan. Keputusan telah menunjukkan tiada perbezaan yang signifikan dalam kandungan AA di antara cv. B2 dan cv. B17. Antara kultivar-kultivar tersebut, cv. B17 mengandungi kadar TCC, TPC, komposisi gula, β -karotena dan β -tokoferol yang signifikan manakala cv. B10 hanya mempunyai kandungan yang signifikan ($P < 0.05$) tinggi pada γ - dan δ -tokoferol sahaja. Walaubagaimanapun, cv. B10 dan cv. B17 tidak menunjukkan perbezaan yang signifikan ($P > 0.05$) pada kandungan TCC, TFC, β -karotena dan α -tokoferol. Ini menunjukkan setiap kultivar mempunyai kelebihan sendiri di dalam sebatian bioaktif yang boleh menyumbang kepada aktiviti antioksidan. Oleh itu, aktiviti antioksidan telah digunakan bagi menentukan kultivar yang terbaik dari segi potensi antioksidan. Seperti yang telah dijangka, cv. B10 dan cv. B17 telah menunjukkan aktiviti antioksidan yang signifikan tinggi ($P < 0.05$) daripada cv. B2 bagi kedua-dua aktiviti esei, DPPH dan BCLAM tetapi keputusan BCLAM menunjukkan cv. B17 adalah yang signifikan ($P < 0.05$) tertinggi di kalangan kultivar-kultivar tersebut. Korelasi Pearson dan Partial Least Square (PLS) telah digunakan untuk mengenalpasti kaitan di antara aktiviti antioksidan dan sebatian bioaktif. Belimbing cv. B17 telah dipilih untuk kajian perubahan jumlah sebatian bioaktif dan aktiviti antioksidan pada tahap keranuman buah yang berbeza. Tunas buah belimbing cv. B17 telah ditanda dan dituai pada minggu ke-9, 10, 11, 12 dan 13. Asid askorbik, TCC dan komposisi gula telah meningkat dengan signifikan ($P < 0.05$) manakala TPC, TFC dan β -karotena pula menurun secara signifikan ($P < 0.05$) semasa proses peranakan. Perubahan homolog tokoferol adalah pelbagai

yang mana α - dan β -tokoferol mempunyai nilai meningkat pada minggu ke-12 sebelum nilai itu menurun pada minggu berikutnya. Ini adalah berlawanan dengan γ - dan δ -tokoferol signifikan ($P < 0.05$) menurun secara berperingkat ketika proses peranakan. Buah yang lewat dituai mempunyai masa untuk mensintesis dan mengumpulkan sebatian bioaktif justeru menyebabkan corak nilai yang meningkat. Manakala, corak penurunan oleh sebatian bioaktif disebabkan ia berfungsi sebagai bahan dalam mensintesis sebatian lain. Aktiviti antioksidan berkurang dengan signifikan ($P < 0.05$) seperti yang diukur dengan esei DPPH dan BCLAM berkemungkinan disebabkan penurunan sebatian antioksidan utama iaitu TPC dan TFC. Analisis multivariate mendedahkan perpisahan jurang yang jelas di antara setiap tahap pemasakan disebabkan perubahan transisisebatian biokimia dan antioksidan aktiviti semasa peranakan. Secara kesimpulannya, sebatian bioaktif dan aktiviti antioksidan adalah bergantung kepada kultivar dan peranakan buah. Belimbing cv. B17 adalah yang terbaik dari segi potensi antioksidan dan harus dituai pada minggu ke-9 untuk mendapatkan kebaikan antioksidan dari buah ini.

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I certify that a Thesis Examination Committee has met on 18 October 2013 to conduct the final examination of Mohd Asraf bin Mohd Zainudin on his thesis entitled "Bioactive Compounds and Antioxidant Attribute of Carambola (*Averrhoa carambola* L.) Fruit as Affected by Cultivars and Ripening Stages" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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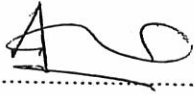
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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENT	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF PLATES	xv
LIST OF ABBREVIATIONS	xvi
LIST OF APPENDICES	xviii

CHAPTER

1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1 Carambola (<i>Averrhoa carambola</i> L.)	
2.1.1 Carambola Cultivars	3
2.1.2 Fruit Development	5
2.1.3 Carambola Nutritional Composition	6
2.1.3.1 Carbohydrate, Protein and Fat	6
2.1.3.2 Fiber	8
2.1.3.3 Vitamin	8
2.1.3.4 Mineral	9
2.1.4 Carambola Health Attribute	9
2.1.4.1 Antioxidant Properties	9
2.1.4.2 Anti-hypercholesteric	15
2.1.4.3 Anti-inflammatory Agent	15
2.1.4.4 Anti-hypertensive	15
2.1.4.5 Hepaprotective Effect	16
2.2 Antioxidant	16
2.2.1 Free Radical Formation and Oxidation Mechanism	16
2.2.1.1 Chemistry of Antioxidant Activity Test	16
2.2.2 Antioxidant and Related Phytochemical Compounds	17
2.2.2.1 Ascorbic Acid	17
2.2.2.2 Carotenoid and Beta-carotene (β -carotene)	18
2.2.2.3 Tocopherols	19
2.2.2.4 Polyphenol Compounds	20
2.2.2.5 Sugars	21
2.3 Factor Influencing Nutritional and Functional Properties of Fruit	22
2.3.1 Preharvest	22
2.3.2. During Harvest	24
2.3.2.1 Maturity Index and Physiological Properties	24

2.3.2.2	Biochemical Changes During Ripening	25
3	METHODOLOGY	28
3.1	Sample Preparation	28
3.1.1	Cultivars Study	28
3.1.2	Carambola cv. B17 Ripening Study	29
3.2	Sample Extraction	29
3.3	Biochemical and Antioxidant Compounds	30
3.4	Antioxidant Activity Assay	32
3.5	Statistical Analysis	33
4	RESULTS AND DISCUSSION	
4.1	Bioactive Compounds and Antioxidant Activity of Three Different Carambola Cultivars (B2, B10 and B17)	34
4.1.1	Ascorbic Acid	34
4.1.2	Total Phenolic Content (TPC)	35
4.1.3	Total Flavonoid Content (TFC)	36
4.1.4	Total Carotenoid Content (TCC) and Beta-carotene (β -carotene)	38
4.1.5	Tocopherols	39
4.1.6	Sugar and Moisture Content	40
4.1.7	Free Radical Scavenging Activity (DPPH) and Beta-carotene-Linoleic Acid Model (BCLAM) Assay	42
4.1.8	Correlation of Bioactive Compounds and Antioxidant Activities	45
4.1.9	Multivariate Analysis between Carambola Cultivars	46
4.2	Effect of Ripening On Bioactive Compounds and Antioxidants Activity of Carambola cv. B17	50
4.2.1	Ascorbic Acid	50
4.2.2	Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)	52
4.2.3	Total Carotenoid Content (TCC) and Beta-carotene (β -carotene)	54
4.2.4	Tocopherols	55
4.2.5	Sugar Content	57
4.2.6	Moisture Content	58
4.2.7	Free Radical Scavenging Activity (DPPH) and β -carotene-Linoleic Acid Model (BCLAM) Assay	59
4.2.8	Multivariate Analysis between Carambola cv. B17 Ripening Stages	61
5	CONCLUSION AND RECOMMENDATIONS	64
	REFERENCES	66
	APPENDICES	86
	BIODATA OF STUDENT	91
	LIST OF PUBLICATIONS	92

LIST OF TABLES

Table		Page
1	Carambola Nutritional Profiling	7
2	Carambola Antioxidant compounds and activity profiling	11
3	Tocopherols content in three different carambola cultivars (B2,B10 and B17)	41
4	Sugar content in three different carambola cultivars (B2, B10 and B17)	42
5	Correlation coefficient between antioxidant compounds and antioxidant activity using Pearson correlation	47

LIST OF FIGURES

Figure		Page
1	Antioxidant role in free radical reaction chain	16
2	Comparison of ascorbic acid content in three different carambola cultivars B2, B10 and B17	35
3	Total phenolic content(TPC) of three different carambola cultivars	37
4	Total flavonoid content (TFC) of three different carambola cultivars	38
5	Total carotenoid content (TCC) and β -carotene of different carambola cultivars	39
6	Antioxidant activity measured using DPPH scavenging activity at inhibition concentration 50% (IC_{50}) of three different carambola cultivars	44
7	Antioxidant activity measured using β -carotene linoleic acid model (BCLAM) assay of three different carambola cultivars	44
8	The score plot between three carambola cultivar	49
9	The loading plot of variables tested with antioxidant activities were the y-variables and the bioactive compounds were x-variables	50
10A	Coefficient correlation between DPPH and the bioactive compounds from three different carambola cultivars	51
10B	Coefficient correlation between BCLAM and the bioactive compounds from three different carambola cultivars	51
11	Ascorbic acid content of carambola cv. B17 at different ripening stages	53
12	Total phenolic compound of carambola cv. B17 at different ripening stages	54
13	Total flavonoid content of carambola cv. B17 at different ripening stages	55
14	Total carotenoid content and β -carotene of carambola cv. B17 at different ripening stages	56
15	Tocopherol content of carambola cv. B17 at different ripening stages	58
16	Sugar content of carambola cv. B17 at different ripening stages	60

17	Moisture content of carambola cv. B17 at different ripening stages	62
18	Antioxidant activity measured using DPPH scavenging activity at inhibition concentration 50% (IC ₅₀) of carambola cultivars B17 at different ripening stages	62
19	Antioxidant activity measured using β -carotene linoleic acid model (BCLAM) of carambola cultivars B17 with concentration 1.25 mg/ml at different ripening stages	63
20A	The score plot of carambola cv. B17 at different ripening stages	65
20B	The loading plot of carambola cv. B17 at different ripening stages	65



LIST OF PLATES

Plate		Page
1	Carambola tree and bagging fruit practices	3
2	Carambola fruit cultivar B2, B10 and B17	4
3	Carambola flower inflorescence formation and bell shape structure	5
4	Carambola maturity indices adopted from SIRIM standard	6
5	Three different carambola cultivars (B10, B2 and B17) at ripening stages 5	29
6	Carambola cv. B17 at different ripening stages starting from week 9 after fruit set until week 13 after fruit set	30

LIST OF ABBREVIATIONS

TPC	Total Phenolic Compound
TFC	Total Phenolic Compound
AA	Ascorbic Acid
AAO	Ascorbate Oxidase
TCC	Total Carotenoid Content
cv	Cultivar
DPPH	2,2 Diphenyl-1-Picrylhydrazyl
BCLAM	β -carotene Linoleic Acid Model
ABTS	2,2'-Azino-Bis-3-Ethylbenzthiazoline- 6-Sulfonic Acid
AEAC	L-Ascorbic Acid Equivalent Antioxidant Capacity
TE	Trolox Equivalents
TEAC	Trolox Equivalent Antioxidant Capacity
ORAC	Oxygen Radical Absorbance Capacity
TRAP	Total Peroxyl Radical-Trapping Antioxidant Parameter
FRAP	Ferric Reducing/Antioxidant Power
BCE	Beta-carotene Equivalent
SPE	Solid Phase Extraction
FRF	Fibre Rich Fraction
TDF	Total Dietary Fibre
IDF	Insoluble Dietary Fibre
AIS	Alcohol Insoluble Solid
WIS	Water Insoluble Solid
WIFF	Water Insoluble Fiber Rich Fraction
CIF	Carambola Insoluble Fiber
HAT	Hydrogen Atom Transfer
ET	Electron Transfer
ROS	Reactive Oxygen Species
BHT	Butylatedhydroxytoluene
BHA	Butylated Hydroxyanisole
MVDA	Multivariate Data Analysis
PCA	Principal Component Analysis
PLS	Partial Least Square Regression
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
SOD	Superoxide Dismutase
GSH	Reduced Gluthathione
AO	Ascorbate Oxidase
APX	Ascorbate Peroxidase
GalURL-	Galacturonic Acid Reductase
GLDase	L-Galactono Dehydrogenase
PAL	Phenylalanine Lyase
PPO	Polyphenol Oxidase
PSY	Phytoene Synthase
γ -TMT	Gamma Methyl Transferase
ANR	Anthocyanin Reductase
LAR1/2	Leucoanthocyanidin Reductase

MIOX	Myo-Inositol Oxygenase
AKR	Aldo/Keto Reductase
ANS	Anthocyanidin Synthase
LCYe	Lycopene ϵ -Cyclase
CRTISO	Carotenoid Isomerase
ZDS	ζ (Zea)-Carotene Desaturase
HYb	β -Ring Hydroxylase
PDS	Phytoene Desaturase
LCYb	Lycopene β -cyclase
g	Gram
mg	Milligram
μ g	Microgram



LIST OF APPENDICES

Plate		Page
1	Ascorbic acid biosynthesis	88
2	Tocopherol and tocotrienol structure.	89
3	Tocopherols and carotenoid biosynthesis pathways in plant	89
4	Data of three different carambola cultivars in table form	90
5	Data of carambola cv. B17 at different ripening stages data in table form	91

CHAPTER 1

INTRODUCTION

In view of the consumer demand and current trend in functional food and nutraceutical industry, the demand for natural dietary antioxidant is increasing due to their medicinal benefits and ability to combat oxidative stress generated free radicals. These free radicals and reactive oxygen species are linked with the incidence of some common diseases like cardiovascular disorders, cancer, aging, and Alzheimer and metabolic syndrome (Maxwell et al., 1997; Tuppo et al., 2001; Hong et al., 2012). Unhealthy life styles in modern world have made free radicals issue more critical. Diet can be an important source of these free radicals but a healthy diet has been linked to a reduction of such diseases. The food pyramid advocates fruits and vegetables as the second most to be consumed after grains and cereals.

Fruits and vegetables contain a wide spectrum of bioactive compounds which have tremendous effect on human health. Many studies support the positive correlation between high intake of fruits and vegetables with reduction of diseases caused by free radicals (Park et al., 2003; O'Bryne et al., 2002; Rautiainen et al., 2012; Marnewick et al., 2011; Mizrahi et al., 2011). Diet consists of fruit had been shown to significantly reduce the risk of metabolic syndrome among adults (Hong et al., 2012). This study had been carried further in another different study which found that diet consists of high antioxidant level fruits for 14 days could lower the cardiovascular diseases (CVD) risk compared to lower antioxidant fruit diet (Franzini et al., 2012). Although many consumers perceive fruits to be expensive due to its low in calories value per price, they misunderstand that in terms of nutritional quality per price, fruits have better score than any other foods or beverages (Darmon et al., 2005). Therefore, enlightenment on selection of fruit that can give maximum nutritional quality per price consumer is pivotal to this group of consumer.

Carambola (*Averrhoa carambola* L.) is one of the popular exotic fruits that are commonly cultivated and consumed in Southeast Asia, in particular, Malaysia. Malaysia is one of the major carambola producers in the world. Other major carambola producers are Israel, Taiwan and Brazil (Othman et al., 2004). Malaysia is one of the largest carambola exporters with production value around RM 54 million in 2012. The amount of carambola production had also increased from 9 106 ton metric in 2007 to 12 999 ton metric in 2012 (MOA, 2012). Ninety eight percent (98%) of the production had been exported to international market while only 2% were used for local consumption. This shows that carambola market is big potential and has great demand internationally. According to Malaysian Dietary Guideline (2010), medium size carambola fruit weighted 261 g is equivalent to one serving size out of two servings per day which had been recommended. Carambola can be categorized as a fruit with high antioxidant capacity based on previous studies (Leong and Shui, 2002; Isabelle et al., 2010, Lim et al., 2007; Mahanattanawee et al., 2006; Charoensiri et al., 2009a).

Despite its high antioxidant attributes compared to other fruit, there is still lack of information on the antioxidant capacity of different carambola cultivars and at

different ripening stages. Cultivars and ripening stages are among the main factors that can affect the antioxidant capacity of fruits (Tlili et al., 2011; Xu et al., 2006; Pinelli et al., 2011; Leonthowicz et al., 2008; Park et al., 2011). Moreover, carambola falls under non-climateric fruit category, implying that the fruit has to be harvested at the right maturity stage (O'hare, 1993). Previous researchers had studied both factors but tend to focus on postharvest physiological quality and processed product such as fresh-cut fruit (Warren et al., 2007; Teixeira et al., 2006; Ding et al., 2007; Bai et al., 2009; Weller 1997; Chin et al., 1999; Ali et al., 2004). However, there was no study that focused on these two factors with respect to antioxidant capacity of carambola fruit. This study is thus instrumental in evaluating the current carambola cultivar used for commercialization. Groups such as farmers, consumers and food producers will benefit from this study. Farmers can select the best cultivar to be cultivated in terms of health benefit in order to fulfill the demand by health-councious consumer. Meanwhile, consumers can get knowledge on the right ripening stage for fresh consumption to gain the optimum health benefit from its antioxidant capacity. This study can also give new view on the current carambola fruit specification that has been used for processed product from carambola fruit or functional food supplement. Therefore, the aims of the study are to;

- compare the bioactive compounds present and antioxidant activities of different carambola (*Averrhoa carambola* L.) fruit cultivars B2, B10 and B17
- evaluate the bioactive compounds and antioxidant activities of carambola cv. B17 at different ripening stages.

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LIST OF PUBLICATIONS

1. Mohd Asraf Mohd Zainudin, Azizah Abdul Hamid, Farooq Anwar, Norshahida Mohamad Shofian And Mohd Sabri Pak Dek (2012) Effect Of Fluorescent Light On Selected Antioxidant Compounds And Antioxidant Activity During Storage Of Fresh-Cut Carambola (*Averrhoa carambola* L.) Fruit. *Pakistan Journal of Botany*, 44(5): 1681-1688.
2. Mohd Asraf Mohd Zainudin, Azizah Abdul Hamid, Farooq Anwar, Azizah Osman and Nazamid Saari(2013). Variation of Bioactive Compounds and Antioxidant Activity Among Three Cultivars (B2, B10 And B17) of Carambola (*Averrhoa carambola* L.) Fruit, *Agrochimica*,57(3), 264-278
3. Mohd Asraf Mohd Zainudin, Azizah Abdul Hamid, Farooq Anwar, Azizah Osman and Nazamid Saari(2014). Variation Of Bioactive Compounds and Antioxidant Activity of Carambola (*Averrhoa carambola*) Fruit at Different Ripening Stages, *Scientia Horticulturae*, 172, 325-331.
4. Azizah Abdul Hamid, Mohd Sabri Pak Dek, Tan Ching Ping, Mohd Asraf Mohd Zainudin, Evelyn Koh Wee Fang. (2014). Changes of major antioxidant compounds and radical scavenging activity of palm oil and rice bran oil during deep-fat frying, *Antioxidant*, Accepted on 15th May 2014.

CONFERENCES

1. 8th Food Science and Technology Seminar (15-16 March 2013), Universiti Sultan Zainal Abidin, Kuala Terengganu. Poster Title; Bioactive Compounds and Antioxidant Capacity Carambola (*Averrhoa carambola* L.) Fruit Cultivars B17 at different ripening stages.
2. 7TH INTERNATIONAL POSTHARVEST SYMPOSIUM 2012 (25-29), Kuala Lumpur. Poster Title; Comparison of Selected Bioactive Compounds and Antioxidant Capacity between Three Different Malaysian Carambola (*Averrhoa carambola* L.) Fruit Cultivars (B2, B10 and B17).
2. NATIONAL BIOTECHNOLOGY SEMINAR 2010, (24-26 May), Kuala Lumpur. Participant
3. SIMPOSIUM BIOLOGI MALAYSIA 2009, Equatorial Hotel, Bangi. Poster Title ; Effect of Fluorescent Light on Selected Antioxidant Compounds and Antioxidant Activity During Storage of Fresh-Cut Carambola (*Averrhoa carambola* L.) Fruit.

AWARD

1. Winner in 2012 Developing Solution For Developing Countries (DSDC) Competition organized by Institute of Food Technology Student Association(IF TSA) in Las Vegas, USA(1st Place)
2. Winner in 2013 Developing Solution For Developing Countries (DSDC) Competition organized by Institute of Food Technology Student Association(IF TSA) in Chicago, USA(1st Place)
2. 3rd Place, Poster Presentation Competition in 8th Food Science and Technology Seminar organized by Unisza and MIFT in KualaTerengganu,





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