

UNIVERSITI TEKNOLOGI MARA

**DEVELOPMENT OF
CHROMATOGRAPHIC
FINGERPRINTS OF BIOACTIVE
COMPOUNDS FOR
DISCRIMINATION OF PINEAPPLE
VARIETIES**

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MSc

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ABSTRACT

Pineapple (*Ananas comosus L.*) is one of the tropical fruit in Malaysia reported to contain a significant amount of phenolic compounds and bromelain, a health inducing enzyme. As there are demands for truthful information including the composition, types, grades and origin of the fruit for international trade, a method for varietal classification of pineapple is essential. Chromatographic fingerprint now been used for classification of fruit varieties as it represents the composition of compounds present in the fruit rather than conventional assessment of fruit varieties by manual sorting that depends very much on human labour and liable to subjectivity. In this study, a comprehensive chromatographic fingerprint of pineapple was achieved by extraction using pressurized liquid extraction (PLE) prior to separation using online solid phase extraction liquid chromatography (online SPE-LC). PLE and online SPE-LC method was developed using selected bioactive compounds presents in pineapple namely; epicatechin, catechin, quercetin, ferulic acid, chlorogenic acid, myricetin and bromelain. Optimization of PLE operating parameters such as extraction temperature and static time conducted using response surface method (RSM) gave optimum extraction temperature of 105 °C with extraction time of 20 minutes using methanol as extraction solvent. Online SPE-LC with diode array detector (DAD) was achieved using two columns: a C18 column (5µm, 4.6 mm x50 mm) for SPE and a longer C18 column (5µm, 4.6 mm x 250 mm) for the separation of compounds. The mobile phase compositions of acidified water, methanol and acetonitrile and column switching time for online SPE-LC were also optimized. Optimization and validation of the developed method gave linearity ranged from 5 to 200 µg/mL ($R^2 = 0.975-0.997$) with RSD ranged from 2.1-4.6 %. The developed method was used to analyse 40 samples of three varieties of pineapple (Morris, Josapine and MD2). From the chromatographic fingerprints, sixteen peaks selected based on peak areas were subjected to chemometric analysis for varietal classification. Cluster analysis (CA) showed three clusters representing the three varieties of pineapple. Using principal component analysis (PCA), the groupings in the score plot were in good agreement with the result obtained from CA. In addition, discriminant analysis (DA) gave 100% correlation coefficient. This study showed that a reliable chromatographic fingerprint obtained using PLE prior to online SPE-LC is a promising approach for classification of pineapple varieties.

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TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF SYMBOLS	xii
LIST OF ABBREVIATIONS	xiii
CHAPTER ONE: INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem Statement	3
1.3 Significance of Study	4
1.4 Scope of Study	4
1.5 Objectives of Study	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 Pineapple	6
2.2 Varieties of Pineapple	7
2.3 Bioactive Compounds in Pineapple	10
2.4 Quality Classification of Fruits using Bioactive Compounds	11
2.4.1 Food Authentication using Quality Classification	12
2.4.2 Chromatographic Fingerprint in Food Authentication	13
2.5 Analysis of Bioactive Compounds in Fruits	14
2.5.1 Extraction of Bioactive Compounds	14
2.5.2 Pressurized Liquid Extraction (PLE)	15
2.5.2.1 <i>Optimization of PLE Operating Conditions</i>	16