## ENZYMATIC METHOD WITH MBTH REAGENT IN SPECTROPHOTOMETRIC DETERMINATION OF POLYPHENOL IN GREEN TEA

SHARIFAH BINTI ABD RASHID

# BACHELOR OF SCIENCE (Hons.) CHEMISTRY FACULTY OF APPLIED SCIENCES UNIVERSITI TEKNOLOGI MARA

**JANUARY 2014** 

#### TABLE OF CONTENTS

		Page	
ACF	KNOWLEDGEMENTS	iii	
TAE	TABLE OF CONTENTS		
LIST	Γ OF TABLES	vi	
LIST	Γ OF FIGURES	vii	
LIST	Γ OF ABBREVIATIONS	viii	
ABS	TRACT	X	
ABS	TRAK	xi	
CHA	APTER 1 INTRODUCTION		
1.1	Background of study	1	
1.2	•	6	
1.3	Significance of study	7	
1.4	Objectives of study	8	
CHA	APTER 2 LITERATURE REVIEW		
2.1	Tea	9	
2.2	Polyphenolic compound	10	
	2.2.1 Classification of polyphenol	11	
	2.2.2 Technique for determination of various types of polyphenol	11	
2.3	Enzyme activities	12	
	2.3.1 MBTH reagent	12	
	2.3.2 pH and temperature	12	
	2.3.3 Inhibition study	13	
2.4	Extraction of phenolic compounds	14	
2.5	Analysis and quantification of phenolics	17	
CHA	APTER 3 METHODOLOGY		
3.1	Materials	22	
	3.1.1 Chemicals	22	
	3.1.2 Apparatus	22	
3.2	Methods	23	
	3.2.1 Preparation of solution	23	
	3.2.1.1 Preparation of reagents	23	
	3.2.1.2 Preparation of tannic acid solution	24	

	3.2.2	Spectrophotometric determination of tannic acid in tyrosinase		
		solution	24	
		3.2.2.1 Study of response time	24	
		3.2.2.2 Spectral study	25	
		3.2.2.3 pH optimization	25	
		3.2.2.4 Reproducibility of the method	26	
		3.2.2.5 Effect of the amount of tyrosinase	26	
		3.2.2.6 Effect of the different concentration of tannic acid	26	
		3.2.2.7 Real sample analysis	27	
СПУ	DTED /	RESULTS AND DISCUSSION		
4.1		uction	28	
4.2		ophotometric determination of tannic acid in tyrosinase solution	28	
7.2		Study of response time	29	
		Spectral study	29	
		oH optimization	31	
		Reproducibility of the method	32	
		Effect of the amount of tyrosinase	33	
		Effect of the different concentration of tannic acid	34	
4.3		ation of study	36	
		5 CONCLUSION AND RECOMMENDATIONS		
5.1	Concl		38	
5.2	Recor	nmendations	39	
REF	ERENC	YES	40	
APPENDICES CURRICULUM VITAE				

#### LIST OF TABLES

Table	Caption	Page
2.1	pH and temperature of different substrates	13
4.1	Determination of tannic acid in green tea sample by developed	
	method and HPLC method	37

#### **ABSTRACT**

### ENZYMATIC METHOD WITH MBTH REAGENT IN SPECTROPHOTOMETRIC DETERMINATION OF POLYPHENOL IN GREEN TEA

Tea is the second most popular beverages after water in the world. Since there is a growing interest in substances that has antioxidant properties, a new method that can provide innovative applications to the tea-beverage industry must be developed. A simple, faster and environmental friendly method for determination of polyphenol in green tea has been developed which are enzymatic method and analysed by using UV-Vis spectrometry. Tannic acid is used as a standard to determine phenolic compound. The optimum condition of enzyme tyrosinase was determined. The obtained optimum condition of tyrosinase enzyme for response time was 30 minutes and pH of 7. The optimum wavelength was 200 to 400 nm. 2 mL of tyrosinase enzyme and  $1.5 \times 10^{-5}$  M tannic acid were also chosen as the optimum condition for tyrosinase enzyme activity. The concentration of polyphenol in green tea was well correlated with the concentration of tannic acid. The percentage relative error between these two methods was 0.11%. The t-test from polyphenol analysis of this method showed that there was no significant difference between the two methods at 95% confidence level.