NUTRITIONAL CHARACTERISTICS EVALUATION OF MALAYSIAN COMMERCIAL PINEAPPLE CULTIVARS

CHONG HANG CHIET

UNIVERSITI TEKNOLOGI MALAYSIA
NUTRITIONAL CHARACTERISTICS EVALUATION OF MALAYSIAN COMMERCIAL PINEAPPLE CULTIVARS

CHONG HANG CHIET

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 2013
To my beloved parents and friends.
ACKNOWLEDGEMENTS

Apart from the efforts of myself, the success of this project depends largely on the encouragement and guidelines of many others. First of all, I would like to convey my sincere gratitude to my supervisor, Dr. Razauden Mohamed Zulkifli for giving me a chance to carry out this meaningful project. I appreciate his friendly helping, dedicated support and patient guidance throughout the period of this research work. I would also like to express my appreciation to my co-supervisor, Dr. Topik Hidayat, who has been very helpful in providing guidance and advices for this project.

In addition, I would like to acknowledge all my labmates who have provided assistance at various occasions by sharing their experiences, views and knowledge. Besides, Malaysia Pineapple Industry Board (MPIB) also deserves special thanks for their assistance in supplying the relevant information and guidance. The guidance and support received was vital for the success of this project and I am grateful for their support and help.

Last but not least, I would like to express my heartfelt thanks to my beloved parents for their blessings and spiritually support throughout my life.
ABSTRACT

Pineapple industry is one of the important agricultural sectors in Malaysia with 76 cultivars planted throughout the country. This study aims to generate useful nutritional information as well as evaluating physicochemical, biochemical and organoleptic properties of ‘Josapine’, ‘Morris’, ‘Sarawak’, ‘MD2’ and ‘Crystal’ pineapple (*Ananas comosus*). The pineapple varieties were collected at commercial maturity stage (20-40% yellowish of fruit peel) and the edible portion of the fruit was used as sample for evaluation. From the results obtained, ‘MD2’ showed highest sweetness and lowest astringency index in terms of physicochemical properties and also had highest content of bioactive compounds, antioxidant capacities and bromelain activity with respect to biochemical properties compared to other cultivars. Furthermore, the highest scores for overall sensory attributes also confirmed the preference of ‘MD2’ over all the other cultivars. Hence, ‘MD2’ compared very well with other pineapple cultivars and has great potential in the commercial market. The bioactive compounds were highly and significantly correlated with antioxidant capacities and bromelain activity suggests that these bioactive compounds have contributed to the antioxidant and enzymatic activities of pineapples. All the mean differences observed between the cultivars were statistically significant.
ABSTRAK

## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>ii</td>
<td></td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
<td></td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
<td></td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
<td></td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vii</td>
<td></td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xii</td>
<td></td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
<td></td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS AND SYMBOLS</td>
<td>xiv</td>
<td></td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xvi</td>
<td></td>
</tr>
</tbody>
</table>

### CHAPTER 1 INTRODUCTION

1.1 Research Background 1
1.2 Problem Statement 3
1.3 Objectives 3
1.4 Scope of Study 4
1.5 Significance of Study 4
CHAPTER 2 LITERATURE REVIEW

2.1 The Role of Fruits in Diet

2.1.1 Fruits Nutrition

2.1.2 The Important Phytochemicals of Fruits in Human Diet

2.2 Free Radicals and Antioxidants

2.2.1 Free Radicals

2.2.1.1 Sources of Free Radicals

2.2.1.2 Reactive Oxygen Species (ROS)

2.2.2 Antioxidants

2.2.2.1 Types of Antioxidants

2.3 Pineapple Plant

2.3.1 Origin

2.3.2 Taxonomy

2.3.3 Morphology

2.3.4 Maturity Index of Pineapple Fruit

2.3.5 Nutrients and Phytochemicals of Pineapple Fruit

2.3.5.1 Cysteine Endopeptidases-Bromelain

2.4 Pineapple Industry of Malaysia

2.4.1 Production and Contribution

2.4.2 Commercial Cultivars in Malaysia

CHAPTER 3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Samples

3.1.2 Chemicals and Reagents
3.1.3 Equipments

3.2 Physicochemical Analysis

3.2.1 Extraction

3.2.2 pH

3.2.3 Total Soluble Solids

3.2.4 Titratable Acidity

3.2.5 Sweetness Index and Astringency Index

3.3 Biochemical Analysis

3.3.1 Vitamin C Content Assay

3.3.1.1 Extraction

3.3.1.2 Determination of Vitamin C Content

3.3.2 Total Phenolic Content Assay

3.3.2.1 Extraction

3.3.2.2 Determination of Total Phenolic Content

3.3.3 Tannin Content Assay

3.3.3.1 Extraction

3.3.3.2 Determination of Tannin Content

3.3.4 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Capacity Assay

3.3.4.1 Extraction

3.3.4.2 Determination of DPPH Radical Scavenging Capacity

3.3.5 Ferric Reducing Capacity Assays

3.3.5.1 Extraction

3.3.5.2 Ferric Reducing Ability of Plasma (FRAP) Assay
CHAPTER 4  RESULTS AND DISCUSSION

4.1  Physicochemical Analysis  44
4.2  Biochemical Analysis  48
  4.2.1  Bioactive Compounds  48
  4.2.2  Antioxidant Capacities  50
    4.2.2.1  DPPH Radical Scavenging Capacity  51
    4.2.2.2  Ferric Reducing Capacity  53
  4.2.3  Bromelain Enzymatic Activity  55
4.3  Organoleptic Analysis  57
4.4  Correlations between the Characteristics of Pineapples Cultivars  60

CHAPTER 5  CONCLUSION AND RECOMMENDATIONS

5.1  Conclusion  63
5.2  Recommendations  64
REFERENCES

APPENDICES A - E
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Types of reactive oxygen species (adapted from Halliwell, 2001)</td>
<td>14</td>
</tr>
<tr>
<td>2.2</td>
<td>Comparison between the classification of Smith and Downs (1979) and the present classification (Coppens d’ Eeckenbrugge &amp; Leal, 2003)</td>
<td>18</td>
</tr>
<tr>
<td>2.3</td>
<td>Features of seven levels of pineapple maturity indices (MPIB)</td>
<td>21</td>
</tr>
<tr>
<td>2.4</td>
<td>Comparison of characteristics among the cultivars (Collins, 1949)</td>
<td>27</td>
</tr>
<tr>
<td>2.5</td>
<td>Malaysian pineapple cultivars and their pulp characteristics (MPIB)</td>
<td>28</td>
</tr>
<tr>
<td>4.1</td>
<td>Physicochemical properties of five pineapple cultivars</td>
<td>44</td>
</tr>
<tr>
<td>4.2</td>
<td>pH value comparison of pineapple cultivars by different studies</td>
<td>45</td>
</tr>
<tr>
<td>4.3</td>
<td>Bioactive compounds of five pineapple cultivars</td>
<td>49</td>
</tr>
<tr>
<td>4.4</td>
<td>DPPH radical scavenging capacity of five pineapple cultivars</td>
<td>52</td>
</tr>
<tr>
<td>4.5</td>
<td>Ferric reducing capacities of five pineapple cultivars</td>
<td>54</td>
</tr>
<tr>
<td>4.6</td>
<td>Protein content, bromelain proteolytic activity and specific activity of five pineapple cultivars</td>
<td>56</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Structure of free radical (Reiter &amp; Robenson, 1995)</td>
<td>12</td>
</tr>
<tr>
<td>2.2</td>
<td>The morphological structures of <em>Ananas comusus</em></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>(Coppens d’ Eeckenbrugge &amp; Leal, 2003)</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>Five pineapple cultivars used in this study</td>
<td>29</td>
</tr>
<tr>
<td>3.2</td>
<td>Hand refractometer handling procedure</td>
<td>32</td>
</tr>
<tr>
<td>3.3</td>
<td>Pineapple samples used for organoleptic analysis</td>
<td>42</td>
</tr>
<tr>
<td>4.1</td>
<td>The structure of DPPH</td>
<td>51</td>
</tr>
<tr>
<td>4.2</td>
<td>The FRAP’s reaction</td>
<td>53</td>
</tr>
<tr>
<td>4.3</td>
<td>Mean values of sensory attributes of pineapple cultivars. Error bars refer to standard error (n=30)</td>
<td>58</td>
</tr>
<tr>
<td>4.4</td>
<td>Overall mean values of all sensory parameters of pineapple varieties. Error bars refer to standard error (n=30)</td>
<td>59</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAE</td>
<td>Ascorbic Acid Equivalents</td>
</tr>
<tr>
<td>AE</td>
<td>Albumin Equivalents</td>
</tr>
<tr>
<td>AI</td>
<td>Astringency Index</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ATC</td>
<td>Automatic Temperature Compensation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-Diphenyl-1-Picrylhydrazyl</td>
</tr>
<tr>
<td>EC</td>
<td>Enzyme Commission</td>
</tr>
<tr>
<td>e.g.</td>
<td>exempli gratia</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>ET</td>
<td>Electron Transfer</td>
</tr>
<tr>
<td>et al.</td>
<td>and Others</td>
</tr>
<tr>
<td>etc.</td>
<td>et cetera</td>
</tr>
<tr>
<td>FAMA</td>
<td>Federal Agricultural Marketing Authority</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric Reducing Ability of Plasma</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GAE</td>
<td>Gallic Acid Equivalents</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen Peroxide</td>
</tr>
<tr>
<td>HAT</td>
<td>Hydrogen Atom Transfer</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HO₂⁻</td>
<td>Hydroperoxyl Radicals</td>
</tr>
<tr>
<td>HOCl</td>
<td>Hypochlorous Acid</td>
</tr>
<tr>
<td>HOBr</td>
<td>Hypobromous Acid</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Concentration providing 50% Inhibition or 0.5 of</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>l</td>
<td>Litre</td>
</tr>
<tr>
<td>lbs</td>
<td>Pound</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>MARDI</td>
<td>Malaysian Agricultural Research and Development Institute</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>MPIB</td>
<td>Malaysian Pineapple Industry Board</td>
</tr>
<tr>
<td>n</td>
<td>Sample Size</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>$^1O_2$</td>
<td>Singlet Oxygen</td>
</tr>
<tr>
<td>$O_2$</td>
<td>Molecular Oxygen</td>
</tr>
<tr>
<td>$O_2^-$</td>
<td>Superoxide Anion</td>
</tr>
<tr>
<td>$O_3$</td>
<td>Ozone</td>
</tr>
<tr>
<td>OH·</td>
<td>Hydroxyl Radicals</td>
</tr>
<tr>
<td>ORAC</td>
<td>Oxygen Radical Absorbance Capacity</td>
</tr>
<tr>
<td>RM</td>
<td>Ringgit Malaysia</td>
</tr>
<tr>
<td>RO·</td>
<td>Alkoxy Radicals</td>
</tr>
<tr>
<td>RO$_2^-$</td>
<td>Peroxy Radicals</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per Minute</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
</tr>
<tr>
<td>SI</td>
<td>Sweetness Index</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>TA</td>
<td>Titratable Acidity</td>
</tr>
<tr>
<td>TAE</td>
<td>Tannic Acid Equivalents</td>
</tr>
<tr>
<td>TPTZ</td>
<td>2, 4, 6-Tri [2-Pyridyl]-S-Triazine)</td>
</tr>
<tr>
<td>TRAP</td>
<td>Total Radical Trapping Antioxidant Parameter</td>
</tr>
<tr>
<td>TSS</td>
<td>Total Soluble Solids</td>
</tr>
<tr>
<td>UV-VIS</td>
<td>Ultraviolet–Visible</td>
</tr>
<tr>
<td>v</td>
<td>Volume</td>
</tr>
<tr>
<td>var.</td>
<td>Variety</td>
</tr>
<tr>
<td>w</td>
<td>Weight</td>
</tr>
<tr>
<td>WW</td>
<td>Wet Weight</td>
</tr>
<tr>
<td>$\mu g$</td>
<td>Microgram</td>
</tr>
<tr>
<td>$\mu l$</td>
<td>Microlitre</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Alpha</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Beta</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Gamma</td>
</tr>
<tr>
<td>$&amp;$</td>
<td>And</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normality Test</td>
<td>86</td>
</tr>
<tr>
<td>B</td>
<td>Analysis of Variance (ANOVA) Test</td>
<td>92</td>
</tr>
<tr>
<td>C</td>
<td>Kruskal-Wallis Test</td>
<td>98</td>
</tr>
<tr>
<td>D</td>
<td>Correlations between Physicochemical, Biochemical and Organoleptic Properties of Pineapple</td>
<td>103</td>
</tr>
<tr>
<td>E</td>
<td>Results Summary of Physicochemical, Biochemical and Organoleptic Properties of Pineapple</td>
<td>104</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Research Background

Agriculture and agro-based industry has made a significant contribution to the national economy and development of modern Malaysia. According to Malaysian Pineapple Industry Board (MPIB), pineapple industry is one of the important agricultural sectors in Malaysia which play a role in country’s earnings as one of the world pineapple suppliers. In 2011, it was reported that the production of fresh fruit and canned pineapple in Malaysia estimated around 96,957 metric tonnes and 17,165 metric tonnes (858,007 standard cases), respectively (MPIB). Furthermore, the export of fresh pineapple, canned pineapple and pineapple juice contributed approximately RM 63.4 millions to the national economy in the same year. The canned pineapple has high market demand in countries include Japan, United States, European countries, Singapore, West Asia and others (MPIB).

Pineapple (Ananas Comosus) contains good aroma, flavour, juiciness, sweetness, texture and high nutritional content such as vitamins, phenolics, fibre and minerals (Brat, Thi-Hoang, Soler, Reynes & Brillouet, 2004). The preference and acceptance of consumers are mainly determined by the general composition and nutritional properties of the commodity. The nature and concentration of phenolic compounds, sugar and
organic acid will largely influence the taste and organoleptic characteristics of the fruit (Kelebek, Selli, Canbas & Cabaroglu, 2009). Phenolic compounds in fruits possess numerous biological activities including antioxidant activity, anti-carcinogenic, anti-inflammatory, and anti-atherosclerotic activities (Chung, Wong, Huang & Lin, 1998). On the other hands, sugar content as soluble solids in the juice not just function as sweetener and also play role in appearance, texture, freezing point, fermentation, preservation and antioxidant activity (Clarke, 1995; Phillips, Carlsen & Blomhoff, 2009). The type of organic acid varies in different fruits. The main organic acids of pineapple fruits are citric acid and malic acid (Belitz, Grosch & Schieberle, 2009). Organic acids composition in the fruit can affect the flavour properties and stability of fruit juices, and the organic acids such as ascorbic acid, citric acid and malic acid can also act as natural antioxidant in the fruit (Houlihan & Ho, 1985; Kelebek et al., 2009). Moreover, pineapple is the best known source of endopeptidase bromelain among the plants of the plant family Bromeliaceae (Kumar, Hemavathi & Umesh Hebbar, 2011) which has wide range of applications in many industries such as food, medical, pharmaceutical and cosmetics industries, etc. (Ketnawa, Chaiwut & Rawdkuen, 2011).

There is variety of Malaysian pineapple cultivars planted in Peninsula and Borneo of Malaysia. These include the ‘Sarawak’, ‘Yankee’, ‘MD2’, ‘Morris’, ‘Morris Gajah’ and ‘Josapine’ for fresh consumption, ‘Gandul’ for canning and juicing, ‘N36’ and ‘Maspine’ for both fresh consumption and canning purposes (MPIB). Several studies had been carried out previously to investigate and compare the phytochemical properties and bioactivities among different pineapple cultivars. For instance, Brat et al. (2004) compared the physicochemical characteristics between the new hybrid ‘FLHORAN41’ and ‘Smooth Cayenne’, Kongsuwan, Suthiluk, Theppakorn, Srilaong and Setha (2009) worked on the bioactive compounds and antioxidant capacities of ‘Phulae’ and ‘Nanglae’, Zulipeli (2007) investigated the bromelain content of ‘Josapine’, ‘Gandul’, ‘Maspine’ and ‘N36’, Wardy, Saalia, Stteiner-Asiedu, Budu and Sefa-Dedeh (2009) compared the physical, chemical and sensory properties of ‘MD2’, ‘Smooth Cayenne’ and ‘Sugarloaf’. The results of these studies showed that different pineapple
cultivars have different phytochemical characteristics and bioactivities from each other (Brat et al., 2004; Kongsuwan et al., 2009; Zulipeli, 2007, Wardy et al., 2009).

1.2 Problem Statement

Pineapples are rich in nutrients and phytochemicals which have multiple benefits to human health. In Malaysia, pineapple industry is one of the important agricultural sectors with 76 cultivars planted throughout this country. According to some previous studies, different pineapple cultivars have different phytochemical characteristics and bioactivities from each other (Brat et al., 2004; Kongsuwan et al., 2009; Zulipeli, 2007, Wardy et al., 2009). However, the physicochemical, biochemical and organoleptic properties evaluation and comparison among the commercial cultivars are yet to be fully accomplished. Hence, this study was performed to analyse the differences among the cultivars with the purpose to generate useful nutritional and health beneficial information of different Malaysian pineapple commercial cultivars in order to provide essential data resource either for future study of the fruit or as reference for commercial activity.

1.3 Objectives

a) To analyse the physicochemical characteristics of the selected Malaysian pineapple cultivars.

b) To analyse the biochemical characteristics of the selected Malaysian pineapple cultivars.

c) To analyse the organoleptic characteristics of the selected Malaysian pineapple cultivars.
d) To evaluate the correlation between physicochemical, biochemical and organoleptic characteristics of pineapples.

1.4 Scope of Study

In this project, pineapple fruits (*Ananas comosus*) of different cultivars were collected at commercial maturity stage (20-40% yellowish of fruit peel) and the edible portion of the fruit was used as sample for evaluation. For physicochemical test, the edible portion of pineapple pulp was homogenized, and then the aliquots of homogenated pulp were analysed for pH and titratable acidity (TA), and total soluble solids (TSS) as degrees Brix at 20°C. For biochemical test, different solvents were used for the extraction of pineapple for different biochemical assays. The phytochemical or bioactive compounds of the fruit extracts were evaluated using ascorbic acid content, total phenolic content, and tannin content assays. Besides, the antioxidant capacities of pineapple fruits were investigated by DPPH radical scavenging capacity and ferric reducing capacity assay. In addition, the enzymatic activity of pineapple fruit was determined by bromelain proteolytic activity and protein content of the samples. For organoleptic test, the evaluation for appearance, flavour, aroma, texture and overall preference involved 30 untrained taste panellist by using 5 point Hedonic scale: 1: Dislike extremely; 2: Dislike; 3: Neither like nor dislike; 4: Like; 5: Like extremely. Lastly, the statistical evaluation was performed by using Minitab version 15 and IBM SPSS (Statistical Package for the Social Sciences) Statistics version 20.

1.5 Significance of Study

Although pineapple is one of the important commodities to national economy, the physicochemical, biochemical and organoleptic characteristics evaluation and
comparison among the commercial cultivars are yet to be fully accomplished. Hence, this study was carried out to determine and compare the physicochemical characteristics, bioactive compounds, antioxidant capacities, enzymatic activity and sensory properties of different pineapple commercial cultivars in Malaysia. The outcome of this study aims to provide relevant nutritional information of different commercial cultivars to consumers, facilitate the promotion of different pineapple cultivars to market with known fruit characteristics and strength, improve the consumption of pineapple due to its nutritional properties, and provide useful information for further hybridization among the pineapple cultivars.
REFERENCES


