

PHYSICOCHEMICAL, BIOCHEMICAL AND ORGANOLEPTIC ANALYSIS OF
PINEAPPLE CULTIVARS MD2 AND MORRIS

IRDLINNA BINTI IDWAR

UNIVERSITI TEKNOLOGI MALAYSIA

PHYSICOCHEMICAL, BIOCHEMICAL AND ORGANOLEPTIC ANALYSIS OF
PINEAPPLE CULTIVARS MD2 AND MORRIS

IRDLINNA BINTI IDWAR

A dissertation submitted in partial fulfilment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JANUARY 2014

To my much-loved parents and friends.

ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude to Allah SWT for giving me His wonderful blessing.

The success and accomplishment of this project were from the support and encouragement from others despite my own effort. I would like to sincerely thank Dr. Razauden Bin Mohamed Zulkifli, my supervisor in this project. His qualities as an extraordinary teacher, counselor and human being will always be remembered.

I would like to extend my appreciation to the members of Nutritional Biochemistry Laboratory members, Nurul Izzah Ismail, Wan Nur Atiqah and Muhd Helmi for their assistance and criticism of this dissertation. Very special thanks go to staffs in Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia for providing materials and equipments during this study.

Next, I would like to express heartfelt appreciation to my parents, Dr. Idwar Bakarudin and Rosenah Hj. Morshidi, for the unconditional support and affection they have given me during the entire course of study.

Finally, thanks to my friends who have helped me throughout this project. Your assistance is gratefully acknowledged.

ABSTRACT

According to Malaysian Pineapple Industry Board (MPIB), pineapple industry is one of the important agricultural sectors in Malaysia thus plays a role in national earnings as one of the world top pineapple suppliers. In Malaysia, pineapple cultivars include Maspine, Morris, Morris Gajah, Gandul, Sarawak, Josapine, N36, Yankee and MD2. Each cultivar has its own physical and phytochemical uniqueness. Before 'MD2' was introduced, 'Morris' was one of the popular cultivars for fresh consumption. This study was geared toward comparing physicochemical, biochemical and organoleptic evaluations of two commercial pineapple cultivars 'MD2' and 'Morris'. Six fruits of each cultivars were purchased at commercial maturity stage which is about 20-40% yellowing of fruit peel and flesh of fruits were used for evaluations. Results of physicochemical analysis showed that 'MD2' is the sweeter and less astringent variety compared to 'Morris' and the same was also observed biochemically where antioxidant capacity, protein content and bromelain enzymatic activity were higher in 'MD2'. For organic acids, 'MD2' had a slightly lower content of oxalic and malic than 'Morris'. Sensory analysis had also supported 'MD2' as the most preferred among the two cultivars where all the attributes such as aroma, flavor, sweetness, off-taste, texture and overall preference scored higher compared to 'Morris'. As a conclusion, 'MD2' compares very well against 'Morris', hence its cultivation in Malaysia for potential local and international market should be encouraged not only due to their higher consumers' preference but nutritional benefits. All the mean differences observed between the cultivars were statistically significant except for protein content and organic acids quantification.

ABSTRAK

Menurut Lembaga Perindustrian Nanas Malaysia (MPIB), industri nanas merupakan salah satu sektor pertanian yang penting di Malaysia dan memainkan peranan penting dalam pendapatan negara sebagai salah satu pembekal nanas terbesar di dunia. Kultivar nanas di Malaysia termasuk 'Maspine', 'Morris', 'Morris Gajah', 'Gandul', 'Sarawak', 'Josapine', 'N36', 'Yankee' dan 'MD2'. Setiap kultivar mempunyai keunikan tersendiri secara fizikal dan fitokimia. Sebelum 'MD2' diperkenalkan, 'Morris' adalah salah satu daripada kultivar yang popular untuk kegunaan segar. Kajian ini menjurus ke arah membandingkan fizikokimia, biokimia dan nilai penilaian deria dua kultivar nanas yang komersial iaitu 'MD2' dan 'Morris'. Enam biji buah bagi setiap kultivar telah dibeli pada peringkat kematangan komersial iaitu kira-kira 20-40% kekuningan kulit buah-buahan dan isi buah-buahan telah digunakan untuk penilaian. Keputusan analisis fizikokimia menunjukkan bahawa 'MD2' adalah lebih manis dan kurang masam berbanding 'Morris' dan yang sama juga diperhatikan secara biokimia dimana kapasiti antioksidan, kandungan protein dan aktiviti enzim bromelain adalah lebih tinggi dalam 'MD2'. Bagi asid organik, 'MD2' mempunyai kandungan oxalic dan malic yang rendah daripada 'Morris'. Analisis deria juga telah menyokong 'MD2' sebagai yang paling digemari di mana semua sifat-sifat seperti aroma, rasa, kemanisan, off-taste, tekstur dan kesukaan secara keseluruhan menjaringkan lebih tinggi berbanding dengan 'Morris'. Kesimpulannya, 'MD2' berbanding dengan baik terhadap 'Morris', oleh itu penanaman 'MD2' di Malaysia untuk potensi pasaran tempatan dan antarabangsa perlu digalakkan bukan sahaja kerana tahap kesukaan pengguna yang lebih tinggi malah faedah pemakanan yang berkhasiat. Semua perbezaan min antara kultivar secara statistik adalah signifikan kecuali kandungan protein dan kandungan asid organik.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	x
	LIST OF FIGURES	xi
	LIST OF ABBREVIATIONS AND SYMBOLS	xiii
	LIST OF APPENDICES	xv
1	INTRODUCTION	1
	1.1 Research Background	1
	1.2 Problem Statement	3
	1.3 Objectives	4
	1.4 Scope of Study	4
	1.5 Significance of Study	5
2	LITERATURE REVIEW	6
	2.1 Introduction	6
	2.2 Pineapple Fruit	6
	2.2.1 Description	6
	2.2.2 Morphology	7
	2.2.3 History of Pineapple	9
	2.2.4 Taxonomy	9
	2.2.5 Commercial Uses	10

	2.2.6 Cultivars	12
2.3	The Importance of Fruits in Diet	13
	2.3.1 Nutritional Benefits	13
2.4	Beneficial Phytochemicals	14
2.5	Antioxidant Capacity	16
	2.5.1 Reactive Oxygen Species (ROS)	16
	2.5.2 Antioxidants Prevent Against Disease	17
	2.5.3 DPPH Radical Scavenging Activity to Determine Antioxidant	18
2.6	Organic Acids in Fruits	19
3	MATERIALS AND METHODS	24
3.1	Experimental Design	24
3.2	Sample	25
3.3	Chemicals and Reagents	25
3.4	Equipments	25
3.5	Physicochemical Analysis Method	26
	3.5.1 Liquid Sample Extraction	26
	3.5.2 pH Measurement	26
	3.5.3 Total Soluble Solids	27
	3.5.4 Titratable Acidity	27
	3.5.5 Sweetness and Astringency Index	28
3.6	Biochemical Analysis	28
	3.6.1 Bromelain Enzymatic Activity Test	28
	3.6.1.1 Extraction of Samples	28
	3.6.1.2 Determination of Bromelain Enzyme Proteolytic Activity	29
	3.6.1.3 Protein Content Quantification	30
	3.6.1.4 Determination of Bromelain Specific Activity	31
	3.6.2 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Capacity Assay	31
	3.6.2.1 Extraction of Sample	32
	3.6.2.2 Determination of DPPH Radical	

	Scavenging Capacity	32
3.6.3	Organic Acid Determination and Quantification	33
3.6.3.1	Extraction of Samples	33
3.6.3.2	Determination and Quantification of Malic and Oxalic Acids	33
3.7	Organoleptic Tests	34
3.8	Statistical Analysis	35
4	RESULTS AND DISCUSSION	36
4.1	Introduction	36
4.2	Physicochemical Analysis of Fruits	36
4.3	Biochemical Analysis of Fruits	40
4.3.1	Antioxidant Capacity	40
4.3.1.1	DPPH Radical Scavenging Capacity	41
4.3.2	Bromelain Enzymatic Activity of Pineapple Cultivars	43
4.3.3	Organic Acid Identification and Quantification	46
4.4	Organoleptic Analysis of Pineapple Cultivars	51
5	CONCLUSION AND RECOMMENDATIONS	54
5.1	Conclusion	54
5.2	Recommendations	55
	REFERENCES	57
	APPENDICES A - E	66-77

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Malaysian Pineapple Cultivars Obtained From MPIB Sources	13
2.2	Nutritional Information of Pineapple	16
4.1	Physicochemical analysis of two Pineapple Cultivars	37
4.2	DPPH Radical Scavenging Capacity of Pineapple Cultivars 'MD2' and 'Morris' indicated in IC ₅₀ (mg/ml)	42
4.3	Comparison of protein content, bromelain proteolytic activity and bromelain specific activity of both 'MD2' and 'Morris' pineapple cultivars	45
4.4	Quantification of oxalic and malic acids in 'MD2' and 'Morris' pineapple cultivars	50

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Morphology of a pineapple plant (<i>A. comosus</i>) (Coppens <i>et al.</i> , 2011)	8
2.2	Pineapple jam produced locally by MPIB	11
2.3	Interconversion reaction of malate and oxaloacetate by MDH enzyme	20
2.4	Interconversion reaction of oxaloacetate to citrate by CS enzyme	20
2.5	A 2 dimensional structure of L-malic acid	21
2.6	Chemical structure of oxalic acid	22
3.1	Experimental design of the overall scope of this study	24
3.2	Different pineapple cultivars for organoleptic test	34
4.1	A schematic drawing of how a typical hand held refractometer works	39
4.2	2D structure of DPPH molecule	42
4.3	Assay by electron-transfer reaction (Source from Huang <i>et al.</i> , 2005)	42
4.4	HPLC of malic acid standard	47
4.5	HPLC of oxalic acid standard	47
4.6	Standard calibration curve of malic acid obtained	48
4.7	Standard calibration curve of oxalic acid obtained	48
4.8	HPLC organic acid profile from 'MD2' fruit sample	49

4.9	HPLC organic acid profile from 'Morris' fruit sample	49
4.10	Graph represents mean values of sensory properties of pineapple cultivars	52
4.11	The mean popularity of pineapple cultivars	53

LIST OF ABBREVIATIONS AND SYMBOLS

AE	:	Albumin Equivalents
AI	:	Astringency Index
cv.	:	Cultivar
DNA	:	Deoxyribonucleic Acid
DPPH	:	2,2-Diphenyl-1-Picrylhydrazyl
EC	:	Enzyme Commission
e.g.	:	exempli gratia
EDTA	:	Ethylenediaminetetraacetic Acid
ET	:	Electron Transfer
et al.	:	and Others
etc.	:	et cetera
g	:	Gram
h	:	Hours
H ₂ O ₂	:	Hydrogen Peroxide
HAT	:	Hydrogen Atom Transfer
IC ₅₀	:	Concentration providing 50% Inhibition or 0.5 of Absorbance
kg	:	Kilogram
L	:	Litre
M	:	Molar
mg	:	Milligram
ml	:	Millilitre
mM	:	Millimolar
MARDI	:	Malaysian Agricultural Research and Development Institute
min	:	Minute

n	:	Sample size
nm	:	Nanometer
ROS	:	Reactive oxygen species
rpm	:	Revolutions per Minute
SEM	:	Standard Error of Mean
SI	:	Sweetness Index
SPSS	:	Statistical Package for the Social Sciences
TA	:	Titrateable Acidity
TSS	:	Total Soluble Solids
UV-VIS	:	Ultraviolet–Visible
v	:	Volume
var.	:	Variety
w	:	Weight
WW	:	Wet weight
μg	:	Microgram
μl	:	Microlitre
°C	:	Degree Celsius
%	:	Percent

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A	T-Test Result From Spss	67
B	Normality Test	70
C	Organoleptic Analysis Data	73
D	Standard Curve	75
E	Organoleptic Survey Form Example	76
F	Retention times (RT) in min for Oxalic and Malic Acids from 'MD2' and 'Morris' Pineapple Cultivars	77

CHAPTER 1

INTRODUCTION

1.1 Research Background

Agriculture and agro-based industry is one of the main contributors to the national economy growth and development and is considered Malaysia's third machine of economic growth. World pineapple trade has shown increasing trend for the past three decades. According to Malaysian Pineapple Industry Board (MPIB), pineapple industry is one of the important agricultural sectors in Malaysia thus plays a role in national earnings as one of the world top pineapple suppliers. Conferring to Economic and Technology Management Review (e-ETMR), Malaysia is listed as top 15 in fresh pineapple exporter and number 9 for canned. Statistics of fresh pineapple production and canned pineapple in Malaysia for 2011 was 96957 metric tonnes and 17165 metric tonnes, respectively (MPIB).

Pineapple (*Ananas comosus*) is a tropical plant with edible multiple fruit and named after its similarity to the pine cone. It is the most economically important fruit in the *Bromeliaceae* family. Rangan (1984) stated that the palatable percentage of the fruit constitutes about sixty percent of their weight and contains approximately eighty five percent water, 0.4% protein, 14% sugar, 0.1% fat and 0.5% fibre. Pineapple is sweet, flavorful, aromatic and has several beneficial properties including an antioxidant property. This fruit contains sugar, vitamin C, and bromelain, a

proteolytic enzyme that breaks down protein that helps fight infections by dissolving layers of slough and bacteria-rich surfaces. Bromelain has proven medicinal uses such as anti-inflammatory effects and subsiding swelling of inflammation such as acute sinusitis, sore throat, arthritis and provide faster recovery from wounds and surgery (Maurer, 2001). Due to its attractive sweet flavor, pineapple is widely consumed as fresh fruit, fruit juice, canned fruit, and as an element in desserts. World production and commercial applications of selected fruits based on Hui (1991) states that pineapple is ranked 8th in the largest 1998 world production (106 ton/year) compared to many other fruits.

Preference of fruits are mainly determined by general composition that greatly influence its flavor and nutritional properties. Since amount and concentration of sugar, organic acid and phenolic compounds greatly influences taste of the commodity (Kelebek *et al.*, 2009), physicochemical and biochemical tests were conducted to improve better knowledge and understanding of consumer's preference. Many sensory characters such as aroma (retronasal sensation observed when chewing and then swallowing), tenderness vs. firmness, saccharinity, astringency and sweetness to acidity ratio are of great importance in terms of preference. However, consumers are now more concerned with the nutritional qualities and benefits of their diet in addition to these general sensory characteristics. Fruit nutrients which are considered important are vitamins, minerals, phenolics, and carotenoids (responsible for the yellow to orange color of fruits). Phenolic compounds contribute toward some useful biological actions for example antioxidative (Larrauri *et al.*, 1997), anti-browning (Chaisakdanugull *et al.*, 2007) and anti-inflammatory (Hale *et al.*, 2005) properties.

The sugar composition of pineapple fruit plays a significant part in its apparent quality, sweetness level and people's acceptability. Sugar level is influenced by weather and fruit maturity stage or conditions. Total soluble solids content (TSS) test the solids concentration of a sucrose containing solution, which is the typical quality attributing for assessing a fruit's sweetness (Delwiche *et al.*, 2008).

Organic acids are present in different concentrations and varieties in different types of fruits. According to Belitz *et al.* (2009), major organic acids found in

pineapple are citric and malic acid. Organic acids are helpful guide of authenticity in fruit product. The organic acid composition of fruits is of great concern because of its significant impact on the sensory characteristics of fruit juices (Kelebek *et al.*, 2009).

Pineapple in Malaysia are planted all across Peninsular and East Malaysia. The most familiar varieties are 'MD2', 'Sarawak', 'Yankee', 'Josapine' and 'Morris Gajah' for fresh fruit intake. 'Sarawak' is also known as 'Smooth Cayenne' in Spanish or 'Kew' in Thailand. Another variety called 'Gandul' is processed for canning and its juice. Varieties 'N36' and 'Maspine' are used for both fresh and canning processes (MPIB). Several research were carried out previously to study and compare the chemical composition, morphology and bioactives of different pineapple varieties. Bartolomé *et al.* (1995) studied morphological features, chemical constituents and sensory analysis of 'Red Spanish' and 'Smooth Cayenne' cultivars while Brat *et al.* (2004) compared the physicochemical characteristics of a new pineapple hybrid named 'FLHORAN41' with 'Smooth Cayenne'. Hossain *et al.* (2011) worked on total phenolic compounds and antioxidant activity of pineapples. The result from many previous studies proved that different cultivars have different phytochemical characteristics.

1.2 Problem Statement

Pineapples are beneficial and nutritious to human due to its phytochemical compositions. The industry is a big contributor to the agricultural sector in Malaysia. Based on previous research, different pineapple varieties contain different chemical compositions. However, physicochemical and biochemical evaluation and comparison of commercially available pineapple cultivars in Malaysia have yet to be fully accomplished. Thus, this study was geared toward analyzing the differences among two different cultivars 'MD2' and 'Morris' to come up with useful information on nutritional values and beneficial health benefits of the two commercial pineapple

cultivars in Malaysia to create vital data resource for further research of the fruit as a reference for commercial use.

1.3 Objectives

- i) To compare physicochemical properties of pineapple cultivars 'MD2' and 'Morris'.
- ii) To analyze the biochemical characteristics of pineapple cultivars 'MD2' and 'Morris'.
- iii) To evaluate organoleptic characteristics of pineapple cultivars 'MD2' and 'Morris'.

1.4 Scope of Study

For this study, six pineapple fruits of two different cultivars 'MD2' and 'Morris' were purchased at commercial maturity stage, which is about 20%-40% yellowing of fruit peel. The edible portions of the fruit were taken as sample for evaluation. For physicochemical tests, the edible portion of fruit pulp were homogenized and the juice was analyzed for pH, titratable acidity (TA) and total soluble solids (TSS) in degrees Brix at 20°C. Biochemical tests composed of determination of DPPH radical scavenging capacity, protein content, bromelain enzyme specific activity and selected organic acids quantification. Different solvents and methods were used for the extraction of pineapple for different tests. Antioxidant capability of pineapple fruits was determined using DPPH radical scavenging ability. Bromelain enzyme proteolytic activity, protein content and specific activity were determined. Organoleptic test included evaluation of appearance, texture, aroma, off-flavor, sweetness and overall preference involving 30 taste respondents by using 5

point Hedonic scale (1: Dislike Extremely; 2: Dislike; 3: Neither like nor dislike; 4: Like; 5: Like Extremely). Lastly, statistical assessment was performed using IBM SPSS 18 Statistics software.

1.5 Significance of Study

Even though pineapple industry is one of the biggest contributor to Malaysian agricultural sector's gain, the physicochemical, biochemical and organoleptic tests and comparison among commercial cultivars are yet to be fully established. Thus, this study was geared to determine, compare and relate the physicochemical and biochemical components, antioxidant ability, enzymatic activity and organoleptic sensory evaluation of two different commercial pineapple cultivars in Malaysia.

REFERENCES

- Abu-Goukh, A. B., Shattir, A. and Mahdi, E. F. (2010). Physico-chemical changes during growth and development of papaya fruit. II: Chemical changes. *Agriculture and Biology Journal of North America*, 1(5), 871-877.
- Alothman, M., Bhat, R. and Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*, 115(3), 785-788.
- Aluko, R. E. (2012). Functional Foods and Nutraceuticals, 99-108. New York, NY: Springer New York.
- Ames, B. M., Shigena, M. K. and Hagen, T. M. (1993). Oxidants, antioxidants and the degenerative disease of aging. *Proceedings of the National Academy of Sciences of the United States of America*, 90, 7915-7922.
- AOAC. (1984). Official Methods of Analysis. (14th ed.). (pp. 579-580). USA: Association of Official Analytical Chemists Inc.
- Bajpai, V. K., Yoon, J. I., and Chul Kang, S. (2009). Antioxidant And Antidermatophytic Activities Of Essential Oil And Extracts Of *Metasequoia Glyptostroboides* Miki ex Hu. *Food And Chemical Toxicology*, 47(6), 1355-1361.
- Bartholomew, D. P., Paull, R. E. and Rohrbach, K. G. (2003). *The pineapple: botany, production and uses*. (eds). (pp. 1-301). CABI Publishing, Wallingford.
- Bartolomé, A., Rupérez, P. and Fúster, C. (1994). Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of Red Spanish and Smooth Cayenne Cultivars. *Food chemistry*, 53, 75-79.

- Beaudry, R., Severson, R., Black, C. and Kays, S. (1989). Banana ripening: implications of changes in glycolytic intermediate concentrations, glycolytic and gluconeogenic carbon flux, and fructose 2, 6-bisphosphate concentration. *Plant physiology*, 91, 1436-1444.
- Belitz, H., Grosch, W., and Schieberle, P. (2009). *Food Chemistry: Aroma Compounds*. (4th ed). (pp. 340-402). Springer Berlin Heidelberg.
- Bio-Rad. Bio-Rad Protein Assay. URL: http://labs.fhcrc.org/hahn/Methods/biochem_meth/biorad_assay.pdf. Accessed 01.10.13.
- Borrelli, F., Capasso, R., Severino, B., Fiorino, F., Aviello, G., De Rosa, G. and Mazzella, M. (2011). Inhibitory effects of bromelain, a cysteine protease derived from pineapple stem (*Ananas comosus*), on intestinal motility in mice. *Neurogastroenterology and motility: The Official Journal Of The European Gastrointestinal Motility Society*, 23(8), 745-e331.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.
- Brat, P., Hoang, L. N. T., Soler, A., Reynes, M. and Brillouet, J. M. (2004). Physicochemical Characterization Of A New Pineapple Hybrid (FLHORAN41 Cv.). *Journal Of Agricultural And Food Chemistry*, 52(20), 6170-7.
- Brien, S., Lewith, G., Walker, A., Hicks, S.M. and Middleton, D. (2004). Bromelain as a Treatment for Osteoarthritis: A Review of Clinical Studies. *Evidence-based Complementary and Alternative Medicine*, 1, 251-257.
- Buck, R. and Rondinini, S. (2002). Measurement of pH. Definition, Standards, and Procedures. *Pure and Applied Chemistry*, 74(11), 2169-2200.
- Cámara, M., Díez, C., Torija, M. and Cano, M. (1994). HPLC determination of organic acids in pineapple juices and nectars. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, 52-56.

- Cámara, M., Diez, C. and Torija, E. (1995). Chemical Characterization Of Pineapple Juices And Nectars. Principal Components Analysis. *Food chemistry*, 54, 93-100.
- Chaisakdanugull, C., Theerakulkait, C. and Wrolstad, R. E. (2007). Pineapple juice and its fractions in enzymatic browning inhibition of banana [Musa (AAA group) Gros Michel]. *Journal Of Agricultural And Food Chemistry*, 55(10), 4252-7.
- Chaiwut, P., Nitsawang, S., Shank, L. and Kanasawud, P. (2007). A Comparative Study On Properties And Proteolytic Components Of Papaya Peel And Latex Proteases. *Chiang Mai Journal of Science*, 34(1), 109-118.
- Choi, Y. and Lee, J. (2009). Antioxidant And Antiproliferative Properties Of A Tocotrienol-Rich Fraction From Grape Seeds. *Food Chemistry*, 114(4), 1386-1390.
- Chong, H. C. (2013). *Nutritional Characteristics Evaluation Of Malaysian Commercial Pineapple Cultivars*. Master. Dissertation. Universiti Teknologi Malaysia; 2013.
- Collins, J. L. (1960) The pineapple: Botany, cultivation, and utilization. New York: Interscience Publishers.
- Coppens d'Eeckenbrugge, G., Leal, F. (2001). Fruits from America: An ethno botanical inventory. URL: http://www.ciat.cgiar.org/ipgri/fruits_from_americas/frutales/Ficha%20Ananas%20comosus.htm; http://www.ciat.cgiar.org/ipgri/fruits_from_americas/frutales/more%20about%20pineapple.htm. Accessed 10.11.13.
- Coppens d' Eeckenbrugge, G. and Leal, F. (2003). Morphology, anatomy and taxonomy. In: D.P. Bartholomew, R.E. Paull, & K.G. Rohrbach (Eds.). *The pineapple: Botany, Production and Uses* (pp. 13-32). Oxon: CABI Publishing.
- Coppens d' Eeckenbrugge, G., Sanewski, G. M., Smith, M. K., Duval, M.-france and Leal, F. (2011). Wild Crop Relatives: Genomic and Breeding Resources. In C.

- Kole (Ed.). *Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits* (pp. 21-41). Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg.
- Davey, M., Sripaoraya, S. and Anthony, P. (2007). Transgenic Crops. *Biotechnology in Agriculture and Forestry*, 60.
- Diakou, P., Svanella, L., Raymond, P., Gaudillere, J. P. and Moing, A. (2000). Phosphoenol-pyruvate carboxylase during grape berry development: protein level, enzyme activity and regulation. *Australian Journal of Plant Physiology*, 27, 221– 229.
- Dull, G. (1971). The Pineapple. In: Hulme AC (3rd Edition). *The Biochemistry of Fruits and Their Products* (pp. 303- 324). Academic Press, New York.
- e-ETMR (Economic and Technology Management Review). *Scenario and Prospect of Malaysia's Pineapple Industry*. URL <http://etmr.mardi.gov.my/index.php/abstract/22-abstract-2vol-4>. Accessed 10.10.13.
- Gailhofer, G., Wilders-Truschnig, M., Smolle, J. and Ludvan, M. (1998). Asthma caused by bromelain: an occupational allergy. *Clinical Allergy*, 18, 445-450.
- Guo, C. and McMartin, K. E. (2005). The cytotoxicity of oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation. *Toxicology*, 208(3), 347-55.
- Hale, L. P., Greer, P. K., Trinh, C. T. and Gottfried, M. R. (2005). Treatment With Oral Bromelain Decreases Colonic Inflammation In The IL-10 Deficient Murine Model Of Inflammatory Bowel Disease. *Clinical Immunology*, 116, 135-142.
- Halliwell, B. and Gutteridge, J. M. C. (1989). Role of free radical and catalytic metals ions in human diseases: An overview. *Methods Enzymologia*, 186, 1–85.

- Hasib, A., Jaouad, A., Mahrouz, M. and Khouili, M. (2002). HPLC Determination of Organic Acids in Moroccan Apricot. *Ciencia y Tecnologia Alimentaria*, 3(4), 207-211.
- Hirai, M. and Ueno, I. (1977). Development of citrus fruits: fruit development and enzymatic changes in juice vesicle tissue. *Plant Cell Physiology*, 18, 791–800.
- Hossain, M. A. and Rahman, S. M. M. (2011). Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Research International*, 44(3), 672-676.
- Huang, D., Ou, B. and Prior, R. L. (2005). The Chemistry Behind Antioxidant Capacity Assays. *Journal Of Agricultural And Food Chemistry*, 53(6), 1841-56.
- Hui, Y. H. (1991). Data sourcebook for Food Scientists and Technologists. VCH Publishers, New York.
- Isabelle, M., Lee, B. L., Lim, M. T., Koh, W. P., Huang, D. and Ong, C. N. (2010). Antioxidant Activity And Profiles Of Common Fruits in Singapore. *Food Chemistry*, 123(1), 77-84.
- Joshi, S., Peck, A. B. and Khan, S. R. (2013). NADPH oxidase as a therapeutic target for oxalate induced injury in kidneys. *Oxidative medicine and cellular longevity*, 2013, 1-18.
- Karadeniz, F. (2004). Main Organic Acid Distribution Of Authentic Citrus Juices In Turkey. *Turkish Journal of Agriculture and Forestry*, 28, 267-271.
- Kelebek, H., Selli, S., Canbas, A. and Cabaroglu, T. (2009). HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. *Microchemical Journal*, 91(2), 187-192. Elsevier B.V.
- Lako, J., Trenerry, V., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S. and Premier, R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry*, 101(4), 1727-1741.

- Larrauri, J., Rupérez, P. and Calixto, F. (1997). Pineapple shell as a source of dietary fiber with associated polyphenols. *Journal of agricultural and Food Chemistry*, 45, 4028-4031.
- Llaudy, M. C., Canals, R., Canals, J. M., Rozés, N., Arola, L. and Zamora, F. (2004). New Method For Evaluating Astringency In Red Wine. *Journal Of Agricultural And Food Chemistry*, 52(4), 742-6.
- Lotz-Winter, H. (1989). On the pharmacology of bromelain: An update with special regard to animal studies on dose-dependent effects. *Planta Medical*, 56, 249-253.
- Maurer, H. (2001). Bromelain : Biochemistry, Pharmacology And Medical Use. *Cellular and Molecular Life Sciences CMLS*, 58, 1234-1245.
- Mhatre, M., Tilak-Jain, J., De, S. and Devasagayam, T.P.A. (2009). Evaluation Of The Antioxidant Activity Of Non-Transformed And Transformed Pineapple: a comparative study. *Food and Chemistry Toxicology*, 47(11), 2696-2702.
- Miller, N. J. and Rice-Evans, C. A. (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chemistry*, 60 (3), 331-337.
- Molyneux, P. (2004). The Use Of The Stable Free Radical Diphenylpicrylhydrazyl (DPPH) For Estimating Antioxidant Activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211-219.
- Morton, J. (1987). Fruits of warm climates. North Carolina: Creative Resource Systems, Inc.
- MPIB (Malaysian Pineapple Industry Board). Official Portal of Malaysian Pineapple Industry Board. URL <http://www.mpib.gov.my/web/guest/home>. Accessed 15.10.12
- Muller, M. L., Irkens-Kiesecker, U., Rubinstein, B. and Taiz, L. (1996). On the mechanism of hyperacidification in lemon. *Journal of Biological Chemistry*, 271, 1916–1924.

- Murachi, T. (1976). Bromelain enzymes. In L. Lorand. (Ed.). *Methods in enzymology* (vol. 1, pp. 475-485). New York: Academic Press.
- Mynott, T., and Ladhams, A. (1999). Bromelain, From Pineapple Stems, Proteolytically Blocks Activation Of Extracellular Regulated Kinase-2 in T Cells. *The Journal of Immunology*, 163(5), 2568-75.
- Nawirska-Olszańska, A., Biesiada, A., Sokół-Łętowska, A. and Kucharska, A. Z. (2013). Characteristics of organic acids in the fruit of different pumpkin species. *Food Chemistry*, 148, 415-419.
- Nasution, M. A. (2010). Genetic Diversity Of Pineapple And Correlation Between Morphological Characters And Fruit Quality Componen With The Bromelain Enzyme. *Jurnal Agrivigor*, 10(1), 62-72.
- Paull, R. E. and Chen, C. C. (2003). Postharvest physiology, handling, and storage of pineapple. In: Bartholomew, D.P., Paull, R., Rohrbach, K.G. (Eds.). *The Pineapple: Botany, Production and Uses* (pp. 253-279). CABI Publishing, Wallingford.
- Purseglove, J.W. (1972). Tropical crops. *Monocotyledons* (pp. 75-91). London: Longman.
- Ruffner, H. P., Possner, D., Brem, S. and Rast, D. M. (1984). The Physiological Role Of Malic Enzyme In Grape Ripening. *Planta*, 160, 444-448.
- Saradhuldhata, P. and Paull, R. E. (2007). Pineapple Organic Acid Metabolism And Accumulation During Fruit Development. *Scientia Horticulturae*, 112(3), 297-303.
- Saura-Calixto, F. and Goñi, I. (2006). Antioxidant capacity of the Spanish Mediterranean diet. *Food Chemistry*, 94(3), 442-447.
- Secor Jr, E. R., Carson, W. R., Cloutier, M., Guernsey, L. A., Schramm, C. M., Wu, C. A. and Thrall, R. S. (2005). Bromelain exerts anti-inflammatory effects in an ovalbumin-induced Murine model of allergic airway disease. *Cellular Immunology*, 237, 68-75.

- Sharma, O. P. and Bhat, T. K. (2009). DPPH Antioxidant Assay Revisited. *Food Chemistry*, 113(4), 1202-1205.
- Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, 40, 945-948.
- Sies, H. (1997). Oxidative stress: Oxidants and Antioxidants. *Experimental Physiology*, 82, 291-295.
- Sigma. Protease Colorimetric Detection Kit (PC0100) – Bulletin. URL <http://www.safcglobal.com/etc/medialib/docs/Sigma/Bulletin/pc0100bul.Par.0001.File.tmp/pc0100bul.pdf>. Accessed 03.08.12.
- Silva, B. M., Andrade, P. B., Valentão, P., Ferreres, F., Seabra, R. M. and Ferreira, M. A. (2004). Quince (*Cydonia Oblonga* Miller) Fruit (Pulp, Peel, And Seed) And Jam: Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, 52, 4705–4712.
- Singleton, V. L. and Gortner, W. A. (1965). Chemical and physical development of the pineapple fruit II. Carbohydrate and acid constituents. *Journal of Food Science*, 30, 19–23.
- Streit, J., Tran-Ho, L. and Königsberger, E. (1998). Solubility of the Three Calcium Oxalate Hydrates in Sodium Chloride Solutions and Urine- Like Liquors. *Monatshefte für Chemie*, 129(12), 1225-1236.
- Thammawong, M. and Arakawa, O. (2010). Starch to Sugar Conversion in “Tsugaru” Apples under Ethylene and 1-Methylcyclopropene Treatments. *Journal of Agricultural Science and Technology*, 12, 617-626.
- Vaclavik, V. and Christian, E. (2008). Vegetables and Fruits. *Essentials of Food Science* (3rd Edition). (pp. 107-141). Springer New York.
- Valko, M., Leibfritz, D. and Moncol, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal Of Biochemistry And Cell Biology*, 39, 44-84.

- Wardy, W. and Saalia, F. (2009). A Comparison Of Some Physical, Chemical And Sensory Attributes Of Three Pineapple (*Ananas Comosus*) Varieties Grown In Ghana. *African Journal of Food Science*, 3(1), 22-25.
- Weinman, E. J., Frankfurt, S. J., Ince, A. and Samson, S. (1978). Renal Tubular Transport of Organic Acids. *The Journal of Clinical Investigation*, 61(3), 801-806.
- Zulkipeli, N. L. (2007). Screening For High Bromelain Content In Different Species Of Pineapple In Malaysia. In PSM Presentation (Bioprocess Engineering Dept). URL http://eprints.utm.my/3171/1/NUR_LINA_ZULKIPELI.pdf. Accessed 10.12.13.