# CONTENTS

Page

Acknowledgements	c
Abstract in Thai	d
Abstract in English	e
List of Tables	k
List of Figures	1
List of Abbreviations	0
List of Symbols	q
Chapter 1 Introduction	1
1.1 Overview	1
1.2 Antioxidant and its important	2
1.3 Method for estimation of antioxidant activity	2
1.4 Colorimetric analysis	6
1.4.1 Camera based colorimetric analysis	6
1.4.2 Colorimetric analysis on well plate platform	10
1.5 Research objectives	16
Chapter 2 Experimental	17
2.1 Chemicals	17
2.2 Materials and instruments	17

2.3 Software	18
2.4 Preparation of standard solution and reagents	18
2.4.1 Standard solution of DPPH and Trolox	
2.4.2 Reagent solution	18
2.4.2.1 Buffered medium	18
(methanol : 10 mM Tris buffer pH 7.5, 1:1 v/v)	
2.4.2.2 Ethanol-buffer solution	18
(ethanol: 10 mM Phosphate-buffered saline pH 7.4, 1:1 v/v	v)
2.5 The study improve sensitivity on reaction of DPPH radical with	19
Trolox in batch method	
2.6 Analytical procedure of a simple colorimetric detection system	19
on well plate	
2.7 Batch spectrophotometric procedure	20
2.8 Apparatus and instrument setup	20
2.8.1 Photographic unit	20
2.8.2 96-well plate 3D design	21
2.8.3 Lab on paper	21
2.9 Computer program for antioxidative assay	22
2.9.10 Optimization of Antioxidative Assay	23
2.9.10.1 Back screen of photographic unit	23
2.9.10.2 Focal length of the mobile phone camera	23
2.9.10.3 Lighting control	23
2.9.10.4 Performance of smart phone camera	23
2.9.10.5 Study sensitivity of colors	24
2.9.10.6 Effect of the reaction time	24
2.9.10.7 Effect of DPPH volume	24
2.11 Analytical characteristics of the procedure	24
2.11.1 Calibration curves and limit of detection	24
2.11.2 Precision study	24
2.11.3 Accuracy study	25
2.12 Preparation of sample	25

2.12.1 Application of the method to real samples	25
2.12.2 Sample analysis	25
Chapter 3 Results and Discussion	26
3.1 Overview of the research	26
3.2 The study to improve sensitivity on reaction of DPPH radical	27
with Trolox	
3.2.1 The effect of the solution media on absorption wavelength o	f 26
DPPH radical with Trolox	
3.2.2 The effect of the solution media on reaction kinetic of	29
DPPH radical with Trolox	
3.2.3 Concentration-response curve for DPPH	29
3.3 Colorimetric analytical system based on camera detection	30
3.3.1 Study of interior wall in the photographic unit	29
3.3.2 Study focal length of the mobile phone camera	31
3.3.3 Lighting control of photographic unit	32
3.3.4 ISO mode of smart phone camera	33
3.3.5 Colors sensitivity of pantone papers	34
3.3.6 Effect of the reaction time	35
3.3.7 Effect of DPPH volume	36
3.4 Analytical characteristics of the procedure	37
3.4.1 Precision study	38
3.4.2 Stability of colorimetric method	39
3.4.3 Real sample analysis	39
Chapter 4 Conclusion	41
References	42
List of publication	48
Appendix A	49

Appendix B	50
Appendix C	54
Appendix D	56
Appendix E	57
Appendix E Appendix F The relevance of the research work to Thailand Curriculum Vitae	59
The relevance of the research work to Thailand	60
Curriculum Vitae	61
<b>ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่</b> Copyright <sup>©</sup> by Chiang Mai University All rights reserved	

### LIST OF TABLES

Table 1.1	Methods for antioxidative assay associated with electron and radical scavenging.	5
Table 1.2	Summary of camera applications used for image capture and analysis of some foods and beverages	9
Table 1.3	Methods for colorimetric analysis on well plate platform	12
Table 1.4	Summary of methods for colorimetric analysis based on smartphone camera	13
Table 3.1	Effect of the reaction time on antioxidative assay	36
Table 3.2	Effect of DPPH volume on antioxidative assay	36
Table 3.3	The optimum condition for antioxidant capacity array by DPPH method	37
Table 3.4	Calibration data for antioxidative assay of spectrophotometric and the proposed method	38
Table 3.5 C	The intensity of color obtained antioxidant activity of 10 $\mu$ M Trolox for 11 replicates	39
Table 3.6	summarized Trolox equivalent concentration obtained by proposed method comparing with spectrophotometric method of tea samples.	40

# LIST OF FIGURES

Figure 1.1	Diagram of RGB system	6
Figure 1.2	Schematic of the set-up for wine quality measurements.	7
	Up to six samples could be monitored simultaneously	
Figure 1.3	Schematic diagram (a) and photograph (b) of the system	7
	proposed for determination of total acidity in red wines	
	by using digital image-based titrations	
Figure 1.4	Schematic diagram of allergen testing tool	8
Figure 1.5	Image analysis and quantification of IgG image paper-based	10
	ELISAs results with Windows- and Android-based tablets	
Figure 1.6	Experimental arrangement to collect and analyze images	11
	to determinate the concentration of glucose, triglycerides,	
	creatinine,total cholesterol and total proteinin blood serum	
Figure 2.1	Photographic system in a light controlled box	20
Figure 2.2	96-well plate 3D design	21
Figure 2.3	Procedure of computer program for antioxidative assay.	22
	First step, Input Image and region of interest plate segmentation.	
	Second step, edge detection and image	
Figure 3.1	Absorption spectra of DPPH in ethanol-phosphate buffer	27
	solution; 50, 100 and 250 $\mu$ M	
Figure 3.2	Absorption spectra of DPPH in Tris buffered medium;	28
	50, 100 and 250 µM	

Figure 3.3	Figure 3.3 Absorbance-Time plot indicating reaction kinetic between DPPH radical (350 $\mu$ M) and Trolox standard concentrations, dashed line 10 and solid line 70 $\mu$ M in (a) ethanol-phosphate buffer and (b) Tris buffer solutions	29
Figure 3.4	Calibration data for antioxidative assay of spectrophotometric and the proposed methods	30
Figure 3.5	Figure 3.5 Histogram showing tonal distribution of a digital image of interior in the box with (a) white paper (b) black paper on interior wall of the box, and photographs corresponded to the histograms (c) white paper (d) black.	31
Figure 3.6	For more details see text Peak area under the graph of tonal histogram at various distance between the mobile phone camera and well plate (a) 10-23 cm and at various distances	32
Figure 3.7	between behind wall of the box and well plate (b) 2.5-4.5 cm Image histograms of different lighting control procedures (a) LED without filter (b) LED covered with soft box. The conresponded photography of (a) and (b) cases (c) and (d)	33
Figure 3.8	Figure 3.8 (a) 9 detection points on well plate and (b) Relative standard deviation of color intensities on the detection of red green blue papers of different positions of well plate by using various ISO modes: Auto, HDR, ISO 34-39	34
Figure 3.9	Linearity of pantone colors, purple, blue, green, yellow, orange, and red by fixed ISO modes: Auto (a), HDR (b), ISO34 (c), ISO39 (d), ISO44 (e)	35
Figure 3.10	Dynamic range of concentrations-response curve of spectrophotometric and the proposed colorimetric method	38

shown in Figure 3.11(a),(c) and linear range of method shown in Figure 3.11(b),(d), respectively

Figure 5.1	iPhone 5S	49

- Figure 5.2Photograph for samples analysis54
- Figure 5.3 Calibration curve plotting between green intensity and 56 concentrations of Trolox standard 10-130 µM for proposed method



ลิ<mark>ปสิทธิ์มหาวิทยาลัยเชียงใหม่</mark> Copyright<sup>©</sup> by Chiang Mai University All rights reserved

# LIST OF ABBREVIATIONS

ABTS	2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
ACA	aldehyde/carboxylic acid
App	Application
CCD	Charged coupled device
CSPT	Computer screen photoassisted technique
CMOS	Complementary metal oxide-semiconductor
CHT	Circular hough transform
DI	Deionized water
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSLR	Digital single-lens reflex
ELISA	Enzyme-linked immunosorbent assay
FTC	Ferric thiocyanate
FOX	Ferrous oxidation-xylenol orange
FRAP	Ferric reducing antioxidant power
hts A	High throughput screening
ISO	International standards organization
IOS	iPhone OS
MATLAB	Matrix laboratory
MINIPAM	Pulse-amplitude modulation

Light emitting diode LED LOD Limit of detection RGB Intensity of red (R), green (G) and blue (B) color Relative DPPH radical scavenging capacity RDSC RSD Relative standard deviation 2102:23 Standard deviation SD ΤE Trolox equivalents Trolox equivalent antioxidant capacity TEAC 3D Three-dimensional THO MAI

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright<sup>©</sup> by Chiang Mai University All rights reserved

#### LIST OF SYMBOLS

Abs	Absorbance
°C	Degree Celsius
e.g.	Exempli gratia (for example)
g	Gram
i.e.	id est (that is)
к	Kelvin
kg	Kilogram
x	Mean value
Xmea	Measured value
μL	Microliter
μΜ	Micromolar
mm	Millimeter
maaal	Millimolar
	Milliliter by Chiang Mai University
	Minute ghts reserved
R <sup>2</sup>	Square of correlation coefficient
xt	True value
v/v	volume by volume
% w/v	Weight to volume percentage