

STUDY ON CURCUMIN PIGMENT EXTRACTED FROM
TEMU LAWAK (*Curcuma xanthorrhiza* Roxb.) AS
POTENTIAL APPLICATION IN HALAL FOOD
PACKAGING AND COSMETICS

BY

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ABSTRACT

The traditional uses of the family Zingiberaceae have a long history and include everything from folk medicine to culinary applications. Numerous ginger species have been the subject of numerous phytochemical, pharmacological, and molecular studies worldwide. Curcumin is a yellow–orange pigment that occurs naturally in turmeric is insoluble in water and ether but soluble in ethanol, dimethylsulfoxide (DMSO), and acetone. The study of curcumin yellow pigment in kunyit (*Curcuma longa*) have been reported by many researchers but not from other taxon in Zingiberaceae. Therefore, this study was implemented to explore and investigate in greater depth of curcumin extracted from twelve ginger species in a wide range of rhizome flesh colour as a potential natural colourant for food packaging or halal bioactive material for cosmetic in halal market. HPLC analysis was used to identify and quantify curcumin pigment from twelve ginger species using three different extraction methods (chemical, alkaline, and DMSO). DPPH and MIC test were used to measure the antioxidant properties and antimicrobial activities, while UV-Vis spectrophotometer and CIELAB colorimeter were used to measure the curcumin chromaticity stability. The results established that kunyit (*C. longa*), a dark orange-fleshed rhizome, was found to have the highest curcumin content ($578.38 \pm 6.37 \mu\text{g/g DW}$) and demonstrated substantially the highest DPPH scavenging activity of 98.43% inhibition at the concentration of 1 mg/mL. The MIC antimicrobial test established that the highest antimicrobial inhibitory activity was obtained from the extract of dark orange-fleshed rhizome of temu emas (*C. heyneana*) which recorded the lowest MIC values ranged from 2 to 3 $\mu\text{g}/\mu\text{L}$ against all five pathogenic microorganisms. These findings also highlighted that different extraction method and rhizome flesh colour can influence the antimicrobial activities and DPPH scavenging activity. Effects of salinity, pH, ultraviolet and heat on chromaticity stability of curcumin, PLA-curcumin, PVA-curcumin and chitosan-curcumin coatings extracted from temu Lawak (*Curcuma xanthorrhiza* Roxb.) using CIELAB analysis revealed that dark condition exhibited a better and stable colour degradation (approximately 40%) in all curcumin concentrations (1, 2 and 3 mg/mL) as compared to light condition. Overall, the dark condition was preferable and stable for the curcumin brightness and slower the degradation percentage. The chromaticity values showed a maximum in PLA-curcumin coating as compared to curcumin and other packaging materials. The colour of curcumin was more stable at lower concentrations of NaCl (1 mg/mL and 3 mg/mL) as compared to a higher concentration (5 mg/mL). In addition, lower pH (3, 5 and 7) showed the best chromaticity stability than higher pH (9 and 11).

ملخص البحث

لعبت تتمتع الاستخدامات التقليدية لعائلة الزنجبيليات (Zingiberaceae) بتاريخ طويل، وتشمل كل شيء من الطب الشعبي إلى التطبيقات الطهوية. وقد خضعت العديد من أنواع الزنجبيل لدراسات عديدة في مجالات الكيمياء النباتية، والدوائية، والجزئية على مستوى العالم. يُعد الكركمين صبغة صفراء-برتقالية توجد طبيعيًا في الكركم، وهو غير قابل للذوبان في الماء والايثر، ولكنه قابل للذوبان في الإيثانول، وثنائي ميثيل سلفوكسيد (DMSO)، والأسيتون. وقد أفاد العديد من الباحثين عن دراسات تتعلق بصبغة الكركمين الصفراء في نبات الكُنيت (*Curcuma longa*)، إلا أن هناك نقصًا في الدراسات حول أصناف أخرى ضمن فصيلة الزنجبيليات. وعليه، تُفذت هذه الدراسة بهدف استكشاف الكركمين المستخلص من اثني عشر نوعًا من الزنجبيل – والتي تتنوع ألوان لب جذورها – ودراسته بعمق كصبغة طبيعية محتملة لتغليف المواد الغذائية أو كمادة حيوية فعالة حلال للاستخدام في مستحضرات التجميل ضمن سوق الحلال. تم استخدام تحليل الكروماتوغرافيا السائلة عالية الأداء (HPLC) لتحديد كمية صبغة الكركمين وتقديرها في الأنواع الاثني عشر من الزنجبيل باستخدام ثلاث طرق استخلاص مختلفة (الكيميائية، والقلوية، و DMSO) وتم استخدام اختبار DPPH واختبار MIC لقياس الخصائص المضادة للأوكسدة والأنشطة المضادة للميكروبات، بينما استخدم كل من مطياف الأشعة فوق البنفسجية المرئية ومقياس الألوان CIELAB لقياس ثبات الكركمين اللوني. أظهرت النتائج أن نبات الكُنيت (*C. longa*) ذو لبّ بلون برتقالي داكن يحتوي على أعلى تركيز من الكركمين (578.38 ± 6.37 ميكروغرام/غرام وزن جاف)، كما أظهر أعلى نشاط مضاد للأوكسدة بإزالة الجذور الحرة في اختبار DPPH بنسبة تثبيط بلغت 98.43% عند تركيز 1 ملغم/مل. أثبت اختبار

MIC المضاد للميكروبات أن أعلى نشاط تثبيطي تم الحصول عليه كان من مستخلص اللب البرتقالي الداكن لنبات تيمو إماس (*C. heyneana*) ، حيث سُجّلت أدنى قيم MIC تراوحت بين 2 إلى 3 ميكروغرام/ميكرو لتر ضد جميع الكائنات الدقيقة الخمسة المسببة للأمراض. كما أبرزت هذه النتائج أن طريقة الاستخلاص المختلفة ولون لب الجذمور يمكن أن يؤثر على الأنشطة المضادة للميكروبات ونشاط إزالة الجذور الحرة في اختبار DPPH. أظهرت نتائج تحليل تأثيرات الملوحة، والأس الهيدروجيني، والأشعة فوق البنفسجية، والحرارة على ثبات اللون اللوني للكرمين، وطلاءات-PLA كركمين، و-PVA كركمين، و-كيتوزان-كرمين المستخلصة من نبات تيمو لاواك (*Curcuma xanthorrhiza* Roxb.) باستخدام تحليل CIELAB، أن البيئة المظلمة أظهرت تحللاً لونياً أفضل وأكثر استقراراً (حوالي 40%) في جميع تركيزات الكركمين (1، 2، و 3 ملغم/مل) مقارنة بالبيئة المضاءة. بشكل عام، كانت البيئة المظلمة أكثر تفضيلاً واستقراراً من حيث إشراق الكركمين وبطء نسبة تحلله. كما أظهرت قيم اللونية أعلى مستوياتها في طلاء-PLA كركمين مقارنة بالكركمين الخام وغيره من مواد التغليف. وكان لون الكركمين أكثر ثباتاً عند تركيزات منخفضة من كلوريد الصوديوم (1 ملغم/مل و 3 ملغم/مل) مقارنةً بالتركيز الأعلى (5 ملغم/مل). بالإضافة إلى ذلك، أظهرت القيم المنخفضة للأس الهيدروجيني (3، 5، و 7) ثباتاً لونياً أفضل مقارنة بالقيم المرتفعة (9 و 11).

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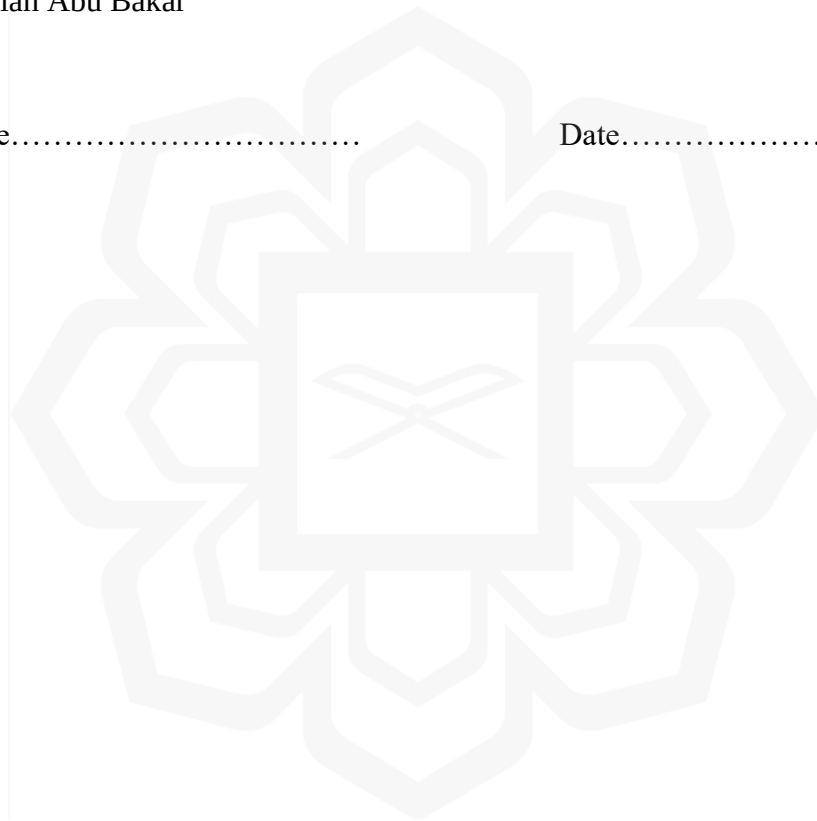
DECLARATION

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TABLE OF CONTENTS

Abstract.....	ii
Abstract in Arabic.....	iii
Approval Page.....	v
Declaration Page.....	vi
Copyright Page.....	vii
Acknowledgements.....	viii
List of Tables.....	xiv
List of Figures.....	xix
List of Symbols.....	xxvii
List of Abbreviations.....	xxviii
CHAPTER ONE: INTRODUCTION.....	1
1.1 Research Background	1
1.2 Statement of the Problem	3
1.3 Research Goal	4
1.4 Research Objectives	4
1.5 Research Questions	5
1.6 Research Hypothesis	5
1.7 Significance of the Study	6
1.8 Scope of the Study	6
CHAPTER TWO: LITERATURE REVIEW.....	7
2.1 Curcumin and Its Properties	7
2.2 Chemical Structures	8
2.3 Functional Groups	9
2.4 Biological Activity of Curcumin	10
2.4.1 Antioxidant Activity	10
2.4.2 Antimicrobial Activity	11
2.4.3 Temu Lawak (<i>Curcuma xanthorrhiza</i> Roxb.)	12
2.4.4 Packaging Material	13
2.4.5 Smart Packaging	14
2.4.6 Active Packaging	16
2.4.7 Criteria of Active Packaging Materials	16
2.4.7.1 Oxygen Scavengers	16
2.4.7.2 Carbon Dioxide Generating System	17
2.4.7.3 Antimicrobials	17
2.4.7.4 Antioxidants	18
2.4.7.5 Intelligent Packaging	18
2.4.7.6 Polymers	19
2.4.7.7 Polylactic Acid (PLA)	20

2.4.7.8 Poly (Vinyl Alcohol) (PVA)	21
2.4.7.9 Chitosan	22
2.5 Curcumin in Polymer-Based Composite Films	23
2.6 Packaging and Colour	23
2.7 Sustainability of Natural and Artificial Colourants	24
2.8 The Halal Concept.....	26
2.9 <i>Halalan-Toyyiban</i> Concept	26
2.10 Malaysia and Halal Recognition.....	27
2.11 Chapter Summary	32
CHAPTER THREE: RESEARCH METHODOLOGY.....	34
3.1 Sample Preparation	34
3.1.1 Chemical Extraction	34
3.1.2 Alkaline Extraction	35
3.1.3 Dimethyl sulfoxide (DMSO) Extraction	36
3.1.4 High-Performance Liquid Chromatography (HPLC) Analysis	36
3.1.5 Standard Preparation	38
3.2 Antioxidant Activity Examination Using 2,2-Diphenyl-1- Picrylhydrazyl (DPPH) Assay	38
3.2.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay of Ascorbic Acid Standard	39
3.2.2 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay of Carotenoid	40
3.2.3 Analysis of Antioxidant Activity	41
3.3 Antimicrobial Activities of Carotenoids Preparation	42
3.4 Assays	43
3.4.1 Sterility	43
3.4.2 Minimum Inhibitory Concentration (MIC)	44
3.5 Inhibitory Status Determination Assay	44
3.5.1 High-Performance Liquid Chromatography (HPLC) Analysis	45
3.5.2 Spectrophotometric Measurements	45
3.6 Curcumin Stability Test	47
3.6.1 Pigments Preparation	47
3.6.2 Analysis of Chromaticity	47
3.6.3 Salinity Test	48
3.6.4 pH Test	48
3.6.5 UV Irradiation Test	49
3.6.6 Heat Test	49
3.7 Polymers and Its Chromaticity Test	50
3.7.1 Colourant Preparation	50
3.7.2 Poly(Lactic Acid) Film Preparation	50
3.7.3 Poly(Vinyl Alcohol) Film Preparation	51
3.7.4 Chitosan Film Preparation	52

3.7.5 Coating Stability Test for PLA, PVA and Chitosan	53
3.7.5.1 Salinity Test	53
3.7.5.2 pH Test	54
3.7.5.3 UV Irradiation Test	54
3.7.5.4 Heat Test	55
3.7.5.5 Light and Dark Condition	55
3.7.6 Chromaticity (ΔE^*) Testing	55
3.8 Soap Making Applications	56
3.8.1 Light vs Dark Condition	56
3.8.2 Chromaticity (ΔE^*)	56
3.9 Multivariate Data Analysis (MDA)	57
3.9.1 Analysis of variance (ANOVA)	57
3.9.2 Principal Component Analysis (PCA)	58
3.9.3 Identification and Removal of Outliers	58
3.9.4 Dataset Transformation	59
3.9.5 Kaiser-Meyer-Olkin (KMO) and Barlett's Test for Sampling Adequacy	59
3.9.3 Pearson's Correlation Coefficient (r^2) Analysis	60
CHAPTER FOUR: QUANTIFICATION OF CURCUMIN CONTENT FROM SELECTED GINGER SPECIES, ANTIOXIDANT PROPERTIES AND ANTIMICROBIAL ACTIVITIES	61
4.1 Introduction.....	61
4.2 Results and Discussions	62
4.2.1 HPLC Analysis of Curcumin Pigment from Selected Ginger Species	62
4.2.2 Antioxidant Properties	67
4.2.3 Antimicrobial Activities	72
4.3 Summary of Results	76
CHAPTER FIVE: CHROMATICITY STABILITY PROPERTIES OF CURCUMIN EXTRACTED FROM TEMU LAWAK (<i>Curcuma xanthorrhiza</i> Roxb.)	77
5.1 Introduction	77
5.2 Results and Discussions	79
5.2.1 Effect of Light, Pigment Concentration and Time of Exposure on Curcumin Stability	79
5.2.2 Effect of NaCl	83
5.2.3 Effect of pH	89
5.2.4 Effect of UV Irradiation	96
5.2.5 Effect of Heating	99
5.3 Multivariate Data Analysis	103
5.3.1 Sampling Adequacy for Multivariate Analysis	103
5.3.2 Correlation Coefficient Matrix (r^2)	105
5.3.3 Principal Component Analysis (PCA)	108

5.4 Summary of the Results	111
CHAPTER SIX: CHROMATICITY STABILITY OF PLA-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS	112
6.1 Introduction.....	112
6.2 Results and Discussions	113
6.2.1 Effect of Light, Pigment Concentration and Time of Exposure on PLA-curcumin Stability	113
6.2.2 Effect of NaCl	118
6.2.3 Effect of pH	124
6.2.4 Effect of UV Irradiation	131
6.2.5 Effect of Heating	134
6.3 Multivariate Data Analysis	139
6.3.1 Sampling Adequacy for Multivariate Analysis	139
6.3.2 Correlation Coefficient Matrix (r^2)	140
6.3.3 Principal Component Analysis (PCA)	143
6.4 Summary of the Results	145
CHAPTER SEVEN: CHROMATICITY STABILITY OF PVA-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS	147
7.1 Introduction.....	147
7.2 Results and Discussions	148
7.2.1 Effect of Light, Pigment Concentration and Time of Exposure on PVA-curcumin Stability	148
7.2.2 Effect of NaCl	152
7.2.3 Effect of pH	158
7.2.4 Effect of UV Irradiation	164
7.2.5 Effect of Heating	167
7.3 Multivariate Data Analysis	171
7.3.1 Sampling Adequacy for Multivariate Analysis	171
7.3.2 Correlation Coefficient Matrix (r^2)	172
7.3.3 Principal Component Analysis (PCA)	175
7.4 Summary of the Results	177
CHAPTER EIGHT: CHROMATICITY STABILITY OF CHITOSAN-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS	179
8.1 Introduction.....	179
8.2 Results and Discussions	180
8.2.1 Effect of Light, Pigment Concentration and Time of Exposure on Chitosan-curcumin Stability	180
8.2.2 Effect of NaCl	184
8.2.3 Effect of pH	190

8.2.4 Effect of UV Irradiation	196
8.2.5 Effect of Heating	199
8.3 Multivariate Data Analysis	203
8.3.1 Sampling Adequacy for Multivariate Analysis	203
8.3.2 Correlation Coefficient Matrix (r^2)	204
8.3.3 Principal Component Analysis (PCA)	207
8.4 Summary of the Results	209
CHAPTER NINE: ASSESSMENT OF CURCUMIN CHROMATICITY STABILITY MODEL SYSTEM IN NATURAL SOAP AS AN ADDITIVE	211
9.1 Introduction.....	211
9.2 Results and Discussions	212
9.2.1 Effect of Light, pigment concentration and time of exposure on curcumin stability in natural soap	212
9.3 Multivariate Data Analysis	217
9.3.1 Sampling Adequacy for Multivariate Analysis	217
9.3.2 Correlation Coefficient Matrix (r^2)	218
9.3.3 Principal Component Analysis (PCA)	221
9.4 Summary of the Results	223
CHAPTER TEN: GENERAL DISCUSSION AND CONCLUSION.....	225
10.1 Research Aim and Objectives	225
10.1.1 Curcumin Properties, Stability and Potential Application	225
10.2 Quantification of Curcumin Content from Selected Ginger Species, Antioxidant Properties and Antimicrobial Activities.....	228
10.3 Chromaticity Stability Properties of Curcumin Extracted from Temu Lawak (<i>Curcuma Xanthorrhiza</i> Roxb.)	230
10.4 Chromaticity Stability of PLA-Curcumin Coating Using CIELab and Principal Component Analysis	231
10.5 Chromaticity Stability of PVA-Curcumin Coating Using CIELab and Principal Component Analysis.....	235
10.6 Chromaticity Stability of Chitosan-Curcumin Coating Using CIELab and Principal Component Analysis.....	241
10.7 Assessment of Curcumin Chromaticity Stability Model System in Natural Soap as an Additive	246
10.8 Conclusion	249
REFERENCES.....	252
APPENDIX I.....	311

LIST OF TABLES

Table 3.1	Interpretation of Correlation Coefficient Values	60
Table 4.1	Different rhizome flesh colour from selected 12 ginger species	63
Table 4.2	The curcumin contents ($\mu\text{g/g DW}$) of the various flesh colours of the rhizomes of the 12 ginger species	64
Table 4.3	Curcumin contents ($\mu\text{g/g DW}$) of the various flesh colours of the rhizomes of the 12 ginger species using three different extraction solvents, namely, acetone and methanol mixture, NaOH, and DMSO	65
Table 4.4	Curcumin concentration and rhizome flesh colours of 7 selected ginger species	68
Table 4.5	Antimicrobial activities of curcumin extracted from seven selected ginger species	73
Table 4.6	The antimicrobial activities of the curcumin extracted from the seven selected ginger species	71
Table 5.1	The CIE $L^*a^*b^*$ colour difference (ΔE^*_{LAB}) assessment	79
Table 5.2	Degradation percentage (%) of s and b^* for different curcumin concentrations in light and dark conditions within four weeks	81
Table 5.3	Degradation percentage (%) of s and b^* for different curcumin concentrations from temu lawak in light and dark conditions within weeks of treatment with 1, 3, and 5 mg/mL of NaCl	84
Table 5.4	Effects of pH on the colour degradation of curcumin pigment concentrations from temu lawak upon exposure to dark and light conditions within 4 weeks	91
Table 5.5	Curcumin colours after 14 days based on CIELab values, when stored at pH 9 and room temperature 25 °C in light and dark condition	92

Table 5.6	Effects of UV radiation for 8 hours on color degradation for different concentrations of curcumin pigment extracted from temu lawak (<i>C. xanthorrhiza</i>)	97
Table 5.7	Effects of heating for 3 hours on colour degradation for different concentrations of curcumin pigment extracted from temu lawak (<i>C. xanthorrhiza</i>)	100
Table 5.8	KMO value	104
Table 5.9	Kaiser-Meyer-Olkin and Bartlett's test for the curcumin pigment chromaticity stability	105
Table 5.10	The r^2 of the curcumin pigment's ΔE^* stability	106
Table 5.11	The strength of the r^2 between the two variables affecting the curcumin pigment's ΔE^* stability	107
Table 5.12	Factor loadings and eigenvalues of the PCA correlation matrix for curcumin pigment ΔE stability	109
Table 6.1	Effects of 4 weeks in light and dark condition on colour degradation of PLA-curcumin	115
Table 6.2	Effects of 4 weeks in light and dark condition on colour degradation of PLA-curcumin	116
Table 6.3	Effects of NaCl exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin	119
Table 6.4	Effects of NaCl exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin	120
Table 6.5	Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin	126
Table 6.6	Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin	127
Table 6.7	Effects of 8 hours of UVB irradiation on colour degradation for different concentrations of PLA-curcumin	132
Table 6.8	PLA-curcumin's CIELAB colour based values after 8 hours of UVB exposure	133

Table 6.9	Effects of 3 hours exposure to different temperatures on PLA-curcumin colour degradation	136
Table 6.10	Effects of temperatures exposure for 4 weeks on colour degradation of PLA-curcumin	137
Table 6.11	Kaiser-Meyer-Olkin and Bartlett's test for the PLA-curcumin pigment chromaticity stability	140
Table 6.12	Correlation coefficient matrix for the PLA-curcumin pigment chromaticity stability	141
Table 6.13	The correlation coefficient (r^2) strength between two variables among PLA-curcumin pigment chromaticity stability test	142
Table 6.14	Principal components factor loading and eigenvalues of the correlation matrix for PLA-curcumin pigment chromaticity stability	143
Table 7.1	Effects of 4 weeks exposure to light and dark conditions on PVA-curcumin colour degradation	150
Table 7.2	Effects of exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin	150
Table 7.3	Effects of 4 weeks exposure to NaCl, light and dark conditions on colour degradation of PVA-curcumin	153
Table 7.4	Effects of NaCl exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin	154
Table 7.5	Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin	159
Table 7.6	Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin	160
Table 7.7	Effects of UVB irradiation after 8 hours exposure on colour degradation for different concentrations of PVA-curcumin	165
Table 7.8	PVA-curcumin's CIELAB colour based values after 8 hours of UVB exposure	165

Table 7.9	Effects of temperature exposure for 3 hours on colour degradation of PVA-curcumin	168
Table 7.10	PVA-Curcumin colours throughout 3 hours based on CIELab values at different concentrations (5, 10, and 20 g/L) incubated in different temperature	169
Table 7.11	Kaiser-Meyer-Olkin and Bartlett's test for the PVA-curcumin pigment chromaticity stability	172
Table 7.12	Correlation coefficient matrix for the PVA-curcumin pigment chromaticity stability	173
Table 7.13	The correlation coefficient (r^2) strength between two variables among PVA-curcumin pigment chromaticity stability test	174
Table 7.14	Principal components factor loading and eigenvalues of the correlation matrix for PVA-curcumin pigment chromaticity stability	175
Table 8.1	Effects of 4 week in light and dark condition on colour degradation of chitosan-curcumin	182
Table 8.2	Effects of 4 week in light and dark condition on colour degradation of chitosan-curcumin	182
Table 8.3	Effects of NaCl exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin	185
Table 8.4	Effects of NaCl exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin	186
Table 8.5	Effects of pH exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin	191
Table 8.6	Effects of pH exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin	192
Table 8.7	Effects of UVB exposure in 8 hours on colour degradation of chitosan-curcumin	197
Table 8.8	Effects of UVB exposure in 8 hours on colour degradation of chitosan-curcumin	197

Table 8.9	Effects of heat exposure in 3 hours on colour degradation of chitosan-curcumin	200
Table 8.10	Effects of heat exposure in 3 hours on colour degradation of chitosan-curcumin	201
Table 8.11	Kaiser-Meyer-Olkin and Bartlett's test for the chitosan-curcumin pigment chromaticity stability	204
Table 8.12	Correlation coefficient matrix for the chitosan-curcumin pigment chromaticity stability	205
Table 8.13	Correlation coefficient (r^2) strength between two variables among chitosan-curcumin pigment chromaticity stability test	206
Table 8.14	The principal components factor loading and eigenvalues of the correlation matrix for chitosan-curcumin pigment chromaticity stability	207
Table 9.1	Effects of exposure for 4 week in light and dark condition on colour degradation of soap-curcumin	214
Table 9.2	Effects of exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin	215
Table 9.3	Kaiser-Meyer-Olkin and Bartlett's test for the PLA-curcumin pigment chromaticity stability	218
Table 9.4	Correlation coefficient matrix for the curcumin pigment in solid bath soap chromaticity stability	219
Table 9.5	Correlation coefficient (r^2) strength between two variables among curcumin pigment in solid bath soap chromaticity stability test	220
Table 9.6	Principal components factor loading and eigenvalues of the correlation matrix for PLA-curcumin pigment chromaticity stability	222

LIST OF FIGURES

Figure 2.1	Functional groups of curcumin (Lestari and Indrayanto, 2014)	10
Figure 3.1	Standard calibration curve of curcumin	37
Figure 3.2	The DPPH assay results of the ascorbic acid standard (concentration in $\mu\text{g/mL}$)	40
Figure 3.3	The DPPH assays preparation of the samples (concentration in $\mu\text{g/mL}$)	41
Figure 3.4	CIELab sphere (Sant' Anna et al., 2013)	48
Figure 4.1	HPLC chromatogram of curcumin extracts from 12 species and combined and injected to HPLC at retention time of 2.667 minutes and at a wavelength of 425 nm	64
Figure 4.2	Antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of kunyit kuning (<i>C. longa</i>) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (98.43% inhibition) at a concentration of 1 mg/mL	68
Figure 4.3	Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of temu lawak (<i>C. xanthorrhiza</i>) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (96.1% inhibition) at a concentration of 2 mg/mL	69
Figure 4.4	Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of temu emas (<i>C. heyneana</i>) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (95.68% inhibition) at a concentration of 1 mg/mL	69
Figure 4.5	Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of temu pauh (<i>C. mangga</i>) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (91.1% inhibition) at a concentration of 5 mg/mL	70

Figure 4.6	Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of lempoyang (<i>Z. zerumbet</i>) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (91.21% inhibition) at a concentration of 3 mg/mL	70
Figure 4.7	Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of kunyit putih (<i>C. zedoaria</i>) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (95.68% inhibition) at a concentration of 3 mg/mL	71
Figure 4.8	Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of halia (<i>Z. officinale</i>) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (95.15% inhibition) at a concentration of 2 mg/mL	71
Figure 4.9	A clear-yellow well indicates the permanent inhibition of growth (cidal) while a dark blue well indicates the recurrence of growth or temporary inhibition	74
Figure 5.1	Chemical structure of demethoxycurcumin	78
Figure 5.2	Chemical structure of bisdemethoxycurcumin	78
Figure 5.3	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values of the different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) in light and dark condition at different exposure times	82
Figure 5.4	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) in light and dark condition for different exposure time after treatment with 1 mg/mL NaCl	85
Figure 5.5	CIE $L^*a^*b^*$ color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (<i>C.</i>	86

	<i>xanthorrhiza</i>) in light and dark condition for different exposure time after treatment with 3 mg/mL NaCl	
Figure 5.6	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) in light and dark condition for different exposure time after treatment with 5 mg/mL NaCl	87
Figure 5.7	Chromaticity (ΔE^*_{Lab}) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) in light and dark condition	93
Figure 5.8	Yellow intensity (b^*) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) in light and dark condition for different pH	94
Figure 5.9	Colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) in light and dark condition for different pH	95
Figure 5.10	CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) and treated with UV radiation for 8 hours	98
Figure 5.11	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) when treated at different temperature for 3 hours	101
Figure 5.12	Yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (<i>C. xanthorrhiza</i>) when treated at different temperature for 3 hours	102

Figure 5.13	BWPs of curcumin pigment's ΔE^* stability variables at different concentrations and exposure times	103
Figure 5.14	Variable plots and correlation biplots of the variables in the curcumin pigment's stability test	110
Figure 6.1	Chemical structure of polylactic acid	113
Figure 6.2	CIE $L^*a^*b^*$ colour difference or Chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PLA-curcumin in light and dark condition for different exposure time	117
Figure 6.3	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L PLA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	121
Figure 6.4	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PLA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	122
Figure 6.5	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PLA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	123
Figure 6.6	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values (%) for 5 g/L PLA-curcumin at different pH in light and dark condition for different exposure time	128
Figure 6.7	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PLA-curcumin at different pH in light and dark condition for different exposure time	129

Figure 6.8	CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PLA-curcumin at different pH in light and dark condition for different exposure time	130
Figure 6.9	CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PLA-curcumin within 8 hours of UVB treatment	133
Figure 6.10	CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PLA-curcumin when treated at different temperature for 3 hours	138
Figure 6.11	Box-and-whisker plots of PLA-curcumin pigment chromaticity stability variables at different concentrations and time of exposure	139
Figure 6.12	Variables plot and correlation biplot for PLA-curcumin pigment stability test variables	145
Figure 7.1	Chemical structure illustration pair	148
Figure 7.2	CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PVA-curcumin in light and dark condition for different exposure time	151
Figure 7.3	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L PVA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	155
Figure 7.4	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PVA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	156

Figure 7.5	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PVA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	157
Figure 7.6	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L PVA-curcumin at different pH in light and dark condition for different exposure time	161
Figure 7.7	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PVA-curcumin at different pH in light and dark condition for different exposure time	162
Figure 7.8	CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PVA-curcumin at different pH in light and dark condition for different exposure time	163
Figure 7.9	CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PVA-curcumin when treated with UV radiation for 8 hours	166
Figure 7.10	CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PVA-curcumin upon exposure to different temperature for 3 hours	170
Figure 7.11	Box-and-whisker plots of PVA-curcumin pigment chromaticity stability variables at different concentrations and time of exposure	171
Figure 7.12	Variables plot and correlation biplot for PVA-curcumin pigment stability test variables	177

Figure 8.1	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of Chitosan-curcumin in light and dark condition for different exposure time	183
Figure 8.2	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L chitosan-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	187
Figure 8.3	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L chitosan-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	188
Figure 8.4	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L chitosan-curcumin at different concentrations of NaCl in light and dark condition for different exposure time	189
Figure 8.5	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L chitosan-curcumin at different pH in light and dark condition for different exposure time	193
Figure 8.6	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L chitosan-curcumin at different pH in light and dark condition for different exposure time	194
Figure 8.7	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L chitosan-curcumin at different pH in light and dark condition for different exposure time	195
Figure 8.8	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for	198

	different concentrations of chitosan-curcumin after treated with UV radiation for 8 hours	
Figure 8.9	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of chitosan-curcumin when treated with different temperature for 3 hours	202
Figure 8.10	Box-and-whisker plots of chitosan-curcumin pigment chromaticity stability variables at different concentrations and time of exposure	203
Figure 8.11	Variables plot and correlation biplot for chitosan-curcumin pigment stability test variables	209
Figure 9.1	CIE $L^*a^*b^*$ colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin in light and dark condition for different exposure of time in natural soap	216
Figure 9.2	Box-and-whisker plots of curcumin pigment in solid bath soap chromaticity stability variables at different concentrations and time of exposure	217
Figure 9.3	Variables plot and correlation biplot for PLA-curcumin pigment stability test variables	223

LIST OF SYMBOLS

%	Percentage
<	Less than
>	More than
µg/g	Microgram per gram
µL	Microlitre
µm	Micrometre
a*	Red-green component of a colour
b*	Yellow-blue component of a colour
CFU	Colony forming unit
cm	Centimetre
CO ₂	Carbon dioxide
g	Gram
kg	Kilogram
L*	Difference in lightness or darkness
lux	Luminous flux per unit area
M	molar
mL	Millilitre
mm	Millimetre
MΩ	A unit of resistance, equal to one million ohms
O ₂	Oxygen
°C	Degree celcius
Pa	Pascal
r ²	Coefficient of determination
v/v	Volume per volume
Δ	Delta

LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
c.d.b.	Conjugated double bonds
CFR	Code of Federal Regulations
CIE	International Commission on Illumination
DAD	Diode-array detection
DMSO	Dimethyl Sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dried weight
EU	European Union
EV	eigenvalues
FDA	Food and Drug Administration
FL	Factor loading
FTIR	Fourier transform infrared
HC	halal cosmetics
HFP	halal food packaging
HPLC	High-Performance Liquid Chromatography
MBC	Minimum Bacterio Concentration
MCC	Minimum Candido Concentration
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
NA	Nutrient agar
PCs	Principal components
PLA	Polylactic acid
ppm	Part per million

PVA	Poly(vinyl alcohol)
ROS	Reactive Oxygen Species
SDA	Sabouraud Dextrose agar
SOD	Superoxide Dismutase
US	United States of America
USFDA	United States Food and Drug Administration
UV	Ultraviolet



CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Since the beginning of time, natural options always prevailed over artificial or synthetic materials because those were the only alternatives available to humans. Additionally, organically produced materials were given precedence over manufactured ones because the former offered better quality. Colour has always been a significant factor in the development of many human cultures worldwide. The public has been aware of issues related to eco-preservation, eco-safety and health problems; moreover, there has been awareness of the sustainability of non-toxic and environmentally friendly colourants derived from natural resources. The perception of colour manifests because light sources emit a limited range of frequencies in the electromagnetic spectrum (Delgado-Vargas and Paredes-Lopez, 2003). Colourants are substances that alter an object's perceived colour or can be used to add colour to objects that would otherwise be colourless. Additionally, the definition of natural includes the following: *"present in or produced by nature; not artificial or man-made"*, as well as *"not altered, treated, or disguised."* Additionally, a colourant may refer to a chemical that is naturally derived from plants, animals, or minerals and can give apparent colour to items or objects that would otherwise be colourless. The United States (US) Food and Drug Administration (FDA) defines a colour additive as *"any material, not exempt under section 201(t) of the act, which contribute to the dye, pigment, or other substance that are made by the process of synthesis or similar artifice, that were extracted, isolated, or otherwise derived, with or without its intermediate and also the final product, that are from vegetable, mineral, animal, or any other substances that can be used to a food, drug, or cosmetic or to the human body that are reacted and can be helped in imparting the colour."* (CFR, 2016a). This definition can be related to human nature (*fitrah*)

and the enduring attraction to vibrant colours and exquisite details. The use of natural colourants evolved in the second half of the 1800s with the development of synthetic colourants, which can be helpful in a variety of appealing cases (Ul-Islam, 2017). Consumers pay close attention to contentious issues surrounding the safety of synthetic colouring. Because of its economically significant qualities, such as low cost, resilience to light, oxygen (O₂), and pH fluctuations, as well as great colour stability, food makers employ synthetic food colouring more often than natural food colouring (Sharma et al., 2021). Compared to natural hues, synthetic colours are produced in larger quantities at a lesser cost (Mittal, 2020a). Due to the limited and seasonal supply of resources required to make natural hues, the use of natural colourants is limited (Mittal, 2020b). The main reasons for using synthetic dyes are stability, affordability, homogeneity, and colouring properties (Sharma et al., 2021). However, after prolonged use, synthetic colourants can become contaminated and toxic, leading to health issues such as anaemia, urticaria, kidney damage, indigestion, allergic reactions, tumours, and cancerous lesions in the brain, spleen, and liver, abnormalities in offspring, mental retardation, growth retardation, and eye defects that can result in blindness (Sharma et al., 2021). Artificial food colouring has been the subject of debate and scrutiny for a long time. It has been shown to cause allergies, hyperactivity, and cancer (Sharma et al., 2021). Therefore, this study aimed to measure the effectiveness of curcumin active ingredient functional groups towards chromaticity (ΔE^*) stability in halal food packaging (HFP) and halal cosmetics (HC). The presence and reactivity of the hydroxyl functional group will be measured through Fourier transform infrared (FTIR) spectroscopy analysis. Moreover, the mechanism of environmental stress on the ΔE^* stability in poly(lactic acid) (PLA), poly(vinyl alcohol) (PVA), and chitosan-curcumin coating composition over time will be assessed using a chroma (C^*) meter. Antioxidant and antimicrobial activities will also be evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and agar diffusion method in order to promote curcumin as a potential HFP and HC additive to prevent oxidation. The changes in the molecular structure of curcumin under different environmental stresses such as salinity, pH, heat, and ultraviolet (UV) exposure are good criteria for promoting curcumin in HFP and HC applications. For these reasons, the significant output or anticipated benefit from

this research project will be the development of HFP from PLA, PVA, and chitosan. These substances should be able to prevent oxidation and enhance the shelf life of the food products while maintaining their nutritional quality, which supports HC application.

1.2 STATEMENT OF THE PROBLEM

Discovering safer naturally-sourced alternatives to synthetic colourants has been recommended because the use of artificial colourants has been shown to have a detrimental impact on human development and health. Furthermore, synthetic dyes are often substituted by natural colourants to avoid the health side effects of synthetic dyes. However, there is limited research on the extraction of carotenoids, particularly curcumin extracted from *temulawak* (*Curcuma xanthorrhiza* Roxb.), as a possible source of colour and its stability against environmental changes. In conjunction with the trend, the curcumin extracted from *temulawak* can contribute to potential HFP and HC applications. Curcumin, one of the most well-known natural pigments, has received a lot of attention for its excellent functional properties and ability to change colour with changes in pH. Polymers are materials that possess long chains of repeating minor components that are bound together. They can be categorised according to their source, namely, petrochemical or natural (Williams and Hillmyer, 2008). They have a plethora of applications, including plastic-based packages. Nevertheless, although petrochemical-based plastics are easy to use and intended to be disposed of immediately after they have been used, they do not biodegrade. As such, this characteristic has led to environmental issues since the 1940s, which was when they became a popular choice of packaging (Jambeck et al., 2015).

For this reason, the incorporation of PLA, PVA, chitosan, and curcumin as potential or ideal candidates for HFP needs to be explored. Therefore, chemical, thermal, and other physical properties, including antioxidant and antimicrobial aspects, must be evaluated to

understand the behaviour and mechanism and the fundamental concerns regarding the incorporation of curcumin pigment for PLA, PVA, and chitosan. Nevertheless, there are limited studies on carotenoids extracted from *temulawak* and their application in HFP and HC. Therefore, this research introduces the potential application of halal coating substrate integrated into *temu lawak*-based curcumin pigment in the HFP and HC industries.

1.3 RESEARCH GOAL

The present study aimed to explore curcumin pigment extracted from natural resources, specifically *temulawak* rhizomes, for potential applications in halal food packaging (HFP) and halal cosmetics (HC).

1.4 RESEARCH OBJECTIVES

The following objectives were outlined to achieve the aim of the present study:

- i. To determine the curcumin content and composition of 12 species of ginger using extraction methods, namely, chemicals, maceration, and alkaline.
- ii. To determine the antioxidant and antimicrobial properties of the curcumin extracted from the 10 species of ginger.
- iii. To determine the ΔE^* stability of curcumin extracted from *temulawak*.
- iv. To determine the effect of visual indicators, such as colour changes, on curcumin-based packaging materials, such as PLA, PVA, and chitosan, in response to cumulative exposure to stress conditions.

- v. To determine the effect of visual indicators, such as colour changes, on curcumin-based bar soap in response to cumulative exposure to stress conditions.

1.5 RESEARCH QUESTIONS

- i. Which extraction method yields the most curcumin from the 12 species of ginger?
- ii. What are the antioxidant and antimicrobial properties of the curcumin extracted from the 10 species of ginger?
- iii. What is the effect of the ΔE^* stability of curcumin extracted from *temu lawak*?
- iv. What is the effect of visual indicators, such as colour changes, on curcumin-based packaging materials, such as PLA, PVA, and chitosan?
- v. What is the effect of visual indicators, such as colour changes, on curcumin-based bar soap?

1.6 RESEARCH HYPOTHESIS

The present study proposed that naturally-sourced curcumin can, potentially, be used in the halal food packaging (HFP) and halal cosmetics (HC).

1.7 SIGNIFICANCE OF THE STUDY

Synthetic dyes are commonly used as colourants in most industries, including the food and cosmetics sectors. There are several limitations and problems with these artificial colours. These substances are considered xenobiotics, are not environmentally friendly because they are nondegradable and carcinogenic, and feature synthetic hues that are toxic. This is the rationale behind why people favour using natural pigments as colouring agents. Natural colourants are preferred over synthetic dyes in cosmetics due to several problems with the latter category. Present-day cosmetics use a greater number of natural colourants. Biological sources provide natural colourants, which are deemed environmentally benign and non-toxic. Therefore, this research promotes alternatives from natural resources that can contribute to the safe daily usage of products, especially foods and cosmetics, made from sustainable resources, specifically concerning the material used for packaging. Adherence to safety and hygiene standards is important to ensure a good quality (*halalan-toyyiban*) product. Hence, the application of halal food packaging (HFP) is promising because of its natural origin; it can be used as a substrate for different polymers used in various sectors, including the food and cosmetics industries.

1.8 SCOPE OF THE STUDY

The scope of the study pertains to the investigation of curcumin pigment extracted from *temu lawak*. The study sample is specified as *Curcuma xanthorrhiza* Roxb. species, locally bought from *pasar tani* (farmer's market). The study focuses on natural sources because they contain valuable carotenoids and demonstrate potential applications in halal food packaging (HFP) and halal cosmetics (HC).

CHAPTER TWO

LITERATURE REVIEW

2.1 CURCUMIN AND ITS PROPERTIES

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] is the main curcuminoid responsible for turmeric's vibrant yellow hue. This carotenoid, obtained from the rhizomes of *Curcuma longa*, changes its colour in alkaline environments or high-pH solutions (Oglah et al., 2020). Curcumin acts as a potent scavenger of various reactive oxygen species (ROS), such as hydroxyl radicals and superoxide anions, contributing to its antioxidant effects (Hewlings and Kalman, 2017). Additionally, curcumin shows potential in treating cystic fibrosis. While tested only in animal models, particularly baby hamster kidney cells, research indicated that oral curcumin administration promoted the functional appearance of AF508 CFTR protein, the protein impaired in cystic fibrosis (Egan et al., 2016). Curcumin is manufactured by utilising the oleoresin of turmeric as the primary ingredient. Tests have shown that the by-products derived from extracting curcumin also exhibit antibacterial properties against various harmful bacteria like *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Nelson et al., 2017). Research utilising size exclusion chromatography and dynamic light scattering has demonstrated the compound exists as a dimer at pH 7, but transitions to a monomer at pH 2. At pH 7, the dimer is more stable than the monomer at pH 2 (Biswas and Chattopadhyaya, 2014). Delgado et al. (2016) reported that turmeric remained stable in its amorphous form when stored at temperatures below 35°C.

In contrast, Tonnesen et al. (1986a) discovered that curcumin undergoes photodecomposition when exposed to UV radiation, a process observed in both solution

and as a thin solid film. This breakdown involves the loss of two hydrogen atoms from curcumin, leading to its cyclisation. Due to its sensitising nature, curcumin may destabilise mixtures with other compounds. As a diphenolic compound, curcumin is small, hydrophobic, non-toxic, and insoluble in water and ether, but dissolves in ethanol, methanol, and dimethyl sulfoxide (DMSO). Its attractive properties, including enhanced mechanical strength, antioxidant effects, UV protection, and antibacterial activity, make curcumin appealing for use in composite film packaging (Ezati and Rhim, 2021b). Curcumin also showed a significant colour transformation in response to changes in pH. This occurred due to alterations in its main configuration in varied pH environments. Curcumin has a structured crystal form with a chain of seven carbon atoms, an unsaturated diketone segment, and two aromatic rings with ortho-methoxy phenolic-OH groups. The molecular structure of curcumin can be influenced by the pH level of the solution, its polarity, and the temperature. At pH levels that are neutral or alkaline, the α , β -unsaturated β -diketone group acts as a donor of H, causing curcumin to break down and degrade. The β -diketone chain in curcumin exists as a keto-enol tautomer, with its form determined by the solvent's properties. In acidic or neutral pH conditions, curcumin predominantly takes the bis-keto form, while in basic conditions, it shifts to the enolic form. In pH solutions ranging from 1 to 7, curcumin is mostly in its yellow bis-keto state, which has very low solubility in H₂O. As the alkalinity of the environment rises, curcumin's solubility and stability improve. The enolic form acts by donating hydrogen atoms from C-H bonds at the central carbon to nearby oxygen atoms, facilitated by the weak delocalisation of unpaired electrons (Typek et al., 2019).

2.2 CHEMICAL STRUCTURES

Carotenoids' absorption spectra mainly rely on the quantity of conjugated double bonds (c.d.b.) in their molecules. The greater the length of the chromophore, the greater the wavelengths absorbed at maximum. Acyclic carotenoids absorb light at longer wavelengths

than cyclic carotenoids with the same number of double bonds due to the presence of steric strains in the latter. Analysts find the UV/Vis spectrum of carotenoids important as it offers valuable insights into their structure. The spectrum arises because of the existence of the extended chromophore of c.d.b. A carotenoid must have at least 7 c.d.b. in order to have a visible colour. Therefore, a carotenoid having 7 c.d.b. displays a faint yellow hue, while carotenoids possessing 3 and 5 c.d.b. do not exhibit any colour (He et al., 2018).

2.3 FUNCTIONAL GROUPS

Functional groups are added to polymer molecules for several purposes. They can alter the polymer's physical characteristics, such as improving adhesion to substrates or enhancing solubility in specific solvent mixtures. Additionally, functional groups may be crucial for the polymerisation process or for enabling further chemical reactions with other compounds. The success of these functional groups in meeting these objectives depends on their accessibility, as well as potential complications arising from their chemical structures, which may introduce conflicting effects. Curcumin contains a β -diketone (1,3-diketone) moiety, characterized by two ketone (-C=O) groups in the center, which plays a crucial role in its tautomeric stability. Additionally, it possesses phenolic (-OH) groups on both aromatic rings, contributing significantly to its potent antioxidant properties. Furthermore, the presence of methoxy (-OCH_3) groups on both benzene rings enhances its biological activity and influences its solubility and interaction with biomolecules (Figure 2.1).

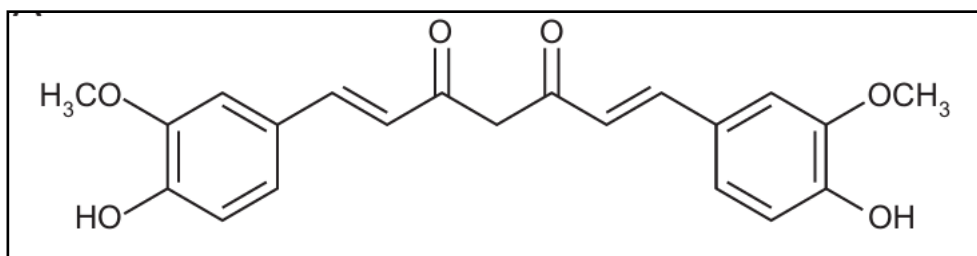


Figure 2.1 Functional groups of curcumin (Lestari and Indrayanto, 2014).

2.4 BIOLOGICAL ACTIVITY OF CURCUMIN

2.4.1 Antioxidant Activity

Different tests have been employed to evaluate the antioxidant properties of curcumin in both in vitro and in vivo environments. The presence of hydroxyl groups in polyphenolic compounds contributes to their antioxidant effect by directly correlating with the number of functional groups and the scavenging activity against free radicals. The existence of one or multiple hydroxyl groups exhibited a marked superiority in antioxidant activity because of the curcumin derivative bis(3,4-dihydroxycinnamoyl)-methane. Curcumin has been known to improve oxidative stress, as evidenced by systemic indicators (Sahebkar et al., 2015). It also aids in raising the levels of antioxidants, such as superoxide dismutase (SOD), in sera (Banach et al., 2015; Menon and Sudheer, 2007; Panahi et al., 2016). Similar to its role in free radical scavenging observed in other compounds like carboxymethyl cellulose (Ezati et al., 2021c), the –OH group plays a crucial part in curcumin. The position of the hydroxyl group significantly influences its activity. In curcumin derivatives, such as bis(2-hydroxycinnamoyl)-methane, the 2-hydroxyphenyl group demonstrated stronger antioxidant activity compared to the 4-hydroxyphenyl group found in curcumin itself. This suggests that the placement of the hydroxyl group is key to enhancing its antioxidant properties. The function of the -OH group in free radical scavenging is the same as that of

other substances, such as carboxymethyl cellulose (Ezati et al., 2021c). Curcumin is most effective when it specifically used to induce pro-oxidant effects in malignant cells, such as cervical cancer cells (Kim et al., 2015). Study have demonstrated that curcumin has a neuroprotective effect as it reduces ROS, which are linked to endoplasmic reticulum (ER) stress and neuronal injury. This effect may be mediated via the control of AMP-activated protein kinase (AMPK) activity (Li et al., 2015).

2.4.2 Antimicrobial Activity

Curcumin is a promising candidate for use in medicine due to its medicinal characteristics. It possesses anti-inflammatory, anticancer, antioxidant, and antibacterial properties (Hewlings and Kalman, 2017). According to the United States (US) Food and Drug Administration (FDA), curcumin is generally safe and non-hazardous. Multiple studies have examined the anti-inflammatory (Hewlings and Kalman, 2017), anticancer (Tomeh et al., 2019), and antibacterial (Praditya et al., 2019a) properties of curcumin. It has been found to exhibit limited bioavailability, poor absorption, and is quickly eliminated by the human body (Cas and Ghidoni, 2019). It is also believed to be unstable in solutions. This, however, depends on the organic solvents that are used to solubilise it, such as ethanol, acetone, methanol, and DMSO (Priyadarsini, 2014). Curcumin demonstrated stronger antifungal properties than demethoxycurcumin. It was stated that the presence of the methoxyl group in curcumin enhances its lipophilicity, allowing for continuous penetration into the fungal cell membrane and inhibiting its growth. Adamczak et al. (2020) investigated how turmeric's antimicrobial properties affect over 100 pathogens across 19 different species. It contains molds, as well as many Gram-positive and Gram-negative bacteria. Research has shown that curcumin exhibits stronger antimicrobial activity towards Gram-positive bacteria than Gram-negative bacteria. The antibacterial efficacy of curcumin has been demonstrated against both Gram-positive and Gram-negative bacteria. This includes strains that cause infections in humans. It has also demonstrated antibiotic

resistance (Praditya et al., 2019b; Moghadamtousi et al., 2014; Zheng et al., 2020; Teow et al., 2016).

2.4.3 Temu lawak (*Curcuma xanthorrhiza* Roxb.)

Medicinal plants have been utilised to treat a variety of human illnesses for centuries (Saida et al., 2018). The Indonesian plant temu lawak is used in traditional medicine and is considered potent due to its medicinal properties (Andini et al., 2015). The rhizome of the temu lawak possesses pharmacological, anti-inflammatory, anti-bacterial, antioxidant, anti-cholesterol, anaemia-inhibiting, appetite-stimulating, and anti-cancer qualities (Kawiji et al., 2011; Ali Rosidi, 2020). Curcuminoid bioactive compounds, which are produced by temu lawak's secondary metabolism, yields health benefits (Cahyono et al., 2011; Ali Rosidi, 2020). Curcumin is an active ingredient that is considered an antioxidant. The curcumin produced by the rhizome of temu lawak also possesses antioxidant qualities. Antioxidants play an important role in improving health (Jamshidi et al., 2018) as they can activate endogenous antioxidant defence mechanisms (Alshubaily and Jambi, 2018). Rao (1995) reported that the curcumin produced by temu lawak is more potent than both beta-carotene or vitamin E (Inayatilah, 2017). Furthermore, according to Purwakusumah (2016), mature temu lawak rhizomes are rich in curcumin and related compounds, all of which possess antioxidant qualities (Purwakusumah et al., 2018). A study on the preparation of temu lawak extract and its subsequent use as an antioxidant revealed a curcumin concentration of 27.19%. A DPPH assay was then conducted to determine its antioxidant activity, which yielded a half-maximal inhibitory concentration (IC_{50}) of 87.01 ppm. Therefore, curcumin was not only present in the temu lawak extract, but it was an active antioxidant (Rosidi et al., 2014).

2.4.4 Packaging Material

Packaging is both an art and a science, involving the application of technology to preserve a product's safety, quality, and integrity. It serves as a vital system for preparing items for transportation, storage, distribution, retail, and eventual use by consumers. Packaging also plays a critical role in ensuring products are safely delivered to buyers, performing a dual function of decreasing shipping costs and boosting sales (Coles and Kirwan, 2011; Pal, Devrani, and Hadush, 2019). With the rise of globalisation and urbanisation, there has been growing consumer demand for safe, high-quality foods. Once packaged, food longevity is reliant on its composition and different external factors, allowing it to last from hours to months, based on its shelf-life. A food item can be exposed to physical, chemical, or biological substances while being handled, prepared, or transported. Food items are wrapped in packaging in order to maintain their quality and freshness. It can help prevent contamination from spreading by avoiding contact with other food products or components that play a role in the food supply chain. Consequently, the current market requires specific criteria and guidelines for packaging and labelling of food products.

Food packaging plays a crucial role in the food supply chain by acting as a protective barrier against contamination, the outside environment, and physical harm during transportation, all of which can harm product quality. It also functions as a preventative measure, necessitating products to be sealed throughout the entire distribution process until they are opened by the consumer, guaranteeing no leaks and monitoring for any tampering problems. Food packaging functions mainly as a means of identification and communication between producers and consumers, displaying important product information like health benefits, nutritional facts, usage and storage instructions, manufacturing location, production date, expiration date, and other essential details. Additionally, effective packaging serves as a marketing tool which can target different consumer demographics based on factors such as gender, age, and culture. The role of packaging also helps optimise purchasing by offering the desired quantity while minimising

waste, making it an essential aspect of both consumer appeal and sustainability efforts (Biji et al., 2015a; Marsh and Bugusu, 2007a; Sohail, Sun, and Zhu, 2018). In 2009, the food packaging industry was the largest packaging industry, representing more than 50% of all packaging industries. Its worth was estimated to be US\$380 billion. Renata Dobrucka (2014) estimated the value of the global active packaging industry to be US\$6.4 billion in 2013, which accounted for 26.9% of all packaging industries. It also estimated intelligent packaging, which is characterised by its ability to actively extend the shelf life of food products, to be US\$2.3 billion. Packaging is described as the act of surrounding food in order to shield it from tampering or contamination originating from chemical, physical, and biological sources. Packaging preserves the advantages of food processing even after completion, ensuring that products can be transported safely over long distances from where they were produced and remain fresh when consumed. Food packaging serves primarily to safeguard food from damage caused by water vapour, oxygen, ultraviolet light, and contamination by chemicals and microorganisms (Drago et al., 2020). Active packaging was initially employed effectively to extend the shelf life of processed foods and satisfy consumer needs by delivering high-quality, fresh, and safe products.

2.4.5 Smart Packaging

Smart packaging is an alternative technology that is used to overcome a variety of issues. It combines both active and intelligent systems to increase shelf-life and communicate the quality of the food contained within to the end consumer. This not only improves the functionality of food packaging but adds additional features to it. Active packaging and intelligent packaging are two types of smart packaging. Active packaging systems extend the shelf life of food products, while intelligent packaging systems communicate the quality of the food contained within to the end consumer. Therefore, smart packaging informs the end consumers of the integrity and authenticity of the food within while actively preventing spoilage. It also enhances food attributes, such as taste and appearance. Apart from that, it

can be used to communicate additional food information to the end consumer as well as help the food adapt to changes to in the environment. The most important aspect of smart packaging is that it can indicate seal integrity and prevent replication. Food packaging is used for the following reasons (Vanderroost et al., 2014):

- i. To prevent potential contamination as well as leaks or breaks.
- ii. To provide cooking directions, thereby, transmitting crucial information about the food and nutritional value.
- iii. To enable end consumers to easily heat or reheat the food within.
- iv. To contain the food within during handling and transportation.

Smart packaging is a comprehensive packaging solution that monitors and adapts to changes in the product's environment or within the product itself (Vanderroost et al., 2014). Extant studies define smart packaging as any packaging that includes both active and intelligent systems. These types of packaging can not only monitor temperature or humidity changes during storage, but slow the degradation of the food within. This is accomplished using compounds, such as antioxidants, emitters of carbon dioxide, humidity, antibacterial agents, O₂ scavengers, and ethylene. (Schaefer and Cheung, 2018; Vanderroost et al., 2014; Latos-Brozio and Masek, 2020; Yucel, 2016). Smart packaging encompasses devices that can be used to heat or cool the food within. They also have electronic displays that show the nutritional information of the food in real time. Device that can change the temperature of the contents of bottles, cans, and cartons have been developed. It works by transferring heat from the contained liquid to a vinyl bag of pressurised H₂O. The heat causes the pressurised H₂O to evaporate and release immediately.

2.4.6 Active Packaging

Active packaging is an innovative packaging in which the package, the product, and the environment interact to prolong the shelf life of the food within as well as enhance its safety and sensory properties while maintaining its quality. This is particularly advantageous for fresh and extended shelf-life food products.

2.4.7 Criteria of Active Packaging Materials

2.4.7.1 Oxygen Scavengers

Molecular O₂ can be decreased by gaining one to four electrons, resulting in the formation of reactive species such as hydroxyl radical, superoxide, hydrogen peroxide (H₂O₂), and H₂O. With the exception of H₂O, these intermediate species are highly reactive. As ROS, they are free radicals, making the oxidative reactions they trigger autocatalytic. Oxygen scavengers offer several benefits (Michiels et al., 2017):

- i. They prevent oxidation, avoiding rancidity in fats and oils, the development of off-odours and off-flavours, colour changes, and the degradation of O₂-sensitive nutrients (vitamins A, C, E, unsaturated fatty acids, etc.).
- ii. They inhibit the growth of aerobic microorganisms.
- iii. They minimise the need for preservatives and antioxidants, supporting claims of “fresh” or “natural” products.
- iv. They provide a cost-effective alternative to modified atmosphere and vacuum packaging.
- v. They decelerate the metabolism of food.

- vi. These systems, used alone or alongside traditional packaging and modified atmospheres, can extend the shelf life of food products.

2.4.7.2 Carbon Dioxide Generating System

As CO₂ has a permeability 3 to 5 times greater than O₂ in many plastic films, it needs to be consistently generated to uphold the preferred concentration in the packaging. The absence of oxygen in the environment by itself is not enough to slow down the growth of *S. aureus*, *Vibrio species*, *E. coli*, *B. cereus*, and *Enterococcus faecalis* at room temperature. Absorbers of oxygen and carbon dioxide hindered the development of *Clostridium sporogenes* (Alehosseini and Jafari, 2019).

2.4.7.3 Antimicrobials

An alternative approach to managing microbial growth involves the use of non-volatile antimicrobial additives. Various preservatives, including benzoic acid, sorbic acid, propionic acid and its salts, bacteriocins like nisin, silver ions, natural spices, and chelators, are incorporated into plastic films and materials for their antimicrobial properties. However, these non-volatile agents need to be in direct contact with the food to exert their antibacterial effects. Incorporating natural plant extracts, particularly essential oils with antioxidant and antimicrobial properties, may increase the efficacy of edible films to maintain post-mortem quality and prolong the shelf life of meat and aquatic products (Sirocchi et al., 2017; Gutiérrez et al., 2017; Yang et al., 2016; Çoban, and Keleştemur, 2016). Aquatic products largely deteriorate due to the proliferation and metabolism of Gram-negative bacteria.

2.4.7.4 Antioxidants

The oxidation of fats is a key process in food spoilage, second only to microbial growth. The shelf-life of food decreases when lipids oxidise, causing changes in taste, odour, texture, functionality of muscle foods, and nutritional quality. The oxidation of food can be prevented by incorporating O₂ scavengers and antioxidant agents into the packaging. This packaging is designed to hinder or delay the oxidation reactions that impact the freshness of food. Apart from compounds that can release or emit CO₂, antioxidants, antimicrobial agents, and flavours, active systems can also comprise compounds that can absorb O₂, C₂H₄, CO₂, flavours, odours, and moisture (Biji et al., 2015b; Realini and Marcos, 2014; Suppakul Miltz, Sonneveld, and Bigger, 2003). According to Shokri et al. (2015), lactoperoxidase, when combined with a whey protein solution, had a unique antimicrobial coating technique that decreased the deterioration of rainbow trout by fighting off spoilage microbes during refrigeration.

2.4.7.5 Intelligent Packaging

Food quality must be continuously monitored during distribution, storage, and selling as well as after the food is received by the end consumer as the microenvironment within the packaging may be altered and only detected after it has been packed. Therefore, this would require biological, physicochemical, and serological testing. Indicators should be simple to activate and display a clear, measurable, and permanent change. Their responses to time and temperature should be consistent and ideally align with or be easily related to the quality of the food, providing insights into the package's condition (Yildirim et al., 2018). Intelligent packaging is a packaging system that monitors the condition of packaged foods and provides information about the quality of the food within during transportation and storage (Silva et al., 2019). Managing the levels of C₂H₄ in storage conditions is crucial for extending the shelf life of various kinds of fresh produce (Hu et al., 2019).

Food packaging is an essential component of the food supply chain as it is a barrier or shield that protect food from external contamination, mechanical damage during transportation, and other factors that may negatively affect the quality of the food. Apart from ensuring that there is no leakage and to determine if the food has been tampered with, food packaging also functions as a preventive throughout the distribution process until it reaches the end consumer. In the goods industry, packaging indirectly decreases waste production (Biji et al., 2015a; Marsh and Bugusu, 2007b; Sohail, Sun, and Zhu, 2018). More specifically, as intelligent packaging can monitor the quality and status of a product, it can help decrease food waste and spoilage, which may allay significant food losses (Müller and Schmid, 2019). However, although it can convey the state of a packaged good, it should not interact with the contained product (Biji et al., 2015b). Its sole purpose is to monitoring the product and provide consumers with information, such as the state of the package, its contents, the manufacturing date, and the storage conditions (Kreibe et al., 2017).

2.4.7.6 Polymers

Polymers are used in various ways, such as for packaging materials in plastics and as implants in the biomedical industry. Yet, these easily accessible petrochemical-derived products, which do not break down naturally and are meant to be disposed quickly, have led to numerous pollution issues since they rose to prominence in the market in the 1940s (Jambeck et al., 2015). As such, multiple studies have examined producing biodegradable polymers as an alternative in every industry (Tsuji, 2014).

2.4.7.7 Polylactic Acid (PLA)

Poly(lactic acid) (PLA) is a biodegradable polymer that has gained significant popularity in multiple fields, such as biomedical, pharmaceutical, and environmental. It is derived from renewable resources, such as corn starch, and is a great alternative to petrochemical-derived polymers. It holds significant promise for many industries, especially the food industry (Krishnan et al., 2015). As curcumin possesses antimicrobial, antioxidant, anti-inflammatory, and other health benefits, efforts should be made to incorporate it into polymers that are food-safe and food packaging. PLA is an eco-friendly polymer made from renewable sources. It is produced through the polymerisation of lactic acid monomers, which come from the fermentation of starch-based feedstocks, and is both biodegradable and compostable (bioplastic). However, its applications in industry are limited due to its lower strength, brittleness, flammability, and high permeability to most gases and solvents when compared to metals and ceramics (Shirai et al., 2013a). Lactic acid is the starting point in the synthesis of PLA. Fish waste, starch, cellulose, and other plant or animal waste products are great sources of $C_3H_6O_3$. PLA also exhibits exceptional repeatability and can decrease reliance on petrochemical-derived products (Vasile et al., 2018; Scaffaro et al., 2018; Battezzato et al., 2018; Avolio et al., 2018; Zhang et al., 2016a; Zare and Rhee, 2019; Espinach et al., 2018; Aghjeh et al., 2018; Yao et al., 2016). It has low molecular chain movability due to the absence of a flexible methylene segment in its backbone chain. It also has a high glass transition temperature (T_g) and a slow crystallisation rate. Therefore, PLA is not hardy, as its hardness depends on its molecular weight and crystallinity (Jin et al., 2018; Supthanyakul et al., 2016; Zhou et al., 2017; Rasal and Hirt, 2009; Yilmaz et al., 2014; Song et al., 2011).

2.4.7.8 Poly (Vinyl Alcohol) (PVA)

Poly(vinyl alcohol) (PVA) is widely used for diverse applications in multiple industries, such as coating, biopolymer film, medical, and food processing. It can also be used as an inhibitor of hydration in the oil and gas industry. As it is a synthetic H₂O-soluble polymer, it comprises C atoms that are biodegradable (Chiellini et al., 2003; Riew et al., 1976; Aina et al., 2014; Halima et al., 2016) under aerobic and anaerobic conditions. As it is derived from single-unit precursor monomers, it is a distinct polymer that it is not produced via polymerisation, but from another polymer (James, 2016). As polymeric materials are lightweight, affordable, and have superior physicochemical qualities, they are often used to replace conventional materials (Abdullah et al., 2017a). PVA is a synthetic polymer that is granular or powdered, semi-crystalline, white, odourless, tasteless, nontoxic, thermostable, and biocompatible (Abdullah et al., 2017b; Saini et al., 2017). It is most frequently employed to produce plastic films (Muller et al., 2020; Zhao et al., 2020). Advancements in science and technology demonstrate the rapid development of plastic film technologies (Geyer et al., 2017). However, plastic films require a long time, possibly hundreds of years, to break down into simple, innocuous molecules, such as CO₂ and H₂O (Ward et al., 2019). Therefore, it is harmful to the ecosystem (Abu-Saied et al., 2017; Jridi et al., 2014). The manner in which a good is transported depends on the packaging used. Most packaging is made of flexible materials, mostly paper and plastic films (Landim, 2016). When utilised in the packaging industry, all the components in PVA H₂O-resistant films must satisfy the fundamental specifications for packaging materials, and all the additives used must be safe and non-hazardous (de Oliveira et al., 2020). There are two types of PVA films; fully hydrolysed and partially hydrolysed. PVA is non-biodegradable. It is a type of H₂O-soluble polymer that is considered a class of H₂O-soluble non-ionic polymers, which belongs to a vinyl group. It is non-hazardous, safe to handle, and relatively environmentally-friendly.

2.4.7.9 Chitosan

After cellulose, chitin is the second most prevalent polysaccharide on Earth. It comprises three different types of reactive functional groups: an amino group at position C-2; primary and secondary hydroxyl groups at positions C-3 and C-6, respectively; and an amino group at position C-2. Although chitin is mostly found in marine invertebrates, it is also present in insects as a significant component of their exoskeletons. It is also found in the cell walls of some fungi. Every year, people eat marine invertebrates, such as crabs, lobsters, prawns and oysters, which are seafoods that are rich sources of protein. However, the non-edible components of these crustaceans, such as their outer shells, make up almost half of their body mass and are disposed of as waste (Hamed, Özogul, and Regenstien, 2016). This waste is one of the main sources of chitin. Commercial chitosan is produced by partially deacetylating chitin, which results in the copolymerisation of *N*-acetylglucosamine and D-glucosamine. Chitosan is a soluble form of chitin that is used in many industrial applications, including food packaging and preservation (Kausar, 2017b; Kumar et al., 2019b; Mihai and Popa, 2015). When the degree of chitin deacetylation reaches 50%, it is soluble in aqueous acidic media and called chitosan. It is considered a functionally versatile biopolymer due to the presence of amino groups that are responsible for the various properties of polymers. Chitosan has been used in various industrial applications due to its properties, such as flexible food packaging and edible food coatings that are comparable to conventional non-biodegradable plastic-based food packaging materials. The barrier properties of a food packaging are important factor as a poor barrier could enable O₂ and H₂O vapours to interact, thereby degrading the quality of the contained food. Chitosan is the only known natural polysaccharide to have antibacterial activity against fungus, yeast, and bacteria. It accomplishes this by forming a layer that resembles cellophane and acts as a barrier against microbial attacks on the food's surfaces (Kumari et al., 2015a). Apart from that, a chitosan coating may decrease the amount of gas exchanged between the contained food and its surroundings. By obstructing the transmission of O₂, the aerobic bacteria in the food are unable to interact with the gas, which results in their removal (Dotto et al., 2015).

2.5 CURCUMIN IN POLYMER-BASED COMPOSITE FILMS

Curcumin can be used to fabricate active and smart packaging materials due to its excellent functionality in polymer systems. The addition of curcumin to a polymer film may improve the functional and barrier properties as well as the mechanical and thermal stability of polymer films. Of the many polymers that can be used to produce active and smart packaging, such as biopolymers, bioplastics, and commercial plastics, biopolymers are the most interesting due to their reproducibility, biodegradability, and excellent functional properties (Rouf and Kokini, 2018a). As curcumin possesses excellent biological activity, it can be used to develop a promising compound for the food packaging industry. Consumers these days are aware of choosing healthy food options. This includes the addition of curcumin as a bioactive substance to protect their food. The advantages of curcumin include its safety, cost effectiveness, and natural source as well as its functional antioxidant and antimicrobial properties and food colouring capabilities. According to Ma et al. (2017b), curcumin's natural colouring and functional properties deem it an ideal alternative synthetic colourant.

2.6 PACKAGING AND COLOUR

Colour plays a significant role in showcasing the impacts of the initial acceptance or rejection of a food item (Manzoor et al., 2021). Consumer preferences have been used to assess colour, which accounts for 62–90% of the total (Teixeira et al., 2022). The colour must be used for maintaining consistent colour between batches, intensifying pigments, and producing coloured products from previously colourless ones (Ranaweera et al., 2020). Moreover, colour is crucial in enhancing the visual appeal of food and highlighting the significance of food safety and nutritional value (Zhou et al., 2022). Packaging plays a crucial role in safeguarding and maintaining food quality and safety (Roy and Rhim, 2021b). Beyond its primary function of protection, food packaging serves as a

communication tool, providing consumers with information about shelf life, nutritional content, manufacturer details, and stowage conditions. With increasing consumer demand for safe, high-quality, and convenient foods, packaging technology has seen significant advancements. The food industry, including both manufacturing and packaging, now emphasises the development of stable, visually appealing, and cost-effective synthetic dyes to enhance food appearance and packaging. However, commonly used artificial colourants, such as Allura Red, Ponceau 4R, Carmoisine, Sunset Yellow, Tartrazine, Quinophthalone, and Quinoline Yellow, have been linked to potential adverse health impacts, including irritability, allergies, hyperactivity, and, in some cases, cancer (Roy and Rhim, 2021b). In conclusion, natural food colourants are an intriguing addition to food packaging and additives to satisfy consumer preferences for safer and healthier food options. According to Ezati and Rhim (2021a) and Ranaweera et al. (2020), natural colourants are not just secure but also possess useful characteristics like antioxidant and antimicrobial properties.

2.7 SUSTAINABILITY OF NATURAL AND ARTIFICIAL COLOURANTS

The US FDA defines a food colourant as *"any dye, pigment, or substance which, when added or applied to a food, drug, or cosmetics, or to the human body, has the ability which is alone or through reactions with other substances, that are used to impart the colour."* (CFR, 2016b; Sigurdson et al., 2017). Furthermore, as the colour of a fruit plays a part in attracting attention, it is the first sensory property that contributes to its commercial worth (Karanjalkar, 2022). Colour is also one of the key attributes and a crucial determinant when selecting a food. Colourants are also employed in food packaging (Echegaray et al., 2022; Gasti et al., 2021). Most manufacturers use synthetic food colouring instead of natural food colouring to achieve uniformity, high colour intensity, low cost, and superior aesthetics. As such, most food and beverages contain excessive amounts of permitted and non-permitted synthetic colourants. This is, primarily, because synthetic colourants are significantly cheaper than natural colourants, especially when required in large quantities (Mittal,

2020b). Furthermore, the availability of natural colourants is significantly affected by the seasons (Mittal, 2020a). In terms of product stability, natural colourants are unable to satisfy the colour, vibrancy, fastness, shades, and hues that manufacturers and consumers require (Mittal, 2020b). Colour is not only the most significant sensory cue for processed food products, but influences consumer preferences, purchasing decisions, and eating impulses as well (Piqueras Fiszman and Spence, 2015; Martins et al., 2016). Both the US and the European Union (EU) utilise food colouring to enhance the appeal of bland and colourless food to customers. This not only makes it easier for customers to identify a product, but makes up for a product's loss of natural colour (Stich, 2016).

However, consumers these days are increasingly concerned about the toxicity, safety, and health of food additives (Gebhardt et al., 2019). There is no process that a natural pigment may go through that will not alter its molecular structure. As such, the naturalness of a pigment is a significantly valued attribute (Kammerer, 2016; Müller et al., 2016; Paliwal et al., 2019). However, natural pigments are more costly than synthetic ones due to their higher raw material requirements. Unlike their natural counterparts, artificial colourants have a stronger tinctorial strength. As such, artificial colourants are preferred to achieve suitable levels of food colouration (Stich, 2016; Sigurdson et al., 2017; Schweiggert, 2018). Natural pigments are also more sensitive to light, O₂, and heat during storage and transit, as such they have significantly shorter shelf lives than manufactured colourants (Schweiggert, 2018; Stich, 2016).

2.8 THE HALAL CONCEPT

The term "halal" originates from an Arabic phrase meaning lawful or permissible under Islamic Law. In the context of food trade and commerce, terms such as "Halal," "Guaranteed Halal," or "Muslim Food," along with other relevant expressions, indicate that a product is suitable for Muslim consumption according to the Trade Description Order of 1975. Halal food must not contain any ingredients prohibited by Islamic Law. In today's technology-driven era, the definition of halal extends to include emulsifiers, gelatin, enzymes, lecithin, glycerin, as well as stabilizers, flavorings, and colorings (Al-Teinaz, 2020).

Islam outlines two fundamental standards for food consumption in Surah al-Baqarah (2:168): halal (permissible according to Shariah) and *toyyib* (of good quality). Additionally, Surah al-Baqarah (2:172-173) emphasizes the duty of every Muslim to consume halal food and refrain from what is prohibited (Md Shariff et al., 2021). Beyond meeting halal requirements, Muslims should also consider the quality of the products they purchase. Prophet Muhammad (PBUH) underscored the significance of consuming good quality food (*toyyib*) in several hadiths, highlighting Islam's emphasis on the well-being of its followers. Every ruling in the Quran carries wisdom and should not be taken lightly (Adekunle and Filson, 2020).

2.9 HALALAN-TOYYIBAN CONCEPT

The concept of halal extends to the consumption and use of food and products that are affected by actions across the globe. The word halal is derived from Arabic word *hala*, which means lawful and permitted. The function of packaging is to help organise supplies and services to ensure that they are handled properly during transportation, delivery, and

distribution as well as protect workers from harm while handling goods, such as liquids and chemicals. The *halalan-toyyiban* principles involve consuming permissible, wholesome, clean, and healthy food (Arif and Sidek, 2015). At a global level, the food packaging field is one of the most advanced industries, which has led consumers to contemplate the economic impact of this sector (Food and Agriculture Organization (FAO), 2014). As consumers are aware of the use of packaging, they tend to purchase food packaging that is high quality but cheap. According to Ambali and Bakar (2014), the term halal refers to cleanliness and hygiene when preparing food. Cleanliness is a significant part of Islam and Allah only allows Muslim consumers to consume products that are hygienic, safe, and halal. The list below outlines the concepts of *halalan-toyyiban*, which includes extending the shelf life and increasing the quality of food products.

2.10 MALAYSIA AND HALAL RECOGNITION

Malaysia is increasingly being recognized as the global hub of the halal industry. Despite being a multi-religious and multi-ethnic nation, establishing its halal sector as a model for others is an ambitious objective (Md Shariff et al., 2021). To achieve this, the Malaysian government allocates a dedicated budget annually for halal development and provides incentives to support and enhance this initiative. While halal products and services are widely available across the country, regulatory oversight remains essential to ensure their authenticity, particularly when non-Muslims are involved in the production, distribution, preparation, or provision of halal goods and services (Ustadi, 2020). Malaysia aspires to be the global benchmark for halal integrity. By 2008, the Malaysian government envisioned the country as a central hub for halal products, service providers, and a key reference for halal standards, research, and development (R&D). Consequently, the Halal Hub Master Plan outlines two key development objectives for Malaysia: prioritizing research and development (R&D) and setting the standard for best practices in products, procedures, standards, and certification.

Malaysia is widely recognized as the most successful halal hub and a global leader in halal certification. The country is acknowledged as an authority in halal standards, research, and training (Mohd Fauzi et al., 2020; Ahmad et al., 2018). The Muslim community is the second-largest worldwide, comprising over two billion people (World Population, 2022). In Malaysia, Muslims made up 61.3% of the total population in 2019, according to the Department of Statistics Malaysia (2019). As a result, the growing demand for halal products in industries such as food and medicine are driven not only by Muslim consumers but also by non-Muslim communities (Majid et al., 2020; Mohamed et al., 2020).

Muslim consumers are becoming increasingly meticulous in selecting halal food products. In making food choices, they must ensure that the products are not only halal but also wholesome, encompassing all essential aspects. Beyond the source and processing of food, there is a growing emphasis on adherence to standards, safety, hygiene, and quality assurance. This ensures that Muslim consumers receive a final product that is fully compliant and guaranteed halal (Pribadi & Fitriana, 2022; Wiryani et al., 2018; Alqudsi, 2014).

Halal logistics management is a specialized branch of the halal supply chain (HSC) that facilitates the handling of both food and non-food products. It involves the strategic planning, management, processing, handling, and implementation of effective procedures for storing goods, material handling, inventory management, services, and information, all in accordance with Shariah principles (Ali et al., 2022; Handayani et al., 2022; Majid et al., 2020; Shariff & Ahmad, 2019; Abdullah et al., 2018; Tieman, 2013). However, maintaining halal compliance throughout the supply chain presents a significant challenge. The risk of cross-contamination arises when manufacturers handle both halal and non-halal products within the same facilities. This contamination can occur at various stages, including sourcing, processing, handling, packaging, storage, and transportation (Li et al., 2021; Mohamed et al., 2020; Supian, 2018; Rasi et al., 2017). Therefore, ensuring a robust halal-

compliant logistics system is essential to guaranteeing that the products reaching consumers remain genuinely halal.

According to the MS 2565:2014 Halal Packaging – Guidelines issued by the Department of Islamic Development Malaysia (JAKIM) (2015b); halal packaging refers to packaging materials that comply with Islamic law. This extends beyond the materials used in production to include the entire manufacturing process and handling procedures (Manual Procedure for Malaysia Halal Certification, 2014; Farah Ayuni et al., 2023b; Ab Talib & Mohd Johan, 2012). The guidelines outline specific criteria that must be met for packaging to be classified as halal. Therefore, stakeholders are responsible for ensuring compliance with Shariah requirements in food packaging production, particularly concerning raw material selection and manufacturing processes, to uphold Islamic principles (Perdana, 2020; Sidek & Ahmad, 2017; Shafii & Khadijah, 2012).

Furthermore, the concept of halalan toyyiban is a fundamental principle in the halal food industry. The root word toyyib means pure and clean from all deficiencies, and according to Al-Qadhi 'Iyad (1998) in his book *Ikmal al-Mu'lim*, toyyib is one of the attributes of Allah. Meanwhile, the term toyyiba in *Tafsir Ibn Kathir* (n.d.) refers to something that is good, beneficial, and excellent in both nature and Shariah, encompassing both spiritual and physical aspects. Linguistically, toyyib means good, delicious, healthy, and safe. According to the Maliki school of thought, al-Toyyibat refers to things that are lawful and inherently good (Qurtubi 2006). Meanwhile, the Hanafi, Shafi'i, and Hanbali schools of thought define al-Toyyibat as things that are considered good according to the taste and perception of the Arabs (Yusuf al-Qaradawi 1978). The determination of al-Toyyibat is based on the argument of the Arabs and the verse from the Qur'an in Surah Al-A'raf (7:157), which emphasizes that what is good is made halal and what is impure is made haram. Ibn Taymiyyah (2005), referring to the consensus of scholars, defines al-Toyyibat as things that are made lawful by Allah and beneficial to the consumer in terms of their faith and well-being. In the context of food and beverages, it is not only essential

to ensure their halal status but also to align with the concept of *toyyib*, which emphasizes food safety and the consumption of wholesome food (Arifah 2022; Azhar & Abdul Wahab 2017).

In Malaysia, all halal food procedures are managed by the Department of Islamic Development Malaysia (JAKIM). JAKIM (2015b) has issued the Manual Procedure for Malaysian Halal Certification 2014, which is used alongside the Malaysian Halal Standard. This manual outlines the definition of halal according to the Trade Descriptions (Halal Definition) Order 2011 and its amendment in 2012, which specifically explains aspects related to halal food, including:

- i. When food or goods are described as halal or labeled with any other term indicating that they can be consumed or used by Muslims, this means that the food or goods:
 - a. Do not consist of or contain any part or substance from animals that are prohibited by shariah law for Muslims to consume or that have not been slaughtered in accordance with shariah law and fatwa;
 - b. Do not contain any substances that are impure (*najis*) according to shariah law and fatwa;
 - c. Are not intoxicating according to shariah law and fatwa;
 - d. Do not contain any part or substance derived from the human body that is not permitted by shariah law and fatwa;
 - e. Are not poisonous or harmful to health;
 - f. Have not been prepared, processed, or manufactured using any equipment contaminated with impurities (*najis*) according to shariah law and fatwa;
 - g. Have not, during preparation, processing, or storage, come into contact, mixed, or been in close proximity to any food that does not comply with sub-paragraphs (a) and (b).

- ii. When a service related to food or goods is described as halal or labeled with any other term indicating that the service is suitable for Muslim consumers, this means that the service is carried out in accordance with shariah law.

Clearly, it is an obligation for every Muslim to seek what is halal and toyyib to ensure that their worship is accepted in alignment with the objectives of Maqasid Shariah (Wahyudi & Ali 2021; Harahap et al., 2020). Islam places great emphasis on purity and cleanliness, as these are closely related to faith and the blessings in a Muslim's life (Farid & Basri 2020; Siti Radhiah & Mohd Shahrim 2013).

The linguistic meaning of maqasid is the plural form of maqsad and maqsid (المقصد), derived from the word qasada (قصد), which means "to head towards a direction." Meanwhile, Shariah originates from the word syara'a (شرع), which means "law" or "regulation" and refers to what Allah has commanded, encompassing aspects of aqidah (faith), rulings, religion, and clear sectarian views (mazhab) (MuftiWP 2019). Generally, Maqasid Shariah refers to the objectives and wisdom set by Allah in legal rulings to establish human well-being (maslahah), addressing the needs of both the worldly and afterlife, material and spiritual aspects, individuals and society, as well as sects and the Muslim ummah across generations (Heroy & Bajalan 2022; Ikhlas et al., 2021).

According to Imam Al-Ghazali Rahimahullah, the pioneer of the concept al-dharuriyyat al-khamsah, there are five main objectives of Shariah: preserving religion (hifz ad-din), life (hifz an-nafs), intellect (hifz al-'aql), lineage (hifz an-nas), and wealth (hifz al-mal). Thus, it can be understood that Maqasid Shariah represents the purpose behind Allah's establishment of laws and regulations to ensure human well-being without harm or detriment (mudarat) (Syahriar & Nafisah 2020; Khatib 2018). One of the key factors in maintaining this well-being is monitoring food intake or anything that enters the body. For Muslims, shaping a good life in both this world and the hereafter requires careful attention

to what is consumed. Food must be guaranteed to be halal, safe, wholesome, nutritious, and of high quality, particularly in daily consumption. In food selection, aside from ensuring the halal status of products, Islam also emphasizes food quality and safety before consumption (Ali & Ahmad 2023; Tahir & Muslih 2023). This is to safeguard the physical, mental, and spiritual well-being of Muslims, as every food that enters the body transforms into blood and flesh, ultimately affecting one's spiritual connection with Allah (Farid & Basri 2020; Nazihah & Arifin 2020). Therefore, adhering to the principle of halalan toyyiban in food consumption is a fundamental requirement for achieving the objectives of Maqasid Shariah (Herindar 2022; Wahyudi & Ali 2021).

The use of bioactive compounds derived from plant sources in food packaging technology innovation serves as an effort to uphold the principles of Maqasid Shariah by ensuring that only halalan toyyiban food reaches Muslim consumers (Farah Ayuni et al., 2023b; Khatijah et al., 2018; Kashim & Husni 2017). With technological advancements, the development of active, intelligent, and bioactive packaging has gained significant attention among researchers. Moreover, safety features and functionality in active, intelligent, and bioactive packaging are crucial for improving and ensuring food products remain free from external or internal contamination. These packaging technologies can also provide information on the packaging surface that directly detects food quality (Halonen et al., 2020; Kuswandi 2020; Janjarasskul & Suppakul 2018; Majid et al., 2018).

2.11 CHAPTER SUMMARY

Curcumin, a natural colourant derived from *Curcuma xanthorrhiza* Roxb., is most effective when combined with other polymers, such as PLA, PVA, and chitosan, that are specifically used for packaging. The use of curcumin, which has a high concentration of biochemical activities, such as antioxidant and antibacterial properties, can be used to combine natural

colourants into packaging materials. The packaging is crucial to increase customer satisfaction with the contents within. The main concern of consumers is the potential health repercussions of using synthetic colourants. For instance, natural colourants have been combined in soaps and observed to determine how natural colourants affect or change their colour under various climatic conditions. When using curcumin with packaging materials and cosmetic applications, it is best to be aware of its qualities. Therefore, researching the properties of curcumin itself is crucial, particularly when it comes to its application in cosmetics and packaging materials.



CHAPTER THREE

RESEARCH METHODOLOGY

3.1 SAMPLE PREPARATION

In this study, the raw compounds derived from the rhizome samples were collected and stored from *Curcuma xanthorrhiza* Roxb. in a freezer at -20°C before they were processed further. Then, the 3kg of the samples were freeze dried by using a freeze dryer ((FDU-1100, EYELA, Tokyo Rikakikai Co., Japan) at -50°C and a vacuum pressure of 6.5 Pa for 3 days. Thereafter, the sample were powdered with the help of a heavy-duty blender (LB20E, Laboratory Blender). To minimise enzymatic reactions, the powdered sample was preserved under suitable conditions at -20°C (Othman, 2009).

3.1.1 Chemical Extraction

The above-mentioned powdered and freeze-dried *Curcuma xanthorrhiza* Roxb. samples (10 g) in 50 mL tubes and rehydrated them using distilled H₂O (1 mL). Thereafter, they added a mixture of acetone and methanol (7:3) solution (5 mL) to every tube. Then, these samples were stored overnight at room temperature in a dark place. Each sample was prepared in triplicates. The samples were vortex mixed and centrifuged at 13,500 x g for 2 min the following day (NU-C200R-E, Nuaire, USA). The supernatant was transferred into graduated polypropylene centrifuge tubes (50 mL). The same solvent (5 mL) was added to the tube and the procedure was repeated until a colourless supernatant sample was acquired (usually two or three times). Next, an equal volume of hexane and distilled H₂O (5 mL) were added to the collected supernatants for carotenoid extraction. The solution was vortex

mixed and kept at room temperature for layer separation. The upper hexane layer contained the carotenoids in the supernatants. This method was repeated using only hexane till acquired a colourless solution. Then, the upper layers were combined and thoroughly dried using a moderate stream of O₂-free nitrogen. After drying, the tubes were covered and sealed using parafilm to prevent any exposure to atmospheric oxygen and immediately stored at -20°C for further examination. For extracting the carotenoid curcumin samples, a mixture containing equal volumes of hexane and distilled water was added to the supernatants. This solution was mixed and placed in a rack for layer separation, where the upper layer contained the carotenoids. Then, the upper layers were combined and thoroughly dried using a moderate stream of O₂-free N (Othman et al., 2017).

3.1.2 Alkaline Extraction

The powdered sample (10 g) is weighed and transferred into a 50 mL tube. Thereafter, the researchers added sodium hydroxide solution (20 mL of 2M solution) to this tube. The solution is mixed and allowed to stand for 30 min. This is followed by the addition of ethyl acetate (20 mL) to the above tube. This solution is vortexed thoroughly and centrifuged at 13,500 rpm for 3 min. The upper ethyl acetate layer is collected with the help of a pipette. This procedure is repeated several times till a colourless upper layer is noted. The collected ethyl acetate layers are combined and concentrated with the help of a rotary evaporator for evaporating the excess ethyl acetate from the samples. The concentrated curcumin sample is then transferred to a universal bottle, and the remaining ethyl acetate solution is evaporated with a nitrogen drying procedure. Finally, the dried curcumin sample is stored in a -20°C freezer until further analysis (Camargo et al., 2016).

3.1.3 Dimethyl sulfoxide (DMSO) Extraction

One gram (1g) of the powdered sample was weighed before 20mL of DMSO solution was added to the tube and allowed to stand for 30 minutes. The solution was mixed well then centrifuged at 13, 500 rpm for 3 minutes. The upper layer was collected using a pipette and the process was repeated until the upper layer was almost colourless. The concentrated curcumin sample is then transferred to a universal bottle, and the remaining DMSO solution is evaporated with a N drying procedure. Finally, the dried curcumin sample is stored in a -20°C freezer until further analysis (Othman et al., 2019b).

3.1.4 High-Performance Liquid Chromatography (HPLC) Analysis

High-performance liquid chromatography (HPLC) that uses a diode-array detection (DAD) is a popular analytical technique that can be used to study the qualitative and quantitative profiles of carotenoid samples. The following process (Othman, 2009; Radzali et al., 2016) was used for HPLC analysis of the carotenoid samples collected in this study. For HPLC analysis, the concentration of carotenoids was determined using the Agilent 1200 series HPLC system (Agilent Technologies, USA). that were extracted from *Curcuma xanthorrhiza* Roxb. This instrument was equipped with a binary pump in addition to micro vacuum degassers, an autosampler injector, and thermostatted column compartment. The researchers used a reverse phase column of ZORBAX Eclipse XDB-C₁₈ end capped (5 µm), with a size of 4.6 x 150 mm. Eluent A included a HPLC grade solution containing acetonitrile: H₂O (9:1, v/v), whereas eluent B included the HPLC-grade C₄H₈O₂ solution. A Millipore S.A.S H₂O purification system was used for acquiring the Ultrapure H₂O 18.0 MΩ in this study.

The following solvent gradient was used for carotenoid analysis: 0-40% eluent B (0-20 min), 40-60% eluent B (20-25 min), 60-100% eluent B (25- 25.1 min), 100% eluent B (25.1-35 min) and 100-0% eluent B (35-35.1 min). The flow rate of the procedure was 1.0 ml min^{-1} while the injection volume of the sample was $10 \text{ }\mu\text{l}$. The column was re-equilibrated using 100% A for 10 min before injecting the next sample. The column was thermostatted at $25 \text{ }^{\circ}\text{C}$, where curcumin would be detected at the maximum absorption wavelength of 423 nm . Curcumin was identified via standard co-chromatography and its spectral characteristics were determined using a photodiode array detector. Meanwhile, its carotenoid concentrations were determined using their standard curves with regards to their structures and properties. The chromatographic peak was identified after comparing the retention times of the peaks and the spectra with a carotenoid standard. The carotenoid concentration was measured in microgram of the compound in 1.0 g dry weight of the sample ($\mu\text{g/g DW}$).

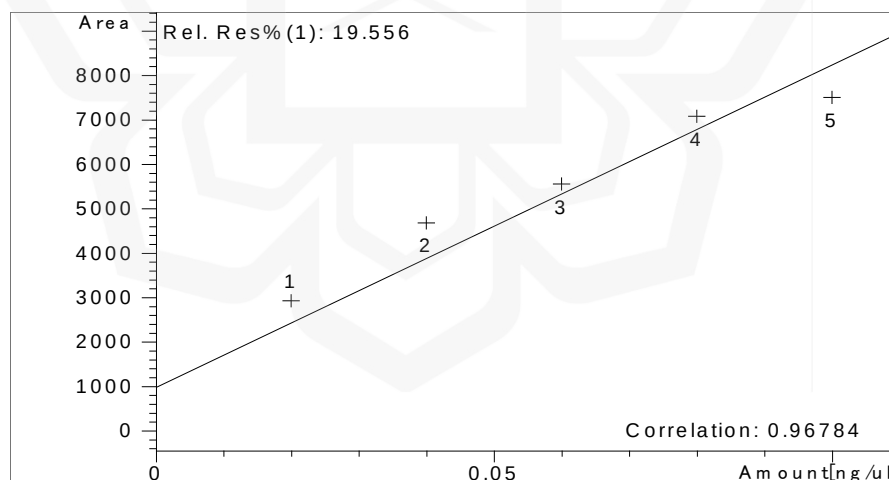


Figure 3.1 The standard calibration curve of curcumin

Using the standard linear regression line of curcumin, a calibration curve was generated (Figure 3.1), which produced an equation equal to $Y=72589.50 \cdot X+986.84$, with a coefficient of determination (R^2) of 0.96784. This equation was used to calculate the curcumin content of the samples in the present study.

3.1.5 Standard Preparation

One milligram of carotenoid, which is curcumin (Sigma-Aldrich Corporation, USA), was dissolved in 1mL of HPLC-grade butyric acid as a standard stock (1 mg/mL). A standard curve was constructed by preparing five points at concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/mL. The data for the standard curcumin curves were subsequently obtained with $R^2 = 0.96784$ (Figure 1). All the sample solutions were filtered using Whatman Mini-UniPrep Syringeless Filters (nylon for curcumin), pore size of 0.20 μm , and placed into a HPLC for analysis.

3.2 ANTIOXIDANT ACTIVITY EXAMINATION USING 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) ASSAY

A DPPH assay (Sigma-Aldrich) was conducted, where the degree of decolourisation was associated with the antioxidant concentration of the sample (Gorinstein et al., 2013). The antioxidant assay was conducted according to the DPPH microplate protocol described by Prieto (2012), with slight modification. The stable radicals displayed a dark purple colour. The calculations used to prepare the 0.2 mM DPPH solution is shown below (Equation 1): 2 mg of DPPH was dissolved in 25 mL of methanol to yield 0.2 mM of DPPH (1)

$$\frac{0.2 \text{ mM}}{1 \text{ L}} = \frac{0.2 \text{ mM}}{1000 \text{ mL}} = \frac{0.2 \text{ mM}}{1 \text{ mL}} = 2 \times 10^{-4} \text{ mM}$$

$$\text{Weight} = \frac{2 \times 10^{-4} \text{ mM}}{1 \text{ mL}} \times 394.32 \text{ g/mol}$$

$$= 0.0788 \text{ g} \times 25 \text{ mL}$$

$$= 1.9716 \text{ mg}$$

DPPH (2.0 mg) has been dissolved in 25 mL methanol to give 0.2 mM DPPH (eqn 1)

3.2.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay of the Ascorbic Acid Standard

One hundred microlitres (100 μ L) of methanol was repeatedly pipetted into a 96-well microplate, from Wells A2 to F2 until Wells A12 to F12 of the 96-well microplate. Next, 200 μ L of ascorbic acid, which had been derived from a 1 μ g/ μ L concentration of stock solution, in CH₃OH was pipetted into wells A1 to F1 and two-fold diluted in matching wells in the second column (wells A2 to F2). This process was repeated until the last column (wells A12 to F12). One hundred microlitres (100 μ L) of the mixtures in the last column were then discarded. One hundred microlitres (100 μ L) of the DPPH-methanol solution were then added into wells A1 to C1, while 100 μ L of methanol were added into wells D1 to F1 until the last column. The final concentrations of the ascorbic acid standard were 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 1.95, 0.975, 0.4875, and 0.24375 μ g/mL (Figure 3.2). Lastly, the microplate was covered with lid to minimise evaporation and stored in the dark at room temperature for 40 minutes to prevent light-related degradation. An absorbance analysis was then conducted using a microplate reader (Varioskan LUX 3020-81526) at 517 nm.

		1	2	3	4	5	6	7	8	9	10	11	12
Ascorbic acid + DPPH	A	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.975	0.4875	0.24375
	B	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.975	0.4875	0.24375
	C	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.975	0.4875	0.24375
Ascorbic acid + MeOH	D	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.975	0.4875	0.24375
	E	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.975	0.4875	0.24375
	F	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.975	0.4875	0.24375

Figure 3.2 The DPPH assay results of the ascorbic acid standard (concentration in $\mu\text{g/mL}$)

3.2.2 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay of the Carotenoid

The present study examined 10 species of ginger, namely, *temulawak* (*Curcuma xanthorrhiza*), *temu emas* (*Curcuma heyneana*), *temu pauh* (*Curcuma mangga*), *halia* (*Zingiber officinale*), *temu kunci* (*Boesenbergia rotunda*), *temu ireng* (*Curcuma aeruginosa*), *lempoyang* (*Zingiber zerumbet*), *kunyit kuning* (*Curcuma longa*), *cekur* (*Kaempferia galanga*), and *kunyit putih* (*Curcuma zedoaria*). The samples were dissolved in analytical-grade methanol to produce a stock solution concentration at $1 \mu\text{g}/\mu\text{L}$. One hundred microlitres ($100 \mu\text{L}$) of methanol was added using a 12-tip multi-pipettor into the 96-well microplate, from wells B1 to B12, wells C1 to C12, and wells H1 to H12. Then, $200 \mu\text{L}$ of the prepared Carotenoid 1 extract was pipetted into wells A1 to A6, while $200 \mu\text{L}$ of the prepared Carotenoid 2 extract was pipetted into wells A7 to A12. All the samples in wells A1 to A12 were then transferred to wells B1 to B12 and mixed four to five times using a pipette. All the samples in wells B1 to B12 up to wells H1 to H12 were then two-fold diluted. An 8-tip multi-pipettor was then used to add $100 \mu\text{L}$ of methanol to wells A4 to A6 and A10 to A12 until the last row of wells H4 to H6 and H10 to H12. Subsequently,

100 μ L of DPPH-methanol solution was added into wells A1 to A3 and A7 to A9, until the last row of wells H1 to H3 and H7 to H9. The final concentrations of the carotenoid 1 and carotenoid 2 extracts were 500, 250, 125, 62.5, 31.3, 15.6, 7.8, and 3.9 μ g/mL (Figure 3.3). The microplate was then covered with a lid and kept in the dark at room temperature for 40 minutes prior to absorbance analysis using a microplate reader (Varioskan LUX 3020-81526) at 517 nm.

	Carotenoid (1) + DPPH			Carotenoid (1) + MeOH			Carotenoid (2) + DPPH			Carotenoid (2) + MeOH		
	1	2	3	4	5	6	7	8	9	10	11	12
A	500	500	500	500	500	500	500	500	500	500	500	500
B	250	250	250	250	250	250	250	250	250	250	250	250
C	125	125	125	125	125	125	125	125	125	125	125	125
D	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
E	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3
F	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6
G	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
H	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9

Figure 3.3 The DPPH assays preparation of the samples (concentration in μ g/mL)

3.2.3 Analysis of Antioxidant Activity

The colour degradation at 517nm wavelength was measured. Equation 2 depicts the inhibition percentage calculation (Dong et al., 2014; Gorinstein et al., 2013), whereby an inhibition of more than 50% was considered good antioxidant activity.

$$\frac{1 - \text{Absorbance sample}}{\text{Absorbance}_{\text{DPPH}}} \times 100 = \% \text{ Inhibition}$$

*Absorbance sample = Absorbance (sample + DPPH) – Absorbance (sample + MeOH)
(eqn 2)

3.3 ANTIMICROBIAL ACTIVITIES OF CAROTENOIDS PREPARATION

All the procedures are conducted in a class 2 biohazard cabinet. The interior surfaces of the cabinet were cleaned using 70% ethanol and exposed to UV light for 20 minutes before use.

- i. **Microbe:** This study selected five target microbes which included the representative microbes from Gram-negative and Gram-positive bacteria, yeast, and fungus: *S. aureus* (ATCC) 25923, *Salmonella typhimurium* ATCC 14028, *E. coli* ATCC 35218, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404. The selected microbial cultures have been purchased from ATCC.
- ii. **Media:** The general and selective media (broth/ agar) based on the manufacturer's instruction. They determined the appropriate media and the necessary amount as per the manufacturer's instruction. All the media were transferred to a Duran glass bottle and dissolved using distilled H₂O. Thereafter, the media were autoclaved at 121 °C for 20 minutes (or based on the specific manufacturer instructions). The autoclaved media were then placed in a water bath (60 °C temperature) before use or stored at 4 °C till further use.
- iii. **Inoculation:** The sterile Circa broth (5-8 mL) were poured aseptically into sterile test tubes. Thereafter, the researchers extracted a microbe-coated-

bead from the cryo-vials and placed them into the above test tubes containing the sterile broth. All tubes were appropriately labelled (i.e., either control or inoculum broth). Both the test tubes (inoculum and control) were incubated in shaking incubators that were set at different temperatures based on the selected microbe, i.e., (i) 37 °C for 18-24 hours (in the case of *S. typhimurium*, *E. coli*, *S. aureus*, and *C. albicans*) and (ii) 26 °C for 48 hours (*A. brasiliensis*). After incubation, turbidity in the broth was regarded as an indicator of microbial growth. However, the control test tube was clear, and showed an absence of any microbial growth.

- iv. **Standardisation:** Then, the above 18-24 hour old culture was adjusted to acquire turbidity that indicated 0.5 McFarland standard (1.5×10^8 CFU/mL) or 0.05-0.1 absorbance value at a wavelength of 600 nm that could be measured using a cell density meter (Biowave C model Co8000).
- v. **Sample stock solution:** All the samples were stored in a freezer at -20 °C and were thawed before use. Then, the samples were pipetted directly into the wells containing some broth (final volume of sample + broth is 50 µL), and a serial dilution of the samples were prepared, which were then exposed to the selected target microorganism.

3.4 ASSAYS

3.4.1 Sterility

The sterility of the media can be maintained when the samples (50 µL) are directly pipetted using a sterile pipette tip onto the nutrient agar (NA) and sabouraud dextrose agar (SDA) plates. The inoculated NA plates were then incubated at 37 °C for 24 h, whereas the inoculated SDA plates were incubated for 72 h at 26 °C. After incubation, all the plates were observed with naked eyes to determine the presence of bacterial and fungal colonies.

The absence of colonies on the NA and SDA plates showed the sample sterility, which made it ideal for antimicrobial assessment.

3.4.2 Minimum Inhibitory Concentration (MIC)

An in vitro quantitative technique was used to assess the inhibitory potential of the sample against a specific microorganism. To serially dilute the sample, different sample volumes (i.e., 50 μ L, 40 μ L, 30 μ L, 20 μ L, and 10 μ L) were pipetted into a 96-well microlitre plate. Then, to acquire a final volume of 50 μ L (sample + broth mixture) in every well, the remaining volumes of the sterile broth (i.e., 0 μ L, 10 μ L, 20 μ L, 30 μ L, and 40 μ L) were added to every well. Finally, a standardised microbiological culture (10^6 CFU/mL; 50 μ L) was added to the well. The microlitre plates were incubated at 37 °C (in the case of *S. aureus*, *E. coli*, *S. typhimurium*, and *C. albicans*) and 26 °C (in the case of *A. brasiliensis*) for 24 h. After incubation, turbidity in the broth was regarded as an indicator of microbial growth. Hence, the minimum concentration of the sample, which remained clear after macroscopic assessment was regarded as minimum inhibitory concentration (MIC).

3.5 INHIBITORY STATUS DETERMINATION ASSAY

After the MIC assessment, the inhibitory activity of every sample (permanent/ cidal) or static/temporary were determined. For this purpose, MTT reagent (20 μ L of 1 mg/ml solution) would be pipetted into MIC wells (clear wells), and the plates are incubated for 30 mins (in the case of the bacterial and *Candida* sp.) and 2-3 h (in the case of *A. brasiliensis*). If the well containing the media-sample-microbe mixture was seen to be clear after incubation, it indicated a permanent or cidal inhibitory activity whereas if any well

containing the media-sample-microbe mixture turned dark purple it indicated a temporary or static inhibitory activity. The minimal bacterio/ candido/ fungicidal concentrations were determined based on the average value of the triplicate samples.

3.5.1 High-Performance Liquid Chromatography (HPLC) Analysis

The curcumin concentrations in the samples were analysed using an Agilent model 1200 series HPLC system, which included a quaternary pump with an autosampler injector, a column compartment with a thermostat, micro degassers, and a diode array detector. An ZORBAX Eclipse XDB-C18 end-capped (5 μ m, 4.6 $\times$ 150 mm) reverse phase column (Agilent Technologies, USA). The following eluents were used in this study, i.e., (A) Acetonitrile: H₂O (9:1 v/v); and (B) butanoic acid. The solvent gradient used for isolating and detecting the carotenoids was: 0-40% eluent B (0-20 min), 40-60% eluent B (20-25 min), 60-100% eluent B (25-25.1 min), 100% eluent B (25.1-35 min) and 100-0% eluent B (35-35.1 min) at the flow rate of 1.0 mL/min. The column was re-equilibrated using 100% eluent A for 10 min before injecting the next sample. The column temperature was maintained at 20 °C, while 10 μ L of every sample would be injected in the column.

3.5.2 Spectrophotometric Measurements

The yellow colour intensity (b^*) of the curcumin samples could be determined with the help of a Remote DRA Cary-50 chromameter that measures the International Commission on Illumination's L^* , a^* , b^* (CIELAB) space's lightness (L^*), bluish-green (-)/red-purple (+) hue component (a^*), b^* = yellow (+)/blue (-) hue component, chroma (C^*) [$(a^{*2} + b^{*2})^{1/2}$], hue angle (h°) from arctangent $b^*/a^* = 0^\circ = \text{red-purple}$, $90^\circ = \text{yellow}$, $180^\circ = \text{bluish-}$

green, 270° = blue). In the CIELAB colour space, the L* ranges between black (0) and white (100), a positive a* denotes a red-purple hue while a negative a* indicates a bluish-green hue. Here, a positive b* refers to a yellow colour while a negative b* shows a blue colour. If a* and b* = 0, an achromatic or grey colour was noted. The CIELAB colour factors (a*, b*, L*, and total variation in CIELAB colour (ΔE^*_{Lab})) would be determined with the help of a UV-Vis spectrophotometer (Shimadzu UV-160), using the technique described by Mochizuki and Takayama (2016) with a few modifications. For calculating the colour variations, the differences noted in the chromatic coordinates (Δa^* and Δb^*), differences in the lightness (ΔL^*), and ΔE^*_{Lab} were estimated with the below equations:

$$\Delta a^* = a^* - a_0^*$$

$$\Delta b^* = b^* - b_0^*$$

$$\Delta L^* = L^* - L_0^*$$

$$\Delta E^*_{Lab} = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2}$$

Here, a_0^* , b_0^* , and L_0^* refer to the primary values before every treatment. The values for every colour factor were presented as the average of 3 values. The relative standard deviation (RSD) was seen to be <0.2% for every value, which indicated a higher repeatability (Mochizuki and Takayama, 2016).

3.6 CURCUMIN STABILITY TEST

3.6.1 Pigments Preparation

The maximum concentration of curcumin obtained by chemical extraction and stored at - 20 °C was used for colour stability tests. Curcumin has been dissolved in ethanol and distilled H₂O to yield three different concentrations: 5, 10, and 20 g/L, respectively. The chromaticity of the untreated sample (ΔE^*) was evaluated as a blank.

3.6.2 Analysis of Chromaticity

A chromameter was used to measure the colour intensity of the extracted curcumin. The CIELAB colour system comprises L*, a*, b*, C*, and h°, with L* ranging from black (0) to white (100), and where a positive a* indicates a hue of red-purple, a negative a* indicates a hue of bluish-green, a positive b* indicates a hue of yellow and a negative b* indicates a hue of blue. The colour is considered achromatic or neutral grey when the points of the a* and b* axes cross and L* = 50. The CIELAB colour parameters, namely, a*, b*, L*, and ΔE^*_{Lab} , were analysed using a Mettler Toledo® UV7 UV-Vis spectrophotometer according to the method proposed by Mochizuki and Takayama (2016). The used of CIELAB system for analysing L*, a*, and b* values of all samples (Figure 3.4). Equation 3 (Yawadio and Morita, 2007; Licón et al., 2012) was used to calculate the 3 measured colour factors where C* represents colour intensity and brightness, h° indicates the colour shade, and ΔE (Licón et al., 2012; Yawadio and Morita, 2007).

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

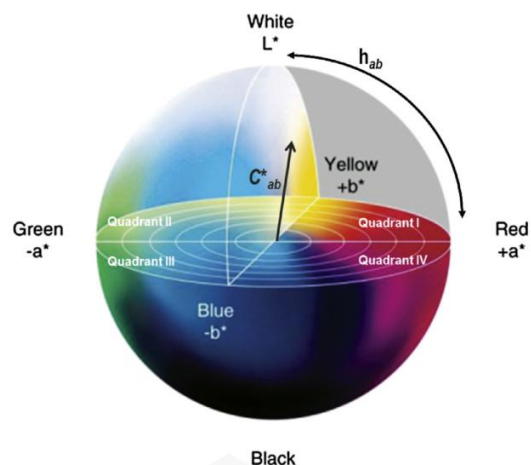


Figure 3.4 CIELab sphere (Sant' Anna et al., 2013)

3.6.3 Salinity Test

Sodium chloride was added to each sample at three different concentrations, resulting in final NaCl concentrations of 1, 3, and 5 mg/mL, respectively. The samples were incubated under both dark and light conditions at room temperature for four weeks. Prepared in triplicate, the samples were analysed hourly to monitor changes in chromaticity. Additionally, the chromaticity of the samples was measured on the first day.

3.6.4 pH Test

The pH of the samples was adjusted to 3, 5, 7, 9, and 11 using hydrochloric acid and sodium hydroxide. Samples were prepared at three different concentrations and exposed to both dark and light conditions (cool light from a fluorescent lamp with an intensity of 3100 lux, positioned perpendicularly 30 cm below) at room temperature for four weeks. The samples

were prepared in triplicate, with chromaticity monitored hourly. Additionally, the chromaticity of the samples was measured on the first day.

3.6.5 UV Irradiation Test

All glass tubes containing samples at three different concentrations were placed in a laminar hood and exposed to UV-A (long wavelength = 365 nm, intensity = 5500 lux) and UV-B (middle wavelength = 312 nm, intensity = 2900 lux) perpendicularly at a distance of 10 cm from the light source for 8 hours at room temperature. The samples were prepared in triplicate, and their chromaticity was analysed hourly to monitor changes. Any colour alterations before and after the experiment were measured accordingly.

3.6.6 Heat Test

Curcumin samples were prepared at concentrations of 1, 2, and 3 mg/mL. All samples were incubated in an oven at seven different temperatures (4, 25, 35, 45, 55, 65, and 75 °C) for 3 hours. The samples were prepared in triplicate, with chromaticity analysed hourly to monitor colour changes. Additionally, the initial colour of the samples was measured before the experiment.

3.7 POLYMERS AND ITS CHROMATICITY TEST

3.7.1 Colourant Preparation

A colourant with a significant curcumin concentration was produced via chemical extraction and stored at -20 °C to be used as the bio-colourants for the PLA, PVA, and chitosan samples. Three concentrations, namely, 5, 10, and 20 g/L, of the curcumin colourant were prepared.

3.7.2 Poly(Lactic Acid) Film Preparation

Poly(lactic acid) (PLA) polymer resin (3051D) and HPLC grade chloroform were used to prepare the PLA film, which had been prepared using the solvent casting technique, namely, solubilisation, casting, and drying. The PLA resins were pre-conditioned in a drying oven at 60 °C for 12 hours to reduce their moisture content and prevent hydrolysis, which can affect the polymer's physical properties (Lim et al., 2008). Five grams of the PLA resin was then weighed and added to a beaker containing 50 mL of chloroform. This was then covered and stirred on a hot plate until completely dissolved. Then, curcumin pigment was added to the PLA solution. Given that;

$$\text{Concentration of curcumin} = \frac{\text{mass of curcumin}}{\text{volume of PLA solution}}$$

$$5 \text{ g/L} = \frac{250 \text{ g of curcumin}}{50 \text{ L of PLA solution}}$$

$$10 \text{ g/L} = \frac{500 \text{ g of curcumin}}{50 \text{ L of PLA solution}}$$

$$20 \text{ g/L} = \frac{1000 \text{ g of curcumin}}{50 \text{ L of PLA solution}}$$

After the curcumin pigment had completely dissolved into the PLA matrix, approximately 4.5 L of the PLA solution was casted onto a glass plate. The diameter of the casted film was 6.2 mm. It was then allowed to dry overnight at room temperature. The PLA film sans curcumin was prepared as a blank. Therefore, a total of four PLA films were prepared, namely, a blank and samples containing 5, 10, and 20 g/L of the curcumin colourant.

3.7.3 Poly (Vinyl Alcohol) Film Preparation

Ninety-nine percent of the PVA polymer resin, which was obtained from Sigma-Aldrich, was hydrolysed and 18 M Ω ultrapure H₂O, from a Merck® Millipore Lab Water Purification System, was used to prepare the films. Much like the preparation of the PLA films, the PVA films were prepared via solvent casting. Four grams of the PVA resin was weighed and added into a beaker containing 100 mL of H₂O. The mixture was then stirred on the hot plate at 70 °C until completely dissolved. Then, a separate 4 grams of NaOH was dissolved into 100 mL of H₂O, whereby 10 mL of the solution was pipetted into the PVA solution. Next, the curcumin pigment was mixed into the PVA solution. Each concentration was prepared using 100 mL of the PVA solution. Given that;

$$\text{Concentration of curcumin} = \frac{\text{mass of curcumin}}{\text{volume of PVA solution}}$$

$$5 \text{ g/L} = \frac{250 \text{ g of curcumin}}{50 \text{ L of PVA solution}}$$

$$10 \text{ g/L} = \frac{500 \text{ g of curcumin}}{50 \text{ L of PVA solution}}$$

$$20 \text{ g/L} = \frac{1000 \text{ g of curcumin}}{50 \text{ L of PVA solution}}$$

After the curcumin pigment had completely dissolved into the PVA matrix, approximately 8.3 mL of the PVA solution was casted onto a disposable petri dish. The diameter of the casted film was 8.4 mm. The casted film was then allowed to dry for three days at room temperature. A PVA film sans curcumin was prepared as a blank. Therefore, a total of four PVA films were prepared, namely, a blank and samples containing 5, 10, and 20 g/L of the curcumin colourant.

3.7.4 Chitosan Film Preparation

For preparing the chitosan films, weighed the chitosan powder (2 g) and transferred it to a beaker. Then, acetic acid (7 mL) is added to this beaker. Then, the mixture is stirred with the help of a magnetic stirrer till it completely dissolves. This is followed by the addition of the ethanolic curcumin solution. This solution (5 mL) is then pipetted in a glass petri dish having a 65 mm diameter. The solution is dried by placing this petri dish under a fume

chamber for 24 h. Various stability tests are conducted for determining the colour stability of curcumin.

$$\begin{aligned} \text{Concentration of curcumin} &= \frac{\text{mass of curcumin}}{\text{volume of chitosan solution}} \\ 5 \text{ g/L} &= \frac{250 \text{ g of curcumin}}{50 \text{ L of chitosan solution}} \\ 10 \text{ g/L} &= \frac{500 \text{ g of curcumin}}{50 \text{ L of chitosan solution}} \\ 20 \text{ g/L} &= \frac{1000 \text{ g of curcumin}}{50 \text{ L of chitosan solution}} \end{aligned}$$

3.7.5 Coating Stability Test for PLA, PVA and Chitosan

3.7.5.1 Salinity Test

Three concentrations of sodium chloride (NaCl) were prepared by dissolving it in distilled H₂O at concentrations of 5, 10, 20, and 30 g/L. Ten milliliters of the NaCl solution were sprayed onto the PLA, PVA, and chitosan films, and another sample without NaCl was prepared as a blank. Thereafter, the samples would be exposed to the light and dark conditions (using the cool light emitted by a fluorescent lamp having a 3, 100 lux light intensity, wherein the sample is placed 30 cm perpendicularly below the lamp) at room

temperature for 4 weeks. All samples were prepared in triplicates and studied every week for monitoring their ΔE^* . The sample colours were measured on Day 1.

3.7.5.2 pH Test

The pH values of distilled H₂O samples were adjusted to 3, 5, 7, 9, and 11 with the help of hydrochloric acid and sodium hydroxide. Then, NaCl solution (10 mL) was sprayed on the PLA, PVA, and chitosan films while the films without NaCl were used as the respective blanks. Thereafter, the samples were exposed to the light and dark conditions (as mentioned in Section 3.7.5.1) at room temperature for 4 weeks. All samples were prepared in triplicate and analysed weekly to monitor their ΔE^* . The ΔE^* of the sample was also measured on Day 1.

3.7.5.3 UV Irradiation Test

All the curcumin-coated films (PLA, PVA, and chitosan) with 3 varying concentrations (5, 10, 20 g/L) were placed in the laminar hood and subjected to UVB exposure (middle wavelength = 312 nm, 2900 lux intensity). These samples were placed 10 cm perpendicularly below the light source and exposed for 8 h at room temperature. All samples were prepared in triplicates and analysed every hour for monitoring their ΔE^* . The change in color of the samples was measured before and after conducting the experiments.

3.7.5.4 Heat Test

The PLA, PVA, and chitosan films were coated with 5, 10, and 20 g/L concentrations of curcumin. The above films were then incubated in an oven at different temperatures (i.e. 10, 20, 30, 40, 50, 60, 70 °C) for 3 h. All samples were prepared in triplicates and analysed every hour for monitoring their ΔE^* . The change in colour of the samples was measured prior to the experiments.

3.7.5.5 Light vs Dark Condition

The PLA, PVA, and chitosan films were coated with 5, 10, and 20 g/L concentrations of curcumin. Thereafter, all the samples were exposed to light and dark conditions (as mentioned in Section 3.7.5.1) at room temperature for 4 weeks. All samples were prepared in triplicates and analysed every week for monitoring their ΔE^* .

3.7.6 Chromaticity (ΔE^*) Testing

The coloured PVA, PLA, and chitosan samples were assessed using a CIELAB colourimeter and Agilent® Cary WinUV. Three readings were taken and the results were presented as mean $\pm$ standard deviations. The results of the salinity, pH, UV irradiation, heat treatment, light vs dark conditions were read using CIELAB colourimeter. Any colour changes in the PLA, PVA, and chitosan coatings were observed and read using CIELAB colourimeter.

3.8 SOAP MAKING APPLICATIONS

The main ingredients used to make the soaps in this study were coconut oil derived from the flesh of coconuts, primarily grown in tropical regions and NaOH. For each preparation, 35 g of NaOH and 100 g of H₂O was combined and stirred then left to cool for 60 minutes. The curcumin colourant was then added at concentrations of 0.25, 0.5, 1.0, 1.5, 2, and 2.5 g and stirred continuously until the soap mixture appeared trace. The mixture was then spun into a mould and left for 24 hours.

3.8.1 Light vs Dark Condition

Soaps containing 0.5, 1, 1.5, 2, 2.5, and 3 g of curcumin were prepared. Thereafter, all the samples were exposed to light and dark conditions (as mentioned in Section 3.7.5.1) for 4 weeks at room temperature. All samples were prepared in triplicates and analysed every week for monitoring their ΔE^* .

3.8.2 Chromaticity (ΔE^*)

Soaps containing 0.5, 1, 1.5, 2, 2.5, and 3 g of curcumin were placed into the CIELAB colourimeter and analysed using Agilent® Cary WinUV. Three readings were taken and the results were presented as mean±standard deviations. The results of the light vs dark condition testing had to be read using the CIELAB colourimeter. Any colour changes in the coloured soaps were observed and read by the CIELAB colourimeter.

3.9 MULTIVARIATE DATA ANALYSIS (MDA)

All the variables and observations were analysed using multivariate data analysis (MDA) to determine the significant variables contributing to the stability of natural colourants at different concentrations, time, and environmental factors. The unbiased standard deviation (n-1) method was employed to transform the standardised variables to a normal distribution. Analysis of variance (ANOVA) was used to analyse the difference between the variables in the present study. Box-and-whisker plots (BWPs) were constructed to determine if each variable conformed to the normal distributions and identify outliers in the dataset. Principal component analysis (PCA) and discriminant analysis (DA) were also used to reduce the large dataset, determine the relationship between the variables, and group the data into clusters. All the statistical analyses were performed using Lumivero® XLSTAT 2024 and Microsoft® Excel® 2017 (Sani et al., 2022).

3.9.1 Analysis of variance (ANOVA)

An ANOVA was conducted to confirm if there was a statistically significant difference in the means of the samples' variables and observations, compare all the means pair-wise, and identify significantly different pairs. The BWPs were used to determine the normality of the distribution of the dataset. The outliers were maintained as they increased the variability of the data and captured valuable information in the studied area (Jim, 2022).

3.9.2 Principal Component Analysis (PCA)

Principal component analysis (PCA) is a statistical approach that is used to identify the underlying dimensions or principal components when investigating the relationships among intercorrelated components. The goal is to split a large dataset into smaller sets of principal components while retaining as much information as possible. Principal component analysis (PCA) can also be used to distinguish sample sources by identifying similarities and determining the components that characterise a sample's sources. Prior to conducting a PCA, it is essential to identify the presence of at least one significant correlation among the components using Bartlett's test of sphericity, at significance level (α) = 0.05 (Ismail et al., 2021b). The sampling adequacy was checked using the Kaiser-Meyer-Olkin (KMO) test, with > 0.5 indicating adequacy. Principal components with eigenvalues (EV) > 1 or cumulative variability $> 75\%$ were selected. Lastly, the biplots were examined to determine the correlation between the components and their contributions to the sample (Sani et al., 2019a). The dataset was then analysed using PCA to identify variables with high factor loadings (FL) that significantly explained the principal components. An FL > 0.75 was considered strong, 0.50-0.75 was considered moderate, 0.30-0.49 was considered weak, and < 0.30 was considered negligible (Rani et al., 2017). Eigenvalues (EV) were used to determine the principal components with more cumulative dataset variations. An EV ≥ 1 was considered significant in the PCA (Le et al., 2017; Zubir et al., 2016b; Yang et al., 2020b).

3.9.3 Identification and Removal of Outliers

An outlier is a data point that deviates significantly from the overall pattern or distribution of the entire dataset. It indicates that a component of interest exhibits an extreme value compared to most of the other values in the dataset. Outlier detection and removal are essential pre-processing steps in data analysis, particularly in MDA. By identifying and

removing outliers, the data becomes more robust and reliable, leading to more accurate and meaningful results (Sani et al., 2019b; Pollet and van der Meji, 2017). The univariate method is one of the most common ways to identify outliers in MDA. It involves constructing BWPs, with $\alpha = 0.05$. The BWP indicates outliers as values that exceeded three times the box's height with an asterisk (*) (Samsudin et al., 2019).

3.9.4 Dataset Transformation

The dataset was transformed after identifying and removing outliers to ensure that it followed a normal distribution before running the PCA. The normality of the dataset was tested using the Shapiro-Wilk test, at $\alpha = 0.01$. The dataset was then transformed using the n-1 method (Sani et al., 2022).

3.9.5 Kaiser-Meyer-Olkin (KMO) and Barlett's Test for Sampling Adequacy

A KMO test was conducted to determine the adequacy of the sampled data and its suitability for MDA. The acceptable sample adequacy was set at > 0.5 (Moyo et al., 2013; Vidal et al., 2020). The KMO categorises dataset adequacy as < 0.5 = inadequate, $0.5-0.7$ = mediocre, $0.7-0.8$ = good, $0.8-0.9$ = very good, and > 0.9 = excellent (Atiqah et al., 2023; Ismail et al., 2021a).

Bartlett's test of sphericity was used to measure the null hypothesis (H_0), namely, whether the original correlation matrix is an identity matrix. The α was set to < 0.05 , which would indicate that the data correlated with the dataset (Shrestha, 2021). The H_1 (alternative

hypothesis) was accepted, while the H_0 was rejected, indicating that the variables in the dataset correlated (Ismail et al., 2021b).

3.9.6 Pearson's Correlation Coefficient (r^2) Analysis

The R^2 , which is measured on a scale from -1 to +1, was used to determine the strength of the correlations between two variables, as described by Guildford's rule of thumb (Guilford, 1973). The strength of the linear relationship can be determined by examining the absolute value of r , with values closer to -1 or +1 indicating a solid relationship and values closer to 0 indicating a weak or no relationship (Suroto et al., 2020) (Table 3.1).

Table 3.1 Interpretation of Correlation Coefficient Values

Size of Correlation	Interpretation
0.90 to 1.00 (-0.90 to -1.00)	Very high positive (negative) correlation
0.70 to 0.90 (-0.70 to -0.90)	High positive (negative) correlation
0.50 to 0.70 (-0.50 to -0.70)	Moderate positive (negative) correlation
0.30 to 0.50 (-0.30 to -0.50)	Low positive (negative) correlation
0.00 to 0.30 (0.00 to -0.30)	Little if any correlation

CHAPTER FOUR

CURCUMIN CONTENT, ANTIOXIDANT PROPERTIES, AND ANTIMICROBIAL ACTIVITIES OF SELECTED GINGER SPECIES

4.1 INTRODUCTION

Native to southern Australia, curcuma has expanded from India through Thailand, Indochina, Malaysia, and Indonesia. In tropical, broad-leaved evergreen forests and tropical forests, the species are enumerated as they occur naturally. The *Curcuma* genus has several uses which are thought to be economically significant. Srivilai et al. (2018) classify the subterranean rhizome of curcuma as a significant source of the colour yellow. The Arabic term “*kurkum*”, which defines yellow, is where the name “*curcuma*” originates (Su et al., 2017). Additionally, plants that are rich in proteins, starch, carbohydrates, vitamins, minerals, and lipids may be referred to as curcuma plants (Roshan and Gaur, 2017). *Curcuma* is a plant that contains various bioactive molecules with pharmacological properties, such as anti-inflammatory (Sikha et al., 2015), antimicrobial (Jagtap, 2015), hypercholesterolaemic (Shafreen et al., 2018), antirheumatic (Abdel-Lateef et al., 2016), antiviral (Pant et al., 2013), antifibrotic (Jose et al., 2014), antihepatotoxic (Jagtap, 2015), antidiabetic (Nwozo et al., 2014), antinociceptive (Pawar et al., 2011), gastroprotective (Jeon et al., 2015), and other beneficial effects on cardiovascular diseases (Nithya and Jayashree, 2017).

The traditional uses of the family Zingiberaceae have a long history and range from folk medicine to culinary applications. Numerous ginger species have been the subject of numerous phytochemical, pharmacological, and molecular studies worldwide. The identification of novel bioactive molecules with various bioactivities, including antioxidant, antiviral, antibacterial, and anti-inflammation activities, has boosted interest in

its therapeutic qualities. Additionally, this invaluable plant is employed as a pesticide, natural dye, and repellent. Therefore, the ultimate aims of this specific chapter are:

- i. To determine the curcumin contents of a wide range of ginger rhizomes based on the germplasms of their flesh colour.
- ii. To evaluate the potential of curcumin pigment as an antioxidant and antimicrobial agent, and as a source of natural colourant.

4.2 RESULTS AND DISCUSSIONS

4.2.1 High-Performance Liquid Chromatography (HPLC) Analysis of Curcumin Pigment from Selected Ginger Species

In this experiment, acetone and methanol mixture, NaOH solution and DMSO were used for solvent extraction of 12 ginger species with different rhizome flesh colour as detailed in Table 4.1. HPLC analysis confirmed that only curcumin was detected at retention time of 2.667 minute and at wavelength of 425 nm (Figure 4.1).

Table 4.1 Different rhizome flesh colour from selected 12 ginger species

Ginger Species	Flesh Colour		Ginger Species	Flesh Colour	
Kunyit (<i>Curcuma longa</i>)	Dark Orange		Temu Lawak (<i>Curcuma xanthorrhiza</i>)	Orange	
Temu Giring/Emas (<i>Curcuma heyneana</i>)	Dark Yellow		Temu Pauh (<i>Curcuma mangga</i>)	Yellow	
Lempoyang (<i>Zingiber zerumbet</i>)	Pale Yellow		Kunyit Putih (<i>Curcuma zedoaria</i>)	Pale Yellow	
Temu Kunci (<i>Boesenbergia rotunda</i>)	Pale Yellow		Halia (<i>Zingiber officinale</i>)	Cream	
Cekur (<i>Kaempferia galanga</i>)	Cream		Lengkuas (<i>Alpinia galanga</i>)	White	
Halia Bara (<i>Z. officinale</i> var. <i>rubrum</i>)	White		Temu Ireng (<i>Curcuma aeruginosa</i>)	Purple	

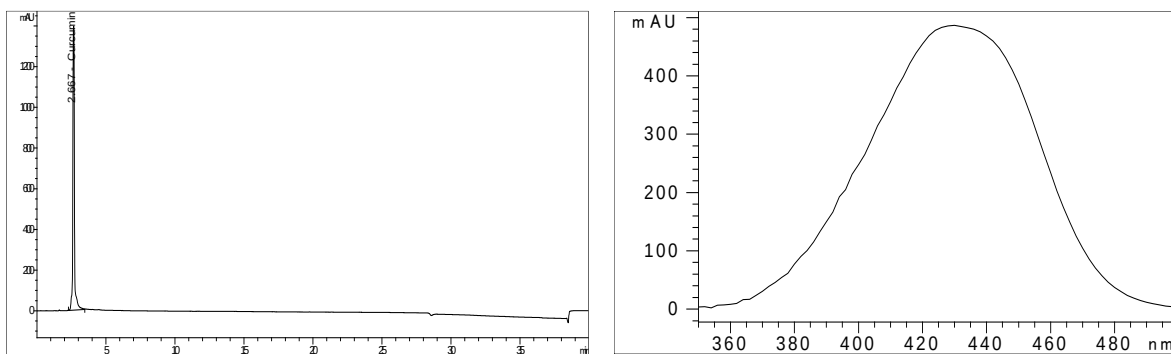


Figure 4.1 HPLC chromatogram of curcumin extracts from 12 species and combined and injected to HPLC at retention time of 2.667 minutes and at a wavelength of 425 nm.

Table 4.2 Curcumin contents ($\mu\text{g/g}$ DW) of the various flesh colours of the rhizomes of the 12 ginger species

Flesh Colour	Total curcumin range ($\mu\text{g/g}$ DW)
Dark Orange	99.33 - 578.38
Orange	115.22 - 222.88
Dark Yellow	47.56 - 97.60
Pale Yellow	21.57 – 82.28
Cream	17.83
White	Non-detectable
Purple	Non-detectable

Table 4.3 Curcumin contents ($\mu\text{g/g DW}$) of the various flesh colours of the rhizomes of the 12 ginger species using three different extraction solvents, namely, acetone and methanol mixture, NaOH, and DMSO

Extraction Method		Chemical	Alkaline	DMSO
Ginger Species	Flesh Colour	Curcumin content ($\mu\text{g/g DW}$)		
Kunyit (<i>Curcuma longa</i>)	Dark Orange	99.33 ± 5.34	578.38 ± 6.37	344.31 ± 0.65
Temu Lawak (<i>Curcuma xanthorrhiza</i>)	Orange	115.22 ± 5.60	222.88 ± 6.56	172.91 ± 0.02
Temu Giring/emas (<i>Curcuma heyneana</i>)	Dark Yellow	47.56 ± 1.18	97.60 ± 6.43	95.51 ± 0.57
Temu Pauh (<i>Curcuma mangga</i>)	Yellow	18.07 ± 0.19	110.67 ± 0.49	30.35 ± 0.09
Lempoyang (<i>Zingiber zerumbet</i>)	Pale Yellow	21.57 ± 0.19	82.28 ± 0.20	32.83 ± 0.67
Kunyit Putih (<i>Curcuma zedoaria</i>)	Pale Yellow	ND	23.85 ± 0.16	ND
Temu Kunci (<i>Boesenbergia rotunda</i>)	Pale Yellow	ND	ND	ND
Halia (<i>Zingiber officinale</i>)	Cream	ND	17.83 ± 0.02	ND
Cekur (<i>Kaempferia galanga</i>)	Cream	ND	ND	ND
Lengkuas (<i>Alpinia galanga</i>)	White	ND	ND	ND
Halia Bara (<i>Zingiber officinale</i> var. <i>rubrum</i>)	White	ND	ND	ND
Temu Ireng (<i>Curcuma aeruginosa</i>)	Purple	ND	ND	ND
ND – non-detectable				

The flesh colours of the rhizomes of the 12 selected ginger species ranged from white, cream, pale yellow, dark orange to purple (Table 4.1). Therefore, the range of their curcumin content was divided into four groups based on colour, namely, dark orange, orange, dark yellow, pale yellow, and cream (Table 4.2). Curcumin was not detected in the purple-fleshed and white-fleshed rhizomes. A positive relationship was observed between

curcumin content, extraction method, and the b^* of the rhizome's flesh. Kunyit kuning, which has a dark orange-fleshed rhizome, had the highest curcumin content ($578.38 \pm 6.37 \mu\text{g/g DW}$) via the alkaline extraction method. Its curcumin content was significantly higher than all the other ginger species examined and extraction methods tested (Table 4.3). Conversely, curcumin was not detected in white- and purple-fleshed ginger species, such as lengkuas, halia bara (*Zingiber officinale* *Vara rubrum*), and temu ireng. Therefore, the examination of the rhizome flesh colours of the 12 ginger species and different extraction methods confirmed that the alkaline extraction method yields the highest curcumin content, as evidenced by higher b^* .

Previous studies stated that the pigment extraction from plants is influenced by the extraction method, the types and amount of solvent, solid-liquid ratio, time and temperature during extraction, amount of sample, and the condition and nature of the matrix. Additionally, the nature of the solvent is the most critical part of attaining extraction efficacy (Jayakumar et al., 2023a; Zannou et al., 2023). These findings highlight the potential to manipulate the curcumin content of ginger rhizomes for genetic manipulation purposes. In this case, the best species for producing yellow pigment is either kunyit kuning, which yields the highest curcumin content via the alkaline and DMSO extraction methods, and temu lawak, which yields the highest curcumin content via the chemical extraction method. Multiple studies have examined the curcumin content of the yellow pigment produced by kunyit kuning. However, not many have examined that of temu lawak. Therefore, the present study explored and investigated the curcumin extracted from temu lawak as a potential natural colourant in greater depth.

4.2.2 Antioxidant Properties

The analysis of antioxidant properties was performed using the DPPH method. The free radicals first generate a violet colour when in methanol and will turn to light yellow after reacting with an antioxidant. This is due to the free radical property being lost because of the breakage of their chain (Ionita, 2021). Varioskan Lux Microplate Reader (Thermo Scientific) at wavelength 517 nm has been used to analyse the antioxidant activity of carotenoid pigment or curcumin properties from seven selected ginger species that were detected with curcumin pigment, as in Figure 4.1. The inhibition percentage of curcumin and ascorbic acid standard for all seven ginger species is represented in Figure 4.2 to 4.8. Examination of DPPH scavenging activity of curcumin from diverse rhizome flesh colours, as summarised in Table 4.4, showed that the concentration of extracts and rhizome flesh colour had a significant effect on the percentage of inhibition. For example, the dark orange of the kunyit kuning rhizome flesh substantially demonstrated the highest DPPH scavenging activity of 98.43% inhibition at a concentration of 1 mg/mL, whereas orange at 96.1% (2 mg/mL), dark yellow at 95.68% (1 mg/mL), yellow at 91.1% (5 mg/mL), pale yellow at 91.21–95.68% (3 mg/mL) and cream at 95.15% (2 mg/mL) were observed to have relatively lower inhibition. It can be concluded that germplasm and their curcumin pigment concentration in rhizomes can influence the DPPH scavenging activity.

Table 4.4 Curcumin concentration and rhizome flesh colours of 7 selected ginger species

Ginger Species	Flesh Colour	Inhibition (%)	Concentration (g/L)
Kunyit (<i>C. longa</i>)	Dark Orange	98.43	1
Temu Lawak (<i>C. xanthorrhiza</i>)	Orange	96.1	2
Temu Giring/emas (<i>C. heyneana</i>)	Dark Yellow	95.68	1
Temu Pauh (<i>C. mangga</i>)	Yellow	91.1	5
Lempoyang (<i>Z. zerumbet</i>)	Pale Yellow	91.21	3
Kunyit Putih (<i>C. zedoaria</i>)	Pale Yellow	95.68	3
Halia (<i>Z. officinale</i>)	Cream	95.15	2

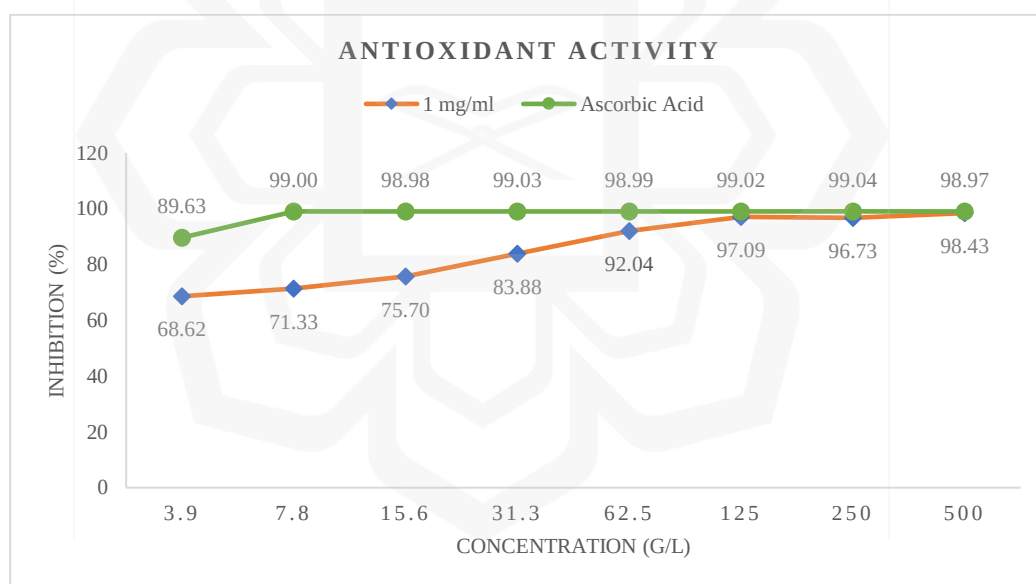


Figure 4.2 Antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of kunyit kuning (*C. longa*) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (98.43% inhibition) at a concentration of 1 mg/mL

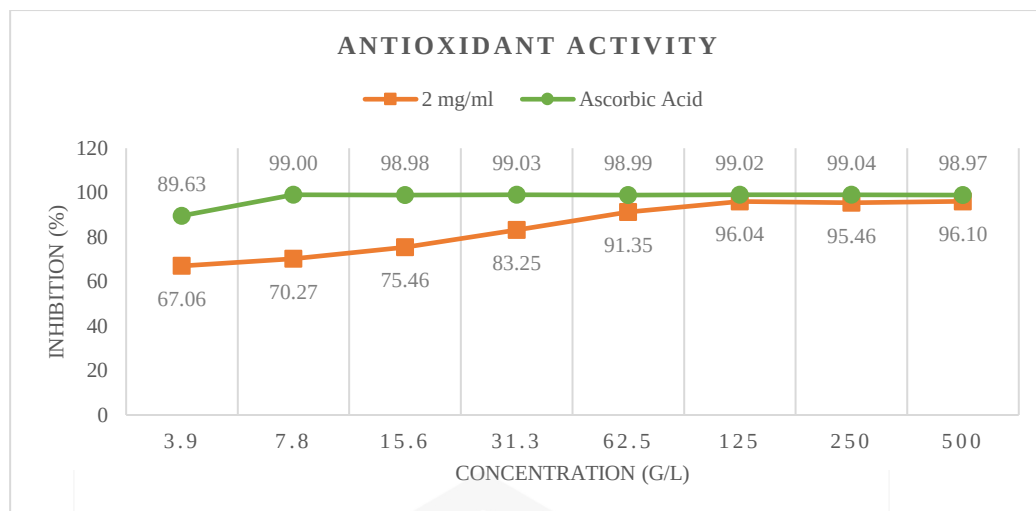


Figure 4.3 Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of temu lawak (*C. xanthorrhiza*) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (96.1% inhibition) at a concentration of 2 mg/mL

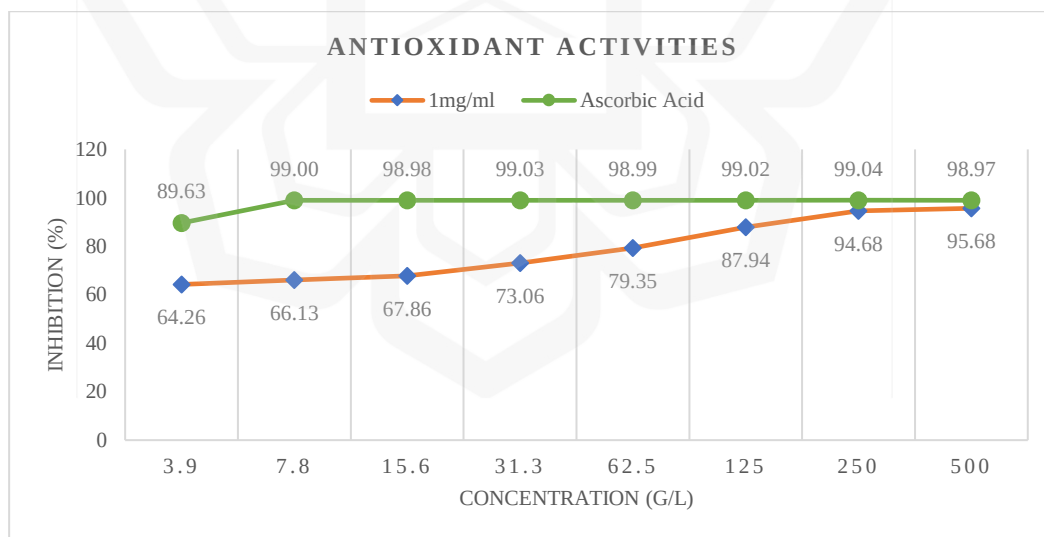


Figure 4.4 Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of temu emas (*C. heyneana*) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (95.68% inhibition) at a concentration of 1 mg/mL

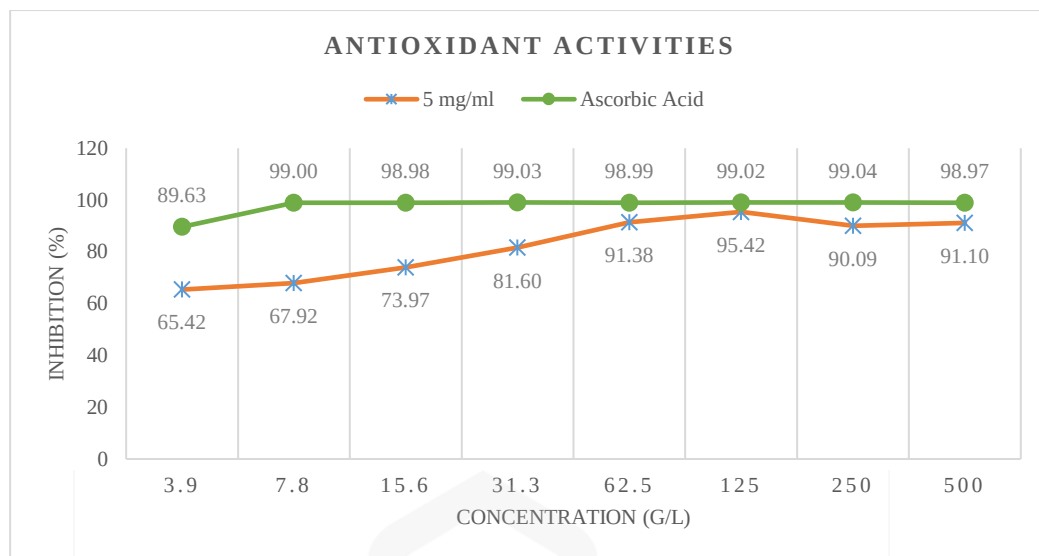


Figure 4.5 Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of temu pauh (*C. mangga*) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (91.1% inhibition) at a concentration of 5 mg/mL

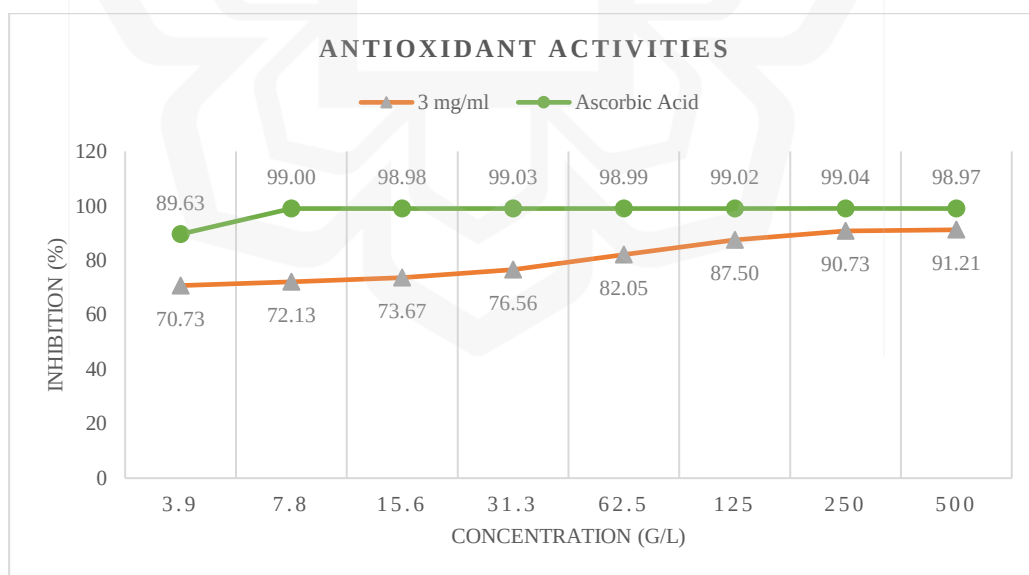


Figure 4.6 Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of lempoyang (*Z. zerumbet*) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (91.21% inhibition) at a concentration of 3 mg/mL

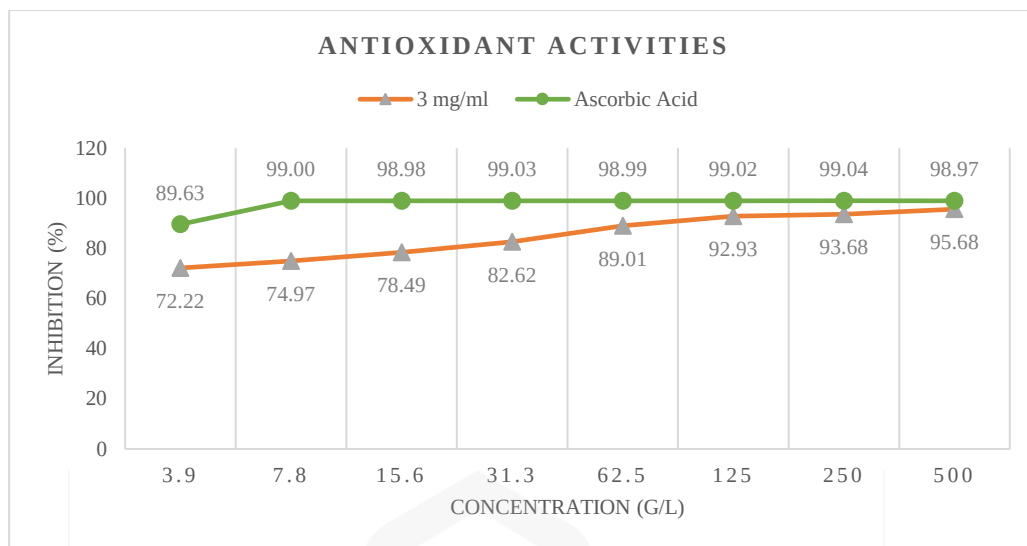


Figure 4.7 Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of kunyit putih (*C. zedoaria*) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (95.68% inhibition) at a concentration of 3 mg/mL

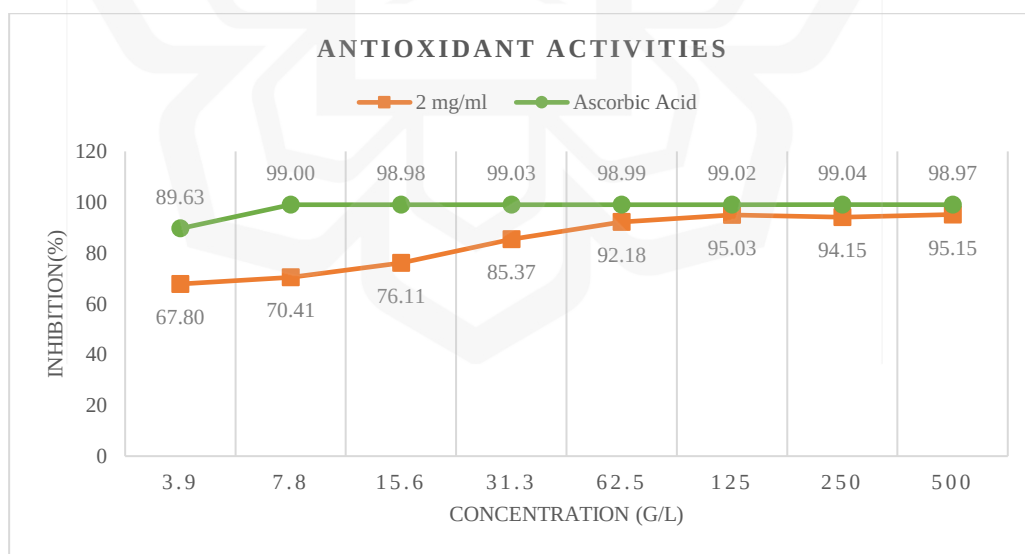


Figure 4.8 Antioxidant activities (inhibition percentage) of curcumin extracted from the flesh of halia (*Z. officinale*) rhizome and ascorbic acid standard with the highest DPPH scavenging activity (95.15% inhibition) at a concentration of 2 mg/mL

A previous study on the scavenging activity of curcumin extracted from *kunyit kuning* had an inhibition percentage of 89.34% at a concentration of 10 mg/ML. (Zulfadhly et al., 2023). It showed a higher antioxidant inhibition percentage for curcumin extracted via the alkaline extraction method, which is 100% DPPH scavenging activity at 0.25 mg/mL (Othman et al., 2019). Apart from that, Karmakar et al. (2018) reported 75% DPPH scavenging activity at 512 µg/mL via the acetone extraction method. The inhibition percentage of the present study was comparable with that reported by Viuda-Martos et al. (2012), with results showing 93% DPPH scavenging activity. The difference in the antioxidant activity of the extracted curcumin correlates with the presence of polyphenols, such as flavonoids and phenolic acids in each pigment, as it helps increase the pigment's scavenging activity (Muflihah et al., 2021; Suchowilska et al., 2020; Quintero Quiroz et al., 2019a). Moreover, the percentage of inhibition may also differ across studies due to various internal and external factors, such as plant maturity and locality, type of solvent and extraction methods used, and the duration and condition of the sample's storage among other things (Hikmawanti et al., 2021; Ahmed et al., 2020; Michiels et al., 2012).

4.2.3 Antimicrobial Activities

Minimum inhibitory concentration (MIC) is a quantitative in vitro assay that is conducted to evaluate the inhibitory potential of a sample against selected microbes. Turbidity is used as an indication of growth, with the lowest concentrations to remain clear under macroscopic evaluation adopted as the MIC. Higher antimicrobial potential correlates with a lower MIC. The inhibitory activity status of each sample, either permanent/cidal or static/temporary, was determined immediately after the macroscopic evaluation of the MIC. The wells that appear as clear-yellow after 20 minutes of incubation at 37 °C indicate permanent inhibition of growth (cidal), whilst the change of mixture colour from clear-yellowish to dark blue indicates recurrence of growth or temporary inhibition (Figure 4.9). The minimum bacterio/candido concentration (MBC/MCC) was recorded as the mean

concentration of triplicates (Table 4.5). Five targeted microbes that represented Gram-negative and Gram-positive bacteria, yeast, and fungus, namely (i) *S. aureus* ATCC 25923, *E. coli* ATCC 35218, *S. typhimurium* ATCC 14028, *C. albicans* ATCC 10231, and *A. brasiliensis* ATCC 16404 were used in the present study. Good antimicrobial activity was indicated by lower MBC/MCC/MFC ($\leq 1000 \mu\text{g}/\mu\text{l}$ if possible) and was worthy of further evaluation. The results were the mean of triplicates with 5 mg/ml concentration used as the maximum concentration, as suggested by Gibbon (2004), where a MIC concentration of $\leq 1000 \mu\text{g}/\text{ml}$ was recommended for the extracts of natural compounds (Rogoza et al., 2010; Thuille et al., 2003).

Table 4.5 Antimicrobial activities of curcumin extracted from seven selected ginger species.

Ginger species	Minimum Bacteriocidal/Yeastocidal/ Fungicidal Concentration ($\mu\text{g} / \mu\text{L}$)				
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 35218	<i>S. typhimurium</i> ATCC 14028	<i>C. albicans</i> ATCC 10231	<i>A. brasiliensis</i> ATCC 16404
Kunyit (<i>C. longa</i>)	>15	>15	>15	>15	>15
Temu lawak (<i>C. xanthorrhiza</i>)	>15	>15	>15	>15	>15
Temu giring/emas (<i>C. heyneana</i>)	3	2	2	2	2
Temu pauh (<i>C. mangga</i>)	9	6	6	6	3
Lempoyang (<i>Z. zerumbet</i>)	9	6	6	6	3
Kunyit putih (<i>C. zedoaria</i>)	9	6	6	3	3
Halia (<i>Z. officinale</i>)	>15	>15	>15	>15	>15

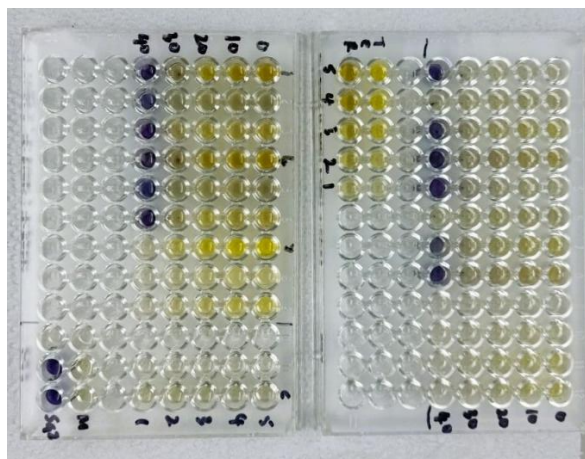


Figure 4.9 A clear-yellow well indicates the permanent inhibition of growth (cidal) while a dark blue well indicates the recurrence of growth or temporary inhibition.

The extract of temu emas was found to have the most effective antimicrobial inhibitory activity compared to six other ginger species and recorded the lowest MIC, ranging from 2-3 $\mu\text{g}/\mu\text{L}$ against all five pathogenic microorganisms. The MIC of temu pauh, lempoyang, and kunyit putih showed moderate antimicrobial activity against all five pathogenic microorganisms except for *A. brasiliensis* (ATCC 16404) with 3 $\mu\text{g}/\mu\text{L}$. Meanwhile, kunyit kuning, temu lawak, and halia were found to have poor antimicrobial inhibitory activity against all the chosen microbes. The MIC from different species of Zingiberaceae extracts was determined using the dilution method. The MIC has a wide range of interpretations (Mann and Markham, 1998). As opposed to the minimum lethal concentration, it is generally accepted that the MIC represents the lowest concentration of a substance that can help to inhibit microbial growth. This is the best method to express the antibacterial activity of a compound or an extract. The MIC is defined as the lowest concentration at which no growth inhibition is observed. According to Saeed and Sabir (2001), some authors have used the serial dilution assay method to examine the zone of inhibition as well as the MICs of extracts and compounds.

According to Eloff (2007), the miscible with H₂O against fungi, which included 45% DMSO, 51% acetone, 30% ethanol, and 32% methanol, influences the average MIC used for extraction. In other cases, polar extracts, such as H₂O, do not extract antimicrobial compounds from many plants, while intermediate polarity compounds contribute to the highest antimicrobial activity (Kotze and Eloff, 2002; Eloff et al., 2017). The antifungal, antibacterial, and anti-inflammatory activities have been reported for several ginger species, such as *C. longa*, *C. zedoaria*, *C. aromatica*, and *C. amada* (Apisariyakul et al., 1995; Yoshioka et al., 1998; Negi et al., 1999; Mujumdar et al., 2000). Due to its anti-inflammatory, anti-carcinogenic, antioxidant, and antimicrobial properties, curcumin has been used as a pharmaceutical agent for many years (Epstein et al., 2010; Naksuriya et al., 2014). It also performs best in terms of anti-ageing and wound healing (Akbik et al., 2014; Lima et al., 2011). Curcumin, a pleiotropic molecule with potent antiviral activity, can aid in fighting various viruses, including hepatitis, influenza, chikungunya or arboviruses, human immunodeficiency virus (HIV), human papillomavirus (HPV), herpes simplex virus 2 (HSV-2), and other sexually transmitted diseases (Praditya et al., 2019b). Curcumin was also found to be effective against 10 strains of *S. aureus* with a MIC range of 125-250 µg/mL. For example, two ATCC MRSA, MRSA standard strains, four MRSA from culture collections, and four MRSA clinical isolates (Mun et al., 2013). The methicillin-susceptible *S. aureus* (MSSA) is also susceptible to curcumin, which has an MIC of 256 µg/ml (Wang et al., 2016a).

One factor affecting the inhibition is the difference in the cell wall structure of bacteria where Gram-positive bacteria consist only of the peptidoglycan layer that causes it to be highly permeable to some substances, while Gram-negative bacteria contain more complex cell walls due to the presence of a lipopolysaccharide layer at the external part of the peptidoglycan layer (Chaturvedi et al., 2023; Ahmed et al., 2020). It can be concluded that the phytochemicals present are more active against the Gram-positive bacteria because of wall permeability, which leads to easy penetration into the cell structure (Chaturvedi et al., 2023; Hochma et al., 2021; Yolmeh, 2016). Apart from that, Perez-Lopez et al. (2008) stated that another factor affecting the MIC is the extraction and storage time of the sample,

in which a shorter duration leads to lower MIC. As stated, lower MIC results from the samples mean higher antimicrobial potential. Meanwhile, the volume of solvent and speed of agitation have no significant impact on MIC. Lastly, Quintero Quiroz et al. (2019b) highlighted that the antimicrobial activities of the pigment extracts were possibly due to the largest content of polyphenols, which were accountable for their antimicrobial activities sequentially.

4.3 SUMMARY OF RESULTS

1. Kunyit kuning, which has a dark orange-fleshed rhizome, yields the highest curcumin content ($578.38 \pm 6.37 \mu\text{g/g DW}$) via the alkaline extraction method, which was significantly higher than all the other examined ginger species and extraction methods tested.
2. A positive correlation was observed between curcumin content, extraction method, and b^* of the rhizome flesh.
3. The dark orange-fleshed rhizome of kunyit kuning had the highest DPPH scavenging activity, with 98.43% inhibition at the concentration of 1 mg/mL.
4. The flesh colour of a rhizome, especially the most pigmented, has a significant effect on the percentage of DPPH scavenging activity.
5. Temu emas yielded the highest antimicrobial inhibitory activity and had the lowest MIC, ranging from 2-3 $\mu\text{g}/\mu\text{L}$, against all five pathogenic microorganisms.

CHAPTER FIVE

CHROMATICITY STABILITY OF CURCUMIN EXTRACTED FROM TEMU LAWAK (*Curcuma xanthorrhiza* Roxb.)

5.1 INTRODUCTION

Temu lawak is a medicinal plant from the *Zingiberaceae* family. At present, most temu lawak cultivated in Indonesia, Malaysia, Thailand, and Philippines, where it thrives in lowlands areas at altitudes of 1500 m above sea level and in tropical forests (Kustina et al., 2020). The use of temu lawak as a traditional medicine has been increasing at an average of 5.4% annually over the last decade (Nihayati et al., 2013). This has been triggered by the rise in public awareness and consumption of alternative therapeutic treatments with fewer side effects. Due to the rising prices of chemical medications in excess of what societies can afford, people have been turning to natural sources for healing (Sumarya et al., 2020). The rhizome of the curcuma plant is nutritious as it contains chemical compounds, such as curcumin, essential oils, saponins, flavonoids, alkaloids, and tannins. It is traditionally used to treat heartburn, diarrhoea, piles, cough, asthma, and canker sores. The properties of temu lawak include increasing appetite, improving digestive function, maintaining and improving liver function, relieving joint and bone pain, reducing blood and fat concentrations in the body, and serving as an antioxidant (Nihayati et al., 2013).

Curcuma very closely correlates with many bioactive compounds, especially curcuminoids, which are a group of phenolic compounds that comprise curcumin, demethoxycurcumin, and bisdemethoxycurcumin as shown in Figure 5.1 and Figure 5.2 (Sari et al., 2013; Anggoro et al., 2015).

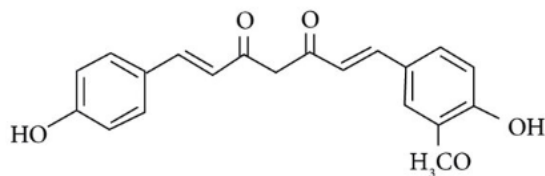


Figure 5.1 Chemical structure of demethoxycurcumin

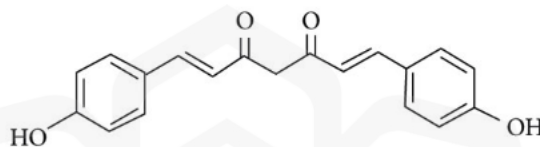


Figure 5.2 Chemical structure of bisdemethoxycurcumin

Due to its safe natural colourant and health benefits, as well as the strong consumer demand for more natural products, *temulawak* has great potential to be cultivated on a large scale in Malaysia. Therefore, it would be beneficial to understand the limitations and unfavourable conditions of curcumin to increase the stability and application of this natural colourant. Therefore, the present chapter aimed to:

1. Evaluate the potential of curcumin extracted from *temulawak* as a natural colourant.
2. Investigate the effects of pH, NaCl, UV irradiation and heat on the stability of curcumin extracted from *temulawak*.

5.2 RESULTS AND DISCUSSIONS

5.2.1 Effects of Light, Pigment Concentration, and Time of Exposure on Curcumin Stability

The main aim of the stability test was to observe the effects of the weather, including exposure to sunlight (UV rays), pH (acidity and alkalinity), NaCl (seawater salinity) and heat (temperature) on curcumin pigment as a potential natural colourant or coating material, as well as to study the causes of degradation. The ΔE^*_{LAB} is used to assess the stabilisation of curcumin pigment particles (Table 5.1), as described by Mokrzyck and Tatol (2011).

Table 5.1 CIE L*a*b* colour difference (ΔE^*_{LAB}) assessment

ΔE^* value	Indication
< 1.0	no colour difference is visible
< 2.0	colour difference is only slightly visible
< 3.5	noticeable colour difference
< 5.0	clear difference in colour is noticed
> 5.0	indicate clearly two different colours

Figure 5.3 presents the ΔE^* of the curcumin pigment at concentrations of 1-3 mg/ml throughout the exposure times of 0-4 weeks in light and dark conditions. The ΔE^* stability test results established that the value was > 5.0, and two different colours were visible for all three concentrations that had been exposed for different periods in response to light and dark conditions. There was a strong correlation between the saturation (s), b^* , and concentration of the yellow colour of the curcumin as well as the period of exposure to light and dark conditions (Table 5.2). Marked variations were observed in terms of the

degradation percentages of b^* and s at all three concentrations (1, 2 and 3 mg/ml) and exposure times (week 0, 1, 2, 3 and 4) for the curcumin developed under light and dark conditions. It was observed that the curcumin at a concentration of 3 mg/ml in dark conditions had the lowest degradation percentages of 36.54% and 38.79% for s and b^* , respectively, whereas the curcumin pigment with the lowest concentration of 1 mg/ml was found to have the highest degradation percentages of 90.38% and 90.14% for s and b^* , respectively. Additionally, the degradation was significantly greater in the presence of light than in the absence of it (Suresh et al., 2013a).

Previous studies have shown that exposure to sunlight causes curcumin to deteriorate (Priyadarsini, 2014a; Priyadarsini, 2009b), and this can be frequently seen in the quick elimination of turmeric stains in the presence of sunlight. The photochemical transformation mechanisms of curcumin have been reported to involve both hydrolytic (photodegradation: breakdown of the heptane chain connecting the two aromatic rings) and oxidative (photooxidation: production of ROS and curcumin radicals and photosensitising molecular O_2) reactions (Tønnesen and Karlsen, 1986, Seidi et al., 2020, Heger et al., 2014). The physical state of curcumin and the circumstances surrounding the light source affect the composition and relative abundance of the ensuing products (Tønnesen et al., 1986a).

Table 5.2 Degradation percentage (%) of s and b* for different curcumin concentrations in light and dark conditions within four weeks

		Degradation percentage (%)					
		Light			Dark		
		Curcumin concentration (mg/mL)					
CIE L*a*b*	Week	1	2	3	1	2	3
Colour saturation (s)	1	30.77	27.27	26.32	24.53	22.22	19.23
	2	57.69	49.09	40.35	35.85	35.19	34.62
	3	78.85	69.09	49.12	37.74	38.89	36.54
	4	90.38	87.27	63.16	41.51	40.74	36.54
Yellow intensity (b*)	1	30.13	26.26	22.66	21.39	20.94	22.46
	2	57.01	47.16	37.83	34.53	34.53	36.39
	3	79.18	69.28	47.19	36.21	37.17	38.67
	4	90.14	86.14	62.17	39.32	39.14	38.79

In addition, a comparison of the s and b* of curcumin in response to light and dark conditions also demonstrated that there was a relatively lower percentage of degradation in dark conditions than in light conditions for all concentrations and times of exposure (Table 5.2). Therefore, although varying the concentration of curcumin influenced the b* and s, exposure to light and the duration of the exposure also influenced the ΔE^* stability.

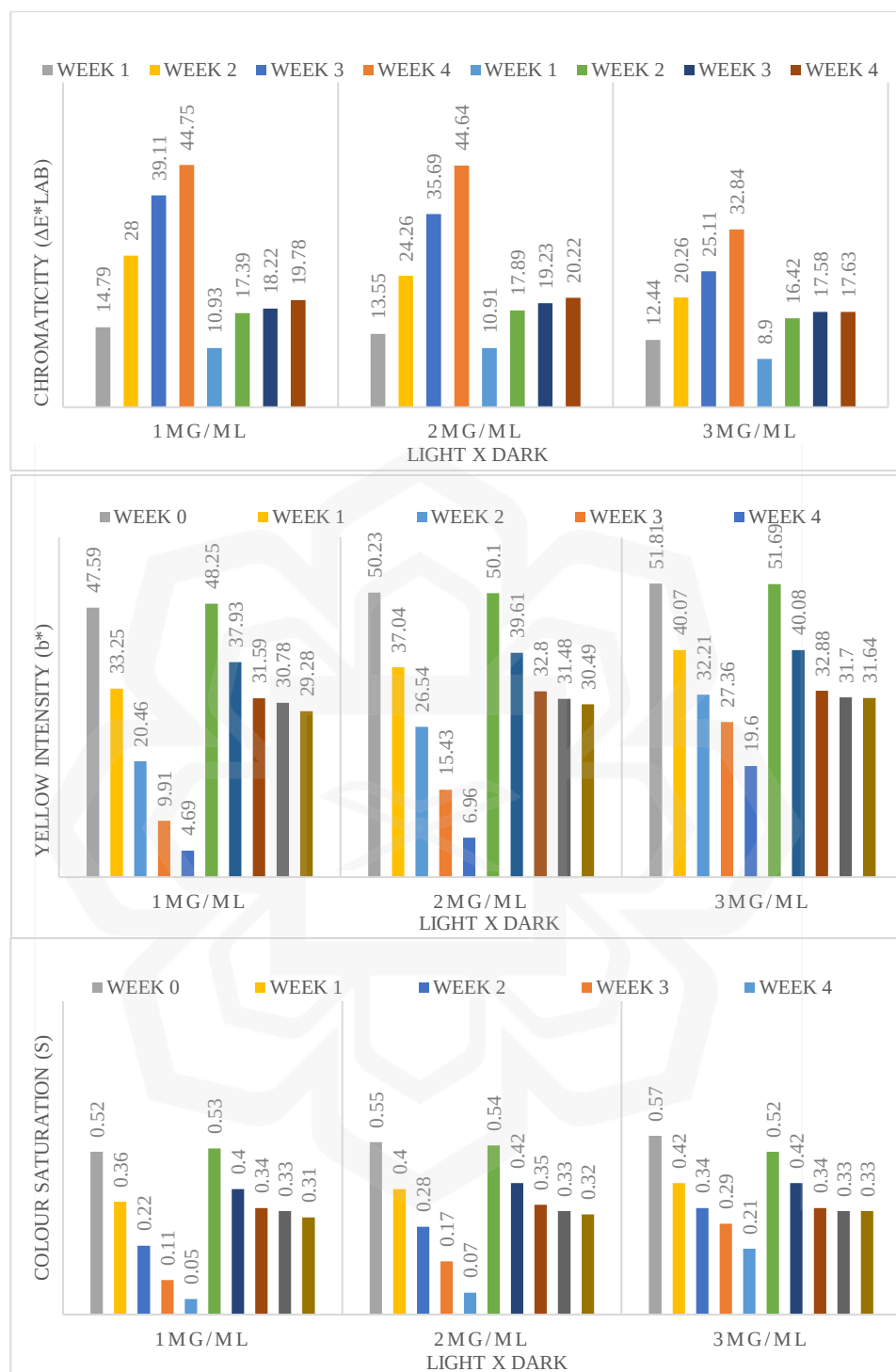


Figure 5.3 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values of the different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) in light and dark condition at different exposure times

5.2.2 Effect of NaCl

The next step in this analysis was to further investigate the relationship between ΔE^*_{Lab} , s , and b^* (%) for different concentrations of curcumin in light and dark conditions for different exposure periods with different concentrations of NaCl (1, 3, and 5 mg/mL). The ΔE^* for the curcumin pigment after treatment with 1, 3, and 5 mg/mL of NaCl established that the ΔE^* was > 5.0 for all three concentrations, except for curcumin concentrations of 1 mg/mL and 2 mg/mL for week 1 (ΔE^* of < 3.5 indicated noticeable colour differences) while being developed in both dark and light conditions (Figure 5.4-5.6). The relative differences in the colour measurements of the curcumin pigments (1, 2, and 3 mg/mL) as well as the duration of exposure (1-4 weeks) during development in light conditions resulted in an approximate doubling of the total ΔE^* compared with curcumin pigments (1, 2, and 3 mg/mL) that were developed in dark conditions (Figures 5.4 to 5.6).

The analysis of the degradation percentages (%) of s and b^* for different concentrations of curcumin in light and dark conditions after four weeks of treatment with 1 mg/ml of NaCl revealed that the longer the time of exposure, the higher the degradation of s and b^* . As shown in Table 5.3 and Figure 5.4, less degradation ($< 40\%$) of s and b^* was found to have occurred in the dark after four weeks, but upon development in the light, the degradation percentage for s was detected to be between 46.43-70.91%, while that of b^* was observed to be between 62.17-90.14 %.

Table 5.3 Degradation percentage (%) of s and b* for different curcumin concentrations from temu lawak in light and dark conditions within weeks of treatment with 1, 3, and 5 mg/mL of NaCl.

		Degradation percentage (%)					
		Light			Dark		
		Curcumin concentration (mg/mL)					
1 mg/mL NaCl							
CIE L*a*b*	Week	1	2	3	1	2	3
Colour saturation (s)	1	1.82	1.79	51.79	0.00	0.00	27.27
	2	27.27	21.43	35.71	21.43	19.64	34.55
	3	45.45	39.29	50.0	32.14	37.50	38.18
	4	70.91	46.43	67.86	41.07	42.86	40.0
Yellow intensity (b*)	1	30.13	26.26	22.66	21.39	20.94	22.46
	2	57.01	47.16	37.83	34.53	34.53	36.39
	3	79.18	69.28	47.19	36.21	37.17	38.67
	4	90.14	86.14	62.17	39.32	39.14	38.79
3 mg/mL NaCl							
CIE L*a*b*	Week	1	2	3	1	2	3
Colour saturation (s)	1	5.36	9.84	33.90	1.79	11.29	22.22
	2	30.36	29.51	40.68	21.43	29.03	31.48
	3	57.14	45.90	55.93	32.14	43.55	37.04
	4	76.79	52.46	72.88	41.07	48.39	38.89
Yellow intensity (b*)	1	3.28	9.70	30.34	1.58	12.65	23.66
	2	23.85	19.43	36.42	13.93	16.89	30.64
	3	53.25	38.70	53.41	25.55	34.61	38.11
	4	73.59	45.21	70.37	35.77	38.88	39.95
5 mg/mL NaCl							
CIE L*a*b*	Week	1	2	3	1	2	3
Colour saturation (s)	1	48.08	27.78	1.89	23.08	22.22	23.64
	2	75.00	33.33	22.64	34.62	35.19	21.82
	3	90.38	66.67	39.62	38.46	38.89	36.36
	4	94.23	77.78	39.62	36.54	38.89	40.00
Yellow intensity (b*)	1	48.37	26.96	24.26	19.90	19.32	23.45
	2	78.48	76.57	26.66	34.82	34.73	25.98
	3	89.26	67.25	43.12	37.64	36.83	36.39
	4	93.68	78.18	41.57	34.47	36.89	38.61

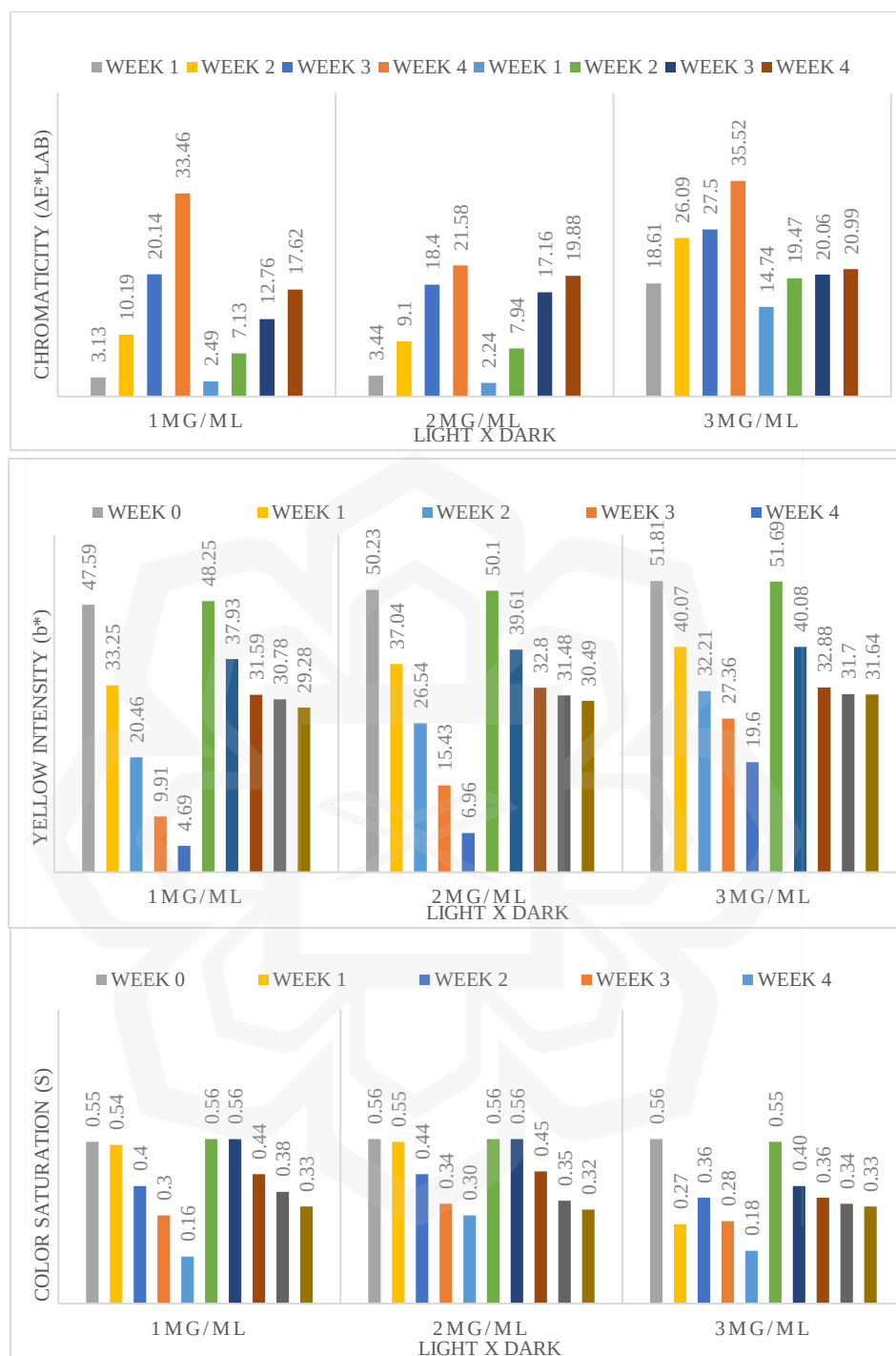


Figure 5.4 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) in light and dark condition for different exposure time after treatment with 1 mg/mL NaCl

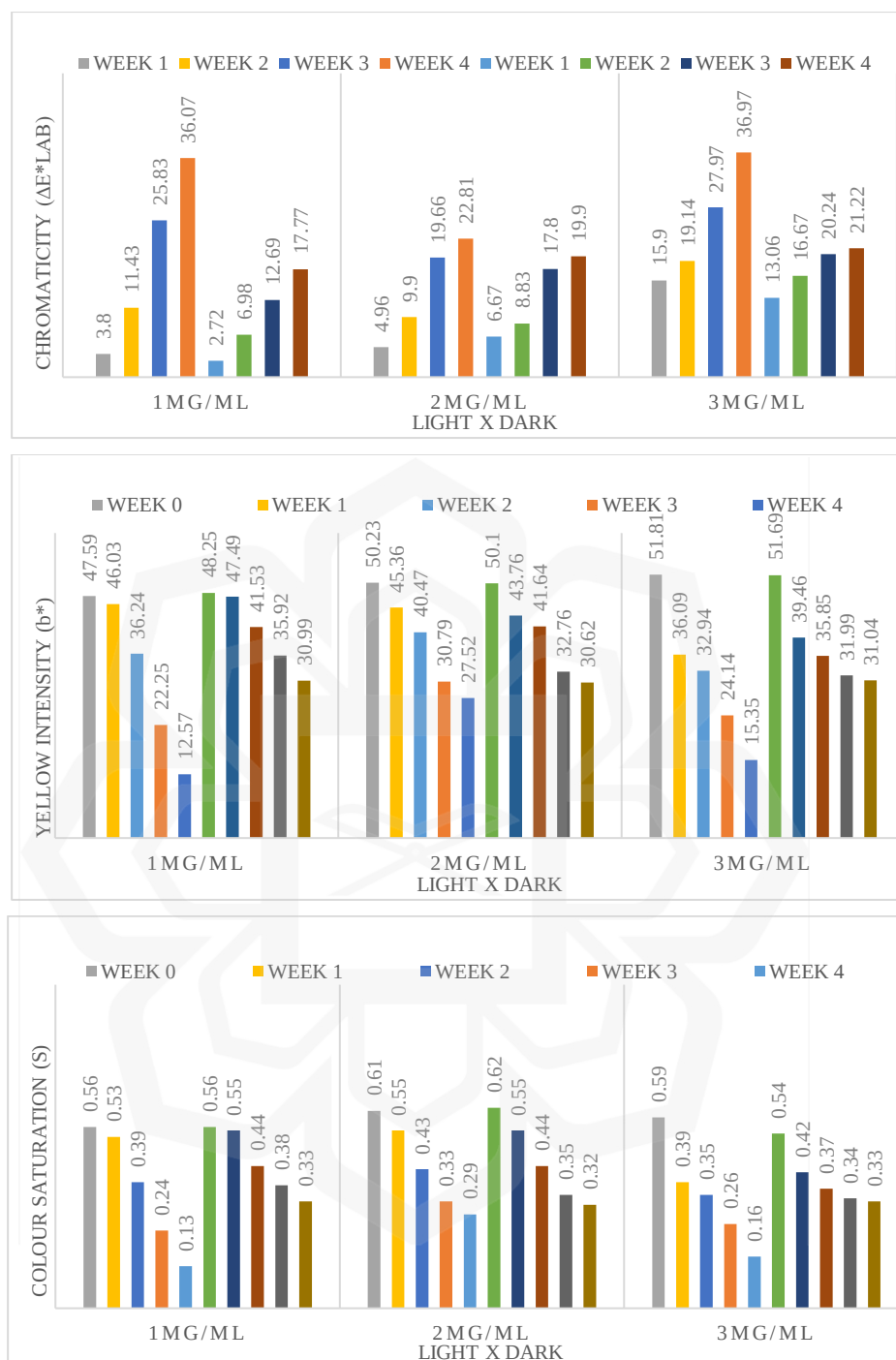


Figure 5.5 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) in light and dark condition for different exposure time after treatment with 3 mg/mL NaCl

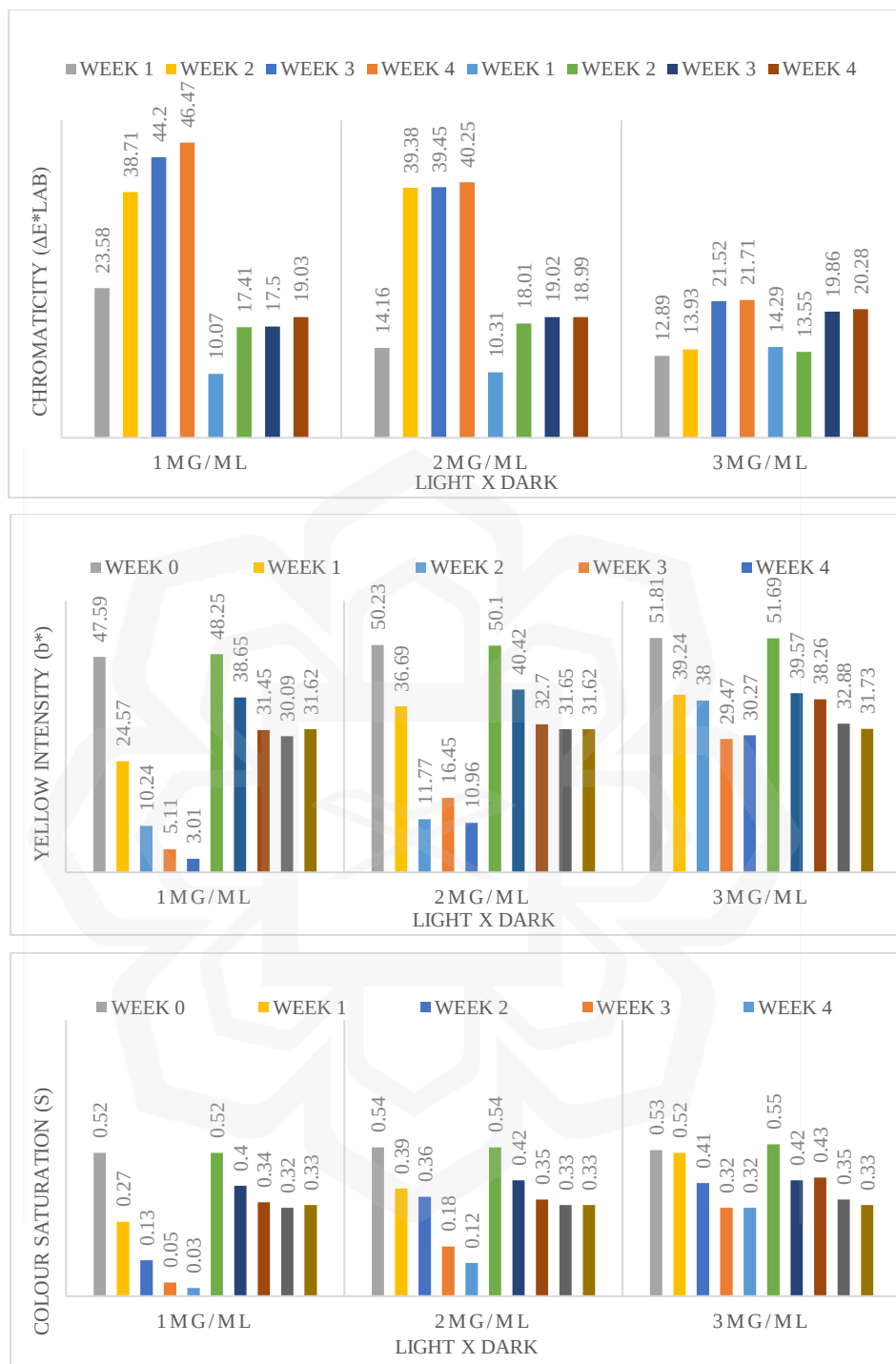


Figure 5.6 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) in light and dark condition for different exposure time after treatment with 5 mg/mL NaCl

The degradation percentages of s and b^* for different concentrations of curcumin in light and dark conditions after four weeks of treatment with 3 mg/mL of NaCl showed that there was less degradation ($< 40\%$) in the dark (Table 5.3). However, the development of curcumin in the light after four weeks resulted in increases in the degradation of s and b^* ranging from 52.46% to 76.79% and 45.21% to 73.59%, respectively. Therefore, light had a significant effect on the stability of the ΔE^* , s , and b^* for different concentrations and durations of exposure of curcumin, as detailed in Table 5.3, Figure 5.5, and Figure 5.6. Exposure to alkaline, UV, or autooxidation conditions caused the curcumin to break down chemically, resulting in a range of reaction products with distinct molecular properties and bioactivities.

As stated in Table 5.3, marked differences were observed in the relative colour degradation measurements of the pigments of the curcumin (1, 2, and 3 mg/mL) post-treatment with 5 mg/mL of NaCl in addition to exposure durations of 1-4 weeks in light conditions. After development under light for 4 weeks, the relative degradation percentages of s and b^* were still very high, ranging from 39.62 to 94.23% and 41.57 to 93.68%, respectively (Table 5.3). In contrast, the s and b^* for curcumin under dark conditions were found to be less degraded as compared to light conditions. The s and b^* degradations for curcumin were observed to be between 36.54 to 40% and 34.47 to 38.61%, respectively. It was discovered that there was a strong association between the curcumin concentration, light conditions, and relative degradation percentages of s and b^* . Salinity is an environmental stressor that may cause the molecular structure of a pigment to become unstable (Mohajer, Taha, and Azmi, 2016). The C^* of a pigment is used to measure its colour intensity and brightness, whereas s is the area of vividness, indicating that it is determined by the ΔE^* to L^* ratio (Nor, Aziz, Mohd Adnan, Taha, and Arof, 2016). This is supported by a study by Tønnesen et al. (2002), especially when a pigment is subjected to light, extreme heat, and an alkaline environment.

The observations and measurements indicated that under dark conditions, the higher the concentration of curcumin and NaCl, the greater the stability of s and b^* . In addition, even though light is a key factor in the degradation and stability of curcumin (the darker the better), the curcumin pigment that had been treated with a higher concentration of NaCl was found to undergo less degradation (15-28%). In addition to its photosensitivity, curcumin can degrade in the dark, and this process is accelerated in a basic medium. It has been shown that the concentration of salt (NaCl) has a significant influence on the degradation process (Mondal et al., 2016).

A study by Elloumi et al. (2020) reported that the salinity level at 10 g/L which is the lowest concentration tested gives the highest carotenoid contents as compared to others salinity level been tested (20 g/L to 60 g/L). Thus, it is possible to validate the results of this study, which show that the treatment with a lower NaCl concentration (1 g/L) resulted in slightly slower color degradation of curcumin pigment compared to higher salinity levels after four weeks of observation under light conditions. This is due to carotenogenesis, which is enhanced by reactive oxygen species (ROS) created by stress conditions such as salinity stress (Elloumi et al., 2020; Tharek et al., 2020). Specifically, there is a complex relationship between carotenoid and salinity stress. This is due to diverse results reported from previous studies. It is believed that the dissimilarity of the results has relation with other factors that contributed to the colour degradation of carotenoid pigment.

5.2.3 Effect of pH

An analysis was conducted to examine the correlation between the ΔE^*_{Lab} , s , and b^* for different concentrations of curcumin in light and dark conditions for different exposure times over a range of pH (3, 5, 7, 9, and 11). Figure 5.7 shows that the ΔE^* was > 5.0 for all three concentrations of curcumin pigment after treatment with solutions with pH values

of 3, 5, 7, 9, and 11 in both light and dark conditions, except for the curcumin at a concentration of 1 mg/mL for the first week of exposure in solutions with pH values of 3, 5 and 7. The curcumin pigments at pH 9 and pH 11 were found to degrade at a faster rate compared to at pH 7, and the degradation was lower when the ΔE^* was > 10.0 . The results depicted in Table 5.4, Figures 5.8 and 5.9 show that increasing the pH, curcumin concentration, time of exposure and light caused greater degradation of s and b^* in the curcumin pigment. The degree of degradation of the s and b^* of the curcumin at pH 3, 5 and 7 was between 22.80% to 39.50% and 22.48% to 51.86% in light conditions, and 20.79% to 32.92% and 22.63% to 40.84% in dark conditions, respectively. This was considerably lower than the degradation of the s and b^* of the curcumin at pH 9 and 11 of 35.12% to 85.09% and 36.05% to 94.54% in light conditions, 37.56% to 90.47% and 42.93% to 96.40% in dark conditions, respectively.

In this case, the curcumin pigment was stable only in highly acidic conditions and unstable at higher pH values. A pH of more than 7 and curcumin concentration of more than 1 mg/mL can cause colour degradation and decrease the stability even in dark conditions (Table 5.5). The results were correlated with those of a previous study by Suresh et al. (2013c), which showed that the stability increased in acidic environments and decreased as the pH increased. The decomposition was pH-dependent and proceeded more rapidly in neutral or basic environments. It has been demonstrated that stability increases in acidic pH and decreases in alkaline pH, which is consistent with the findings of other research, thus indicating that curcumin is more chemically stable in acidic environments (Tonnesen and Karlsen, 1985; Wang et al., 1997b).

Table 5.4 Effects of pH on the colour degradation of curcumin pigment concentrations from temu lawak upon exposure to dark and light conditions within 4 weeks.

		Degradation percentage (%)					
		Light			Dark		
		Curcumin concentration (g/L)					
CIE L*a*b*	pH	1	2	3	1	2	3
Colour saturation (s)	3	22.80	30.01	33.23	20.79	25.22	32.14
	5	23.00	30.05	33.48	20.91	31.87	32.50
	7	24.19	39.15	39.50	20.31	37.12	32.92
	9	35.12	44.02	43.40	37.56	50.26	47.14
	11	64.48	80.25	85.09	65.67	84.04	90.47
Yellow intensity (b*)	3	22.48	31.75	40.72	22.63	31.67	41.65
	5	22.63	31.67	41.65	19.81	32.26	39.70
	7	24.32	41.46	51.86	19.66	37.59	40.84
	9	36.05	54.11	58.47	42.93	66.13	73.95
	11	83.91	92.92	94.54	87.92	95.02	96.40

The studies by Kharat et al. (2017) and Anthony et al. (2022) stated that pure curcumin was unstable towards alkaline ($\text{pH} \geq 7$) and stable in acidic condition ($\text{pH} < 7$). Different pH condition might cause the changes in the molecular structure of the curcumin. Kharat et al. (2017) also proved that curcumin that been stored 30 days in acidic condition only degraded less than 15% while under neutral (pH 7) and alkaline (pH 8) condition, the degradation percentage are about 38 to 47%. The colour shows only minimal changes in acidic but the yellow colour of curcumin was clearly faded after been exposed to alkaline condition. Moreover, Etxabide et al. (2021) mentioned that curcumin also changes to different colour tones such as brown-yellow when exposed to alkaline which in their study at $\text{pH} > 9$. In food packaging industry, curcumin has been used as a colorimetric indicator to notice the existing of alkaline compounds formed during food spoilage as it colours turn from yellow to red when pH changes from acidic to alkaline (Raduly et al., 2021; Luo and Lim 2020; Ma et al., 2020). Thus, it is essential to identify the conditions that regulate the colour changes in curcumin in order to avoid such differences by taking effective strategies

to ensure appropriate stability of curcumin pigment to act as effective pH-indicators (Etxabide et al., 2021).

Table 5.5 Curcumin colours after 14 days based on CIELab values, when stored at pH 9 and room temperature 25 °C in light and dark condition

	LIGHT			DARK		
	pH 9					
DAY0						
	1 mg/mL	2 mg/mL	3 mg/mL	1 mg/mL	2 mg/mL	3 mg/mL
DAY 14						
	1 mg/mL	2 mg/mL	3 mg/mL	1 mg/mL	2 mg/mL	3 mg/mL

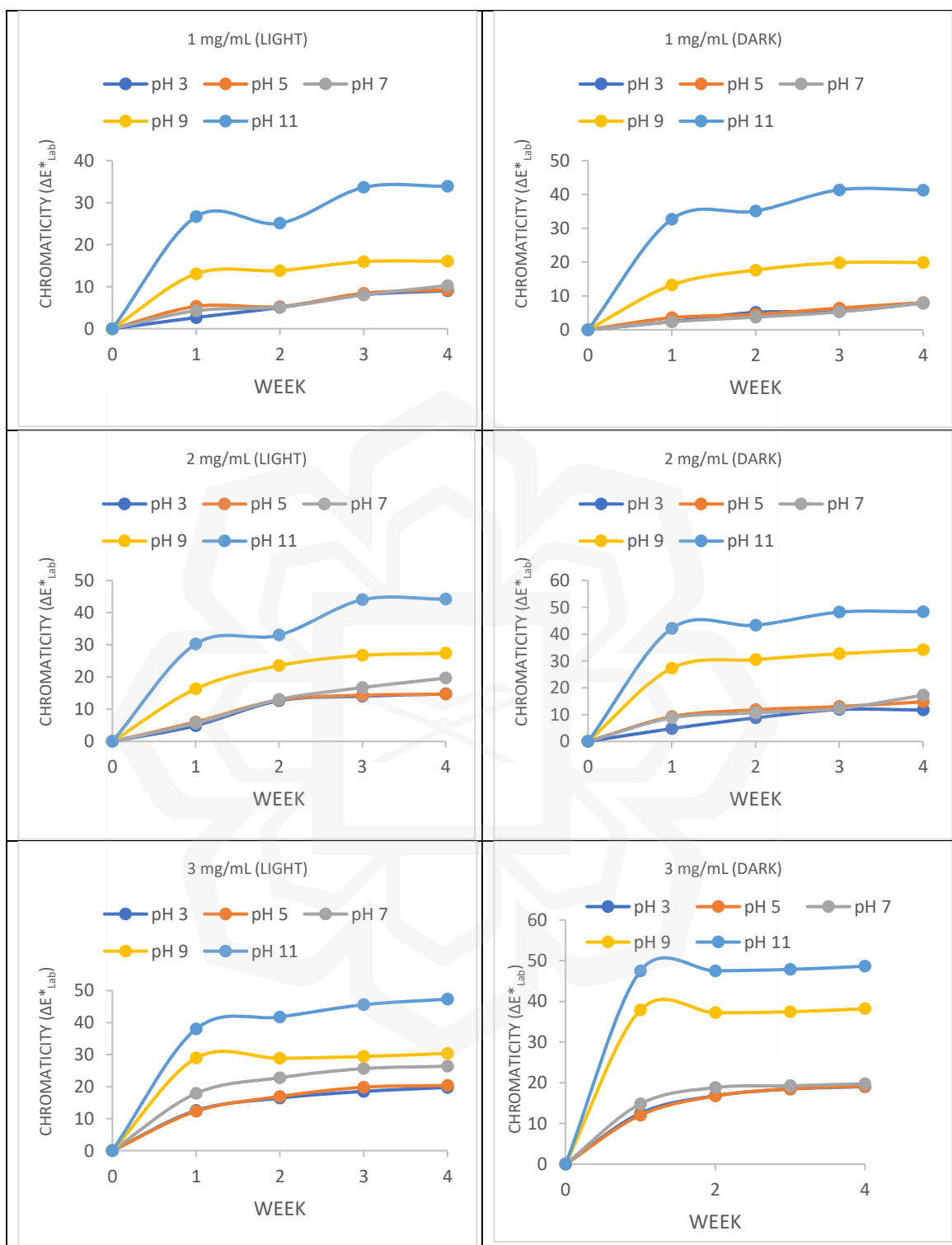


Figure 5.7 Chromaticity (ΔE^*_{Lab}) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) in light and dark condition

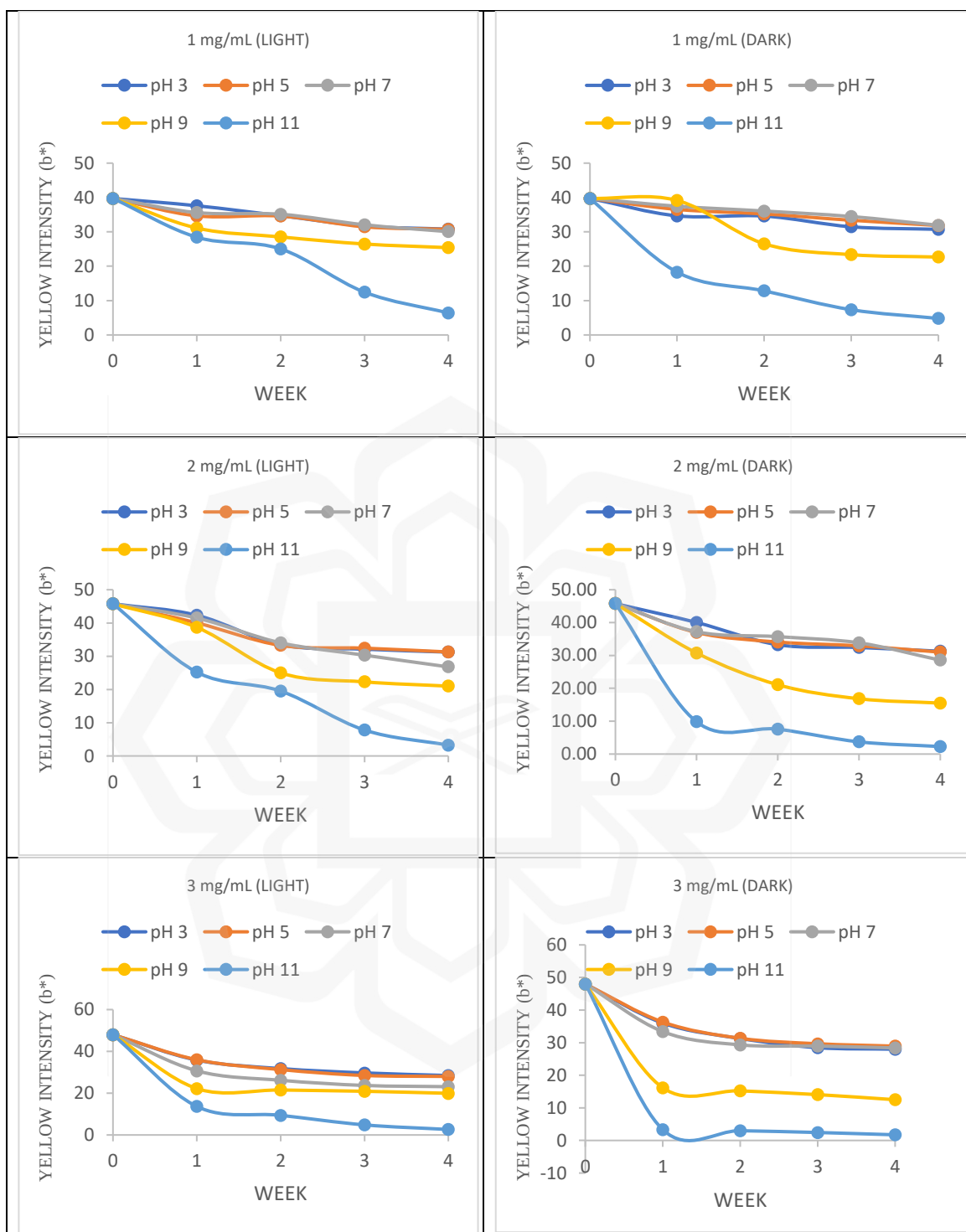


Figure 5.8 Yellow intensity (b^*) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) in light and dark condition for different pH

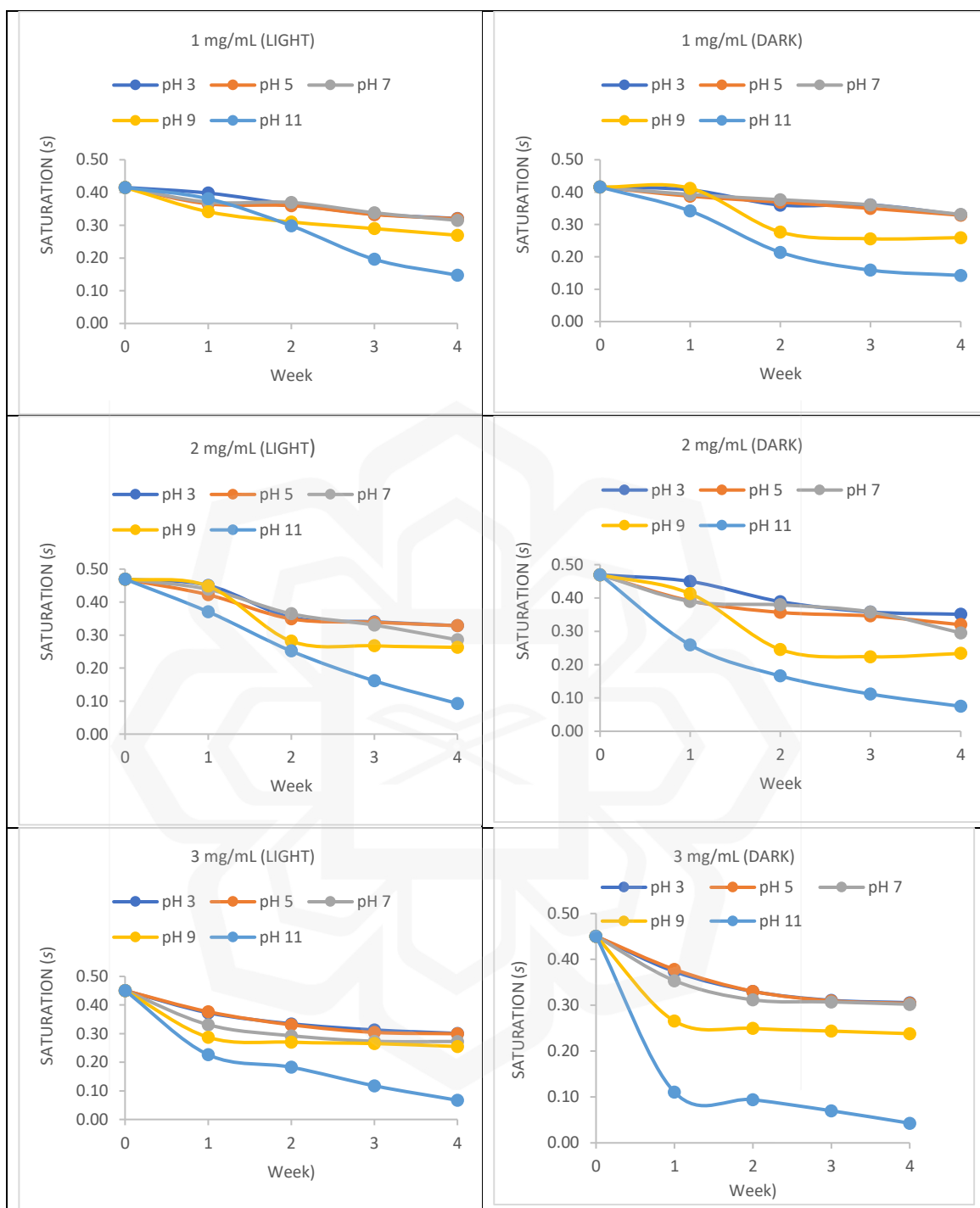


Figure 5.9 Colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) in light and dark condition for different pH

5.2.4 Effect of UV Irradiation

The ΔE^*_{Lab} , s , and b^* (%) for different concentrations of curcumin extracted from *temulawak* and exposed to UV radiation for 8 hours were compared to further investigate the stability of the pigment. The ΔE^* results under different types of UV radiation from the beginning until the end of the exposure are shown in Table 5.6 and Figure 5.10. The ΔE^* corresponds to colour brightness and is generally observed by the colour intensity. In this study, the ΔE^* for the curcumin pigment irradiated under UV-A for 8 hours was > 5.0 , indicating clearly two different colours, whereas under UV-B irradiation, the ΔE^* was < 3.5 , indicating a noticeable difference in colour. The s value is a measure of how colourful an area is visualised by an observer. It is determined as the ratio of ΔE^* to L^* . As depicted in Table 5.6 and Figure 5.10, after 8 hours of exposure, the curcumin pigment exhibited the highest degradation under UV-A as compared to UV-B. At concentrations of 1 mg/mL to 3 mg/mL, its s and yellow intensity degraded from 32.78% to 39.83% and 32.78% to 39.92%, respectively under UV-A, and in contrast, from 8.10% to 3.98% and 6.82% to 5.16%, respectively under UV-B. It was observed that the degradation percentages of s and b^* under UV-B decreased as the concentration of curcumin increased, indicating that the curcumin pigment was more stable under UV-B as compared to UV-A. The stability of carotenoids can be impacted by exposure to light (Boon et al., 2010; Rodriguez-Amaya, 2001) due to the occurrence of *trans-cis* photo-isomerisation (Rodriguez-Amaya, 2001; Yip et al., 2014). The single O_2 production, that creates the excited state of the molecules of the carotenoid, may also be caused by light absorption. A chemical degradation process might then be involved (Boon et al., 2010; Meléndez-Martínez et al., 2006).

Ultraviolet (UV) is one of the solar radiation types which significantly affecting colour stability. UVA which consists longer wavelength is responsible for melanin colour changes while UVB that has shorter wavelength is responsible to damage the protein. The stability of carotenoids can be impacted by light exposure including UV irradiation (Boon et al., 2010; Rodriguez-Amaya, 2001), since *trans-cis* photoisomerization can happen

(Rodriguez-Amaya, 2001; Yip et al., 2014). The single oxygen production that results in the excited state of the carotenoid molecule may also be caused by light absorption. Moreover, each carotenoid consists different number of conjugated double bonds (c.d.b.) which make them a noticeable colour. The longer chromophore of c.d.b., the brighter colour of the pigment. At least 7 c.d.b. are required to have visually be seen while below than 5 c.d.b. are consider as colourless (Mordi et al., 2020; Meléndez-Martínez et al., 2023). For this study, carotenoid from *C. xanthorrhiza* known as curcumin consists at least 7 c.d.b. since it has a yellowish colour in which this organic molecule was able to absorb UV or visible light and led to the rise of colour changes. Previous study confirmed that the delocalization of electrons along the chromophore has contributed to the decrease of molecule energy and lowering the c.d.b. Subsequently, the absorption of UV light is able to affect the chromaticity degradation of curcumin pigment (Maddah et al., 2020; Mordi et al., 2020). Thus, it can be summarised that, UV radiation is one of factors affecting chromaticity degradation as the observation thru colour changes from bright yellow to light yellow.

Table 5.6 Effects of UV radiation for 8 hours on color degradation for different concentrations of curcumin pigment extracted from temu lawak (*C. xanthorrhiza*).

	Degradation percentage (%)					
	UV-A			UV-B		
	Curcumin concentration (mg/mL)					
CIE L*a*b*	1	2	3	1	2	3
s	32.78	38.12	39.83	8.10	6.60	3.98
b*	32.78	38.16	39.92	6.82	6.95	5.16
ΔE^*	> 5.0	> 5.0	> 5.0	< 3.5	< 3.5	< 3.5
< 3.5 - noticeable colour difference;> 5.0 - indicate clearly two different colours						

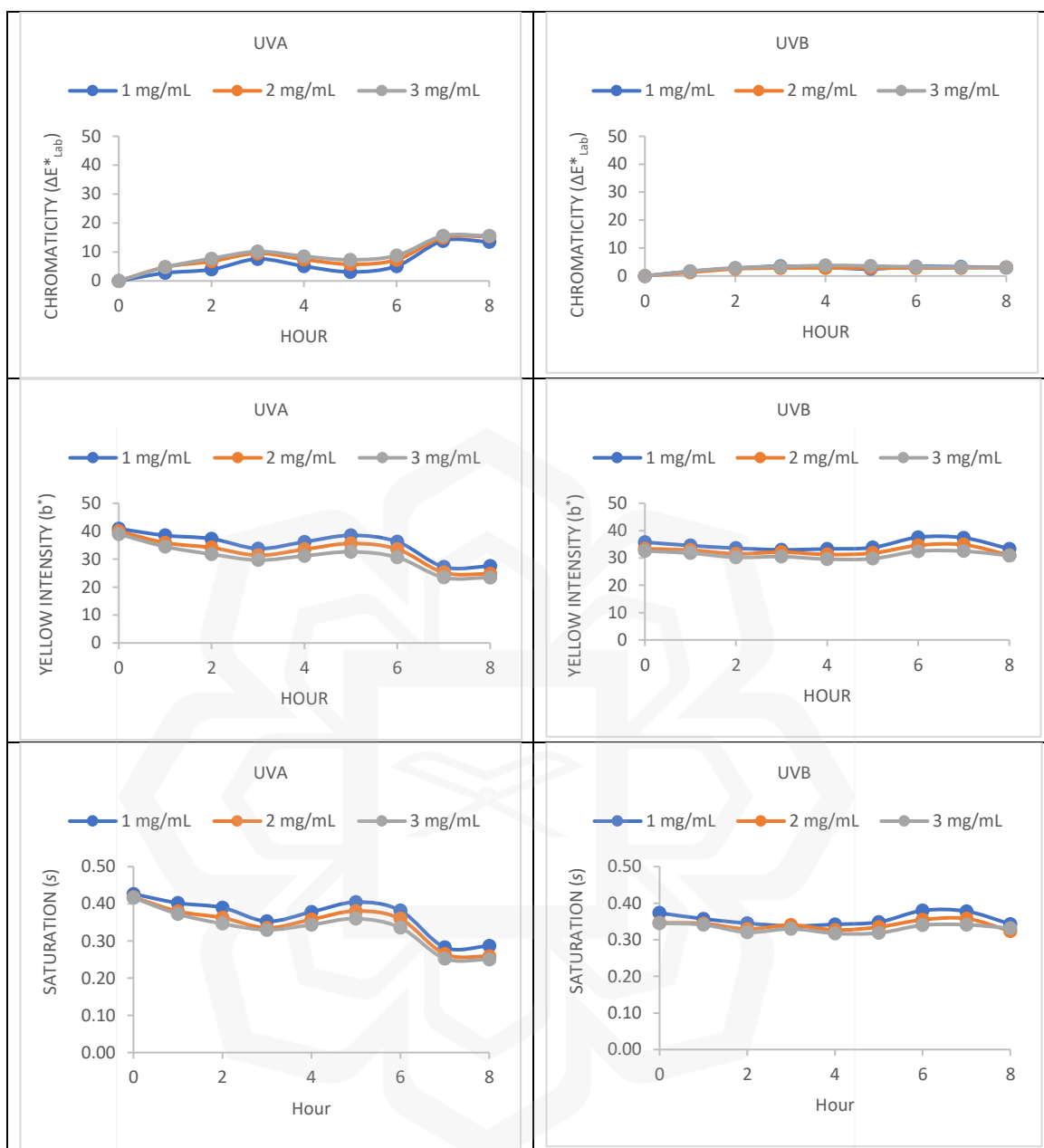


Figure 5.10 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) and treated with UV radiation for 8 hours

5.2.5 Effect of Heating

The results, as indicated in Table 5.7 and Figures 5.11 and 5.12, demonstrate that the higher the temperature and concentration of the curcumin, the higher the ΔE^* compared to the curcumin concentration of 1 mg/mL. In general, the curcumin at a concentration of 1 mg/mL showed a more stable s and b^* compared to the curcumin at higher concentrations. Interestingly, the curcumin at a concentration of 2 mg/mL exhibited the highest s and b^* degradation percentages of 13.9% and 12.8%, respectively at 4 °C, followed by those that were heated to temperatures of 55 °C (s -11.8 and b^* -11.2) and 75 °C (s -7.0 and b^* -7.5). On the other hand, no changes were found to have occurred in the curcumin at a concentration of 3 mg/mL in terms of the degradation percentages of s and b^* , especially from 25 °C to 65 °C, implying that the curcumin performed better with the heat treatment at a higher concentration. According to recent studies (Giménez et al., 2015), curcumin is stable up to 70 °C but is sensitive to heat. Curcumin is thermally degraded by roasting (heating at 180 °C for up to 70 minutes), and the degradation products of curcumin include vanillin, ferulic acid, and 4-vinyl guaiacol (Esatbeyoglu et al., 2015). Curcumin is thermally stable up to 160 °C (Zebib et al., 2010). It is a well-known fact that curcumin breaks down faster under light (Price and Buescher, 1996), at high temperatures (Kharat et al., 2017), in a high O₂ environment (Higaki et al., 2001), and in alkaline conditions (Kharat et al., 2017). Therefore, its stability can be increased by regulating the solution, the surrounding environment, and/or the packaging. As previously reported by Ambati et al. (2014), the pigment is said to be stable in a variety of carriers between 70 and 90 °C, and it is only observed to degrade between 120 and 150 °C due to oxidation, isomerisation, and degradation (Mezzomo and Ferreira, 2016; Mezzomo et al., 2013).

Table 5.7 Effects of heating for 3 hours on colour degradation for different concentrations of curcumin pigment extracted from temu lawak (*C. xanthorrhiza*).

		Degradation percentage (%)		
		Curcumin concentration (mg/mL)		
CIE L*a*b*	°C	1	2	3
Colour saturation (s)	4	2.7	13.9	1.6
	25	0	0	0
	35	2.9	2.7	0
	45	0	0	0
	55	1.8	11.8	0
	65	0	0	0
	75	2.7	7.0	5.4
Yellow intensity (b*)	4	3.0	12.8	1
	25	0	0	0
	35	4	3.2	0
	45	0	0	0
	55	1.6	11.2	0
	65	0	0	0
	75	2.7	7.5	4.0
ΔE^*	4	< 2.0	> 5.0	< 2.0
	25	< 1.0	< 5.0	< 5.0
	35	< 1.0	< 2.0	< 2.0
	45	< 1.0	< 5.0	< 5.0
	55	< 2.0	> 5.0	< 2.0
	65	< 5.0	< 5.0	< 5.0
	75	< 2.0	< 5.0	< 3.5
ΔE^* value	Indication			
< 1.0	No colour difference is visible			
< 2.0	colour difference is only slightly visible			
< 3.5	noticeable colour difference			
< 5.0	clear difference in colour is noticed			
> 5.0	indicate clearly two different colours			

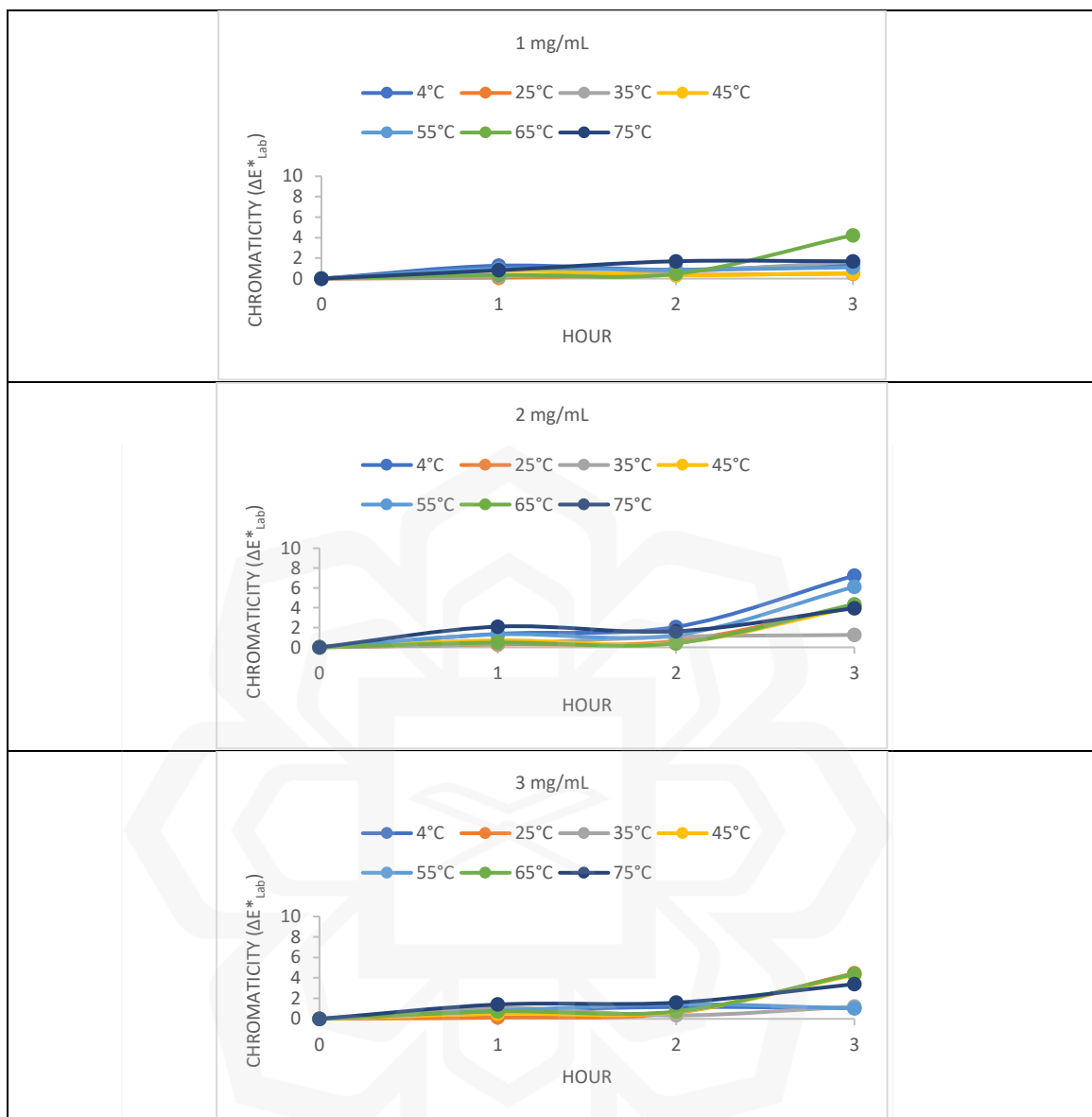


Figure 5.11 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) when treated at different temperature for 3 hours

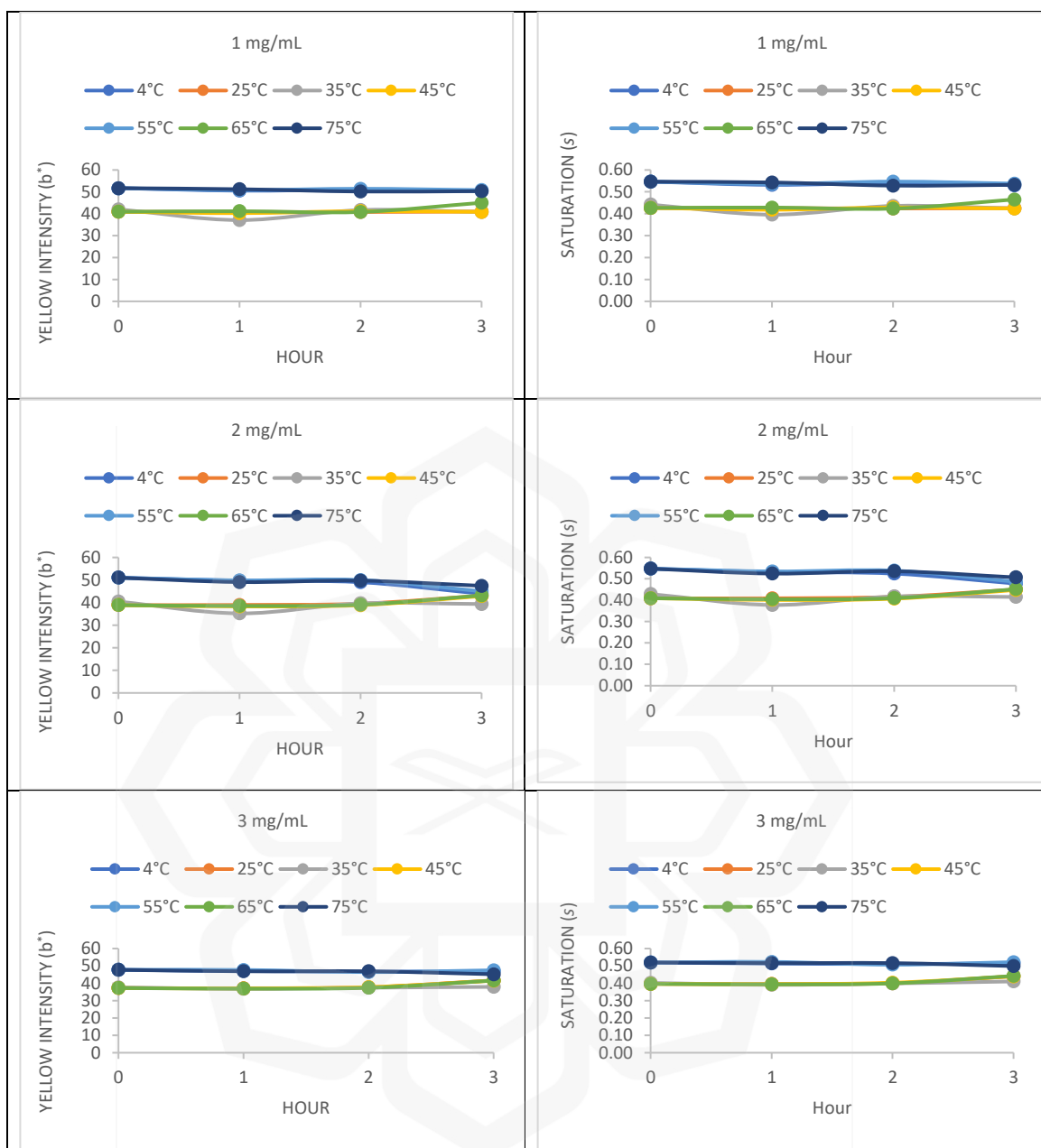


Figure 5.12 Yellow intensity (b*), and colour saturation (s) values for different concentrations of curcumin extracted from temu lawak (*C. xanthorrhiza*) when treated at different temperature for 3 hours

5.3 MULTIVARIATE DATA ANALYSIS

5.3.1 Sampling Adequacy for Multivariate Analysis

The results for the (10 variables) were analysed using MDA to determine those variables that contributed significantly to the ΔE^* stability of the curcumin pigment. Data transformation is a component of the initial preparation of data before a statistical analysis. The entire dataset was normalised through the use of variable transformation. The box and whisker plot in Figure 5.13 shows the graphical comparison of the distributions of the variables to identify outliers. The boxes portrayed the data values in the 25th percentile (lower quartile), median and 75th percentile (upper quartile). The outliers were those values that were less or more than those data values, which were located outside of the boxes. The outliers detected at L^* , a^* , b^* , C^* , H° , and ΔE in this boxplot signified the unusual values that represented the overall data distribution. In this plot, the outliers remained as the data was significant to show the highest values for each variable. The median score of H° was the highest, and s was the lowest.

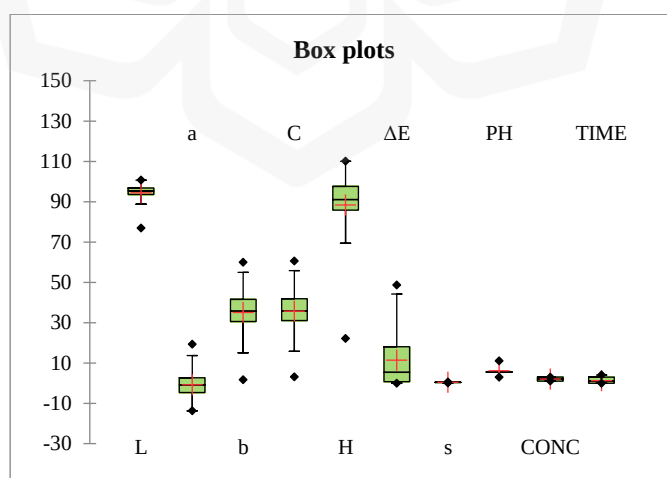


Figure 5.13 BWPs of curcumin pigment's ΔE^* stability variables at different concentrations and exposure times.

The sampling adequacy of the ΔE^* stability data for the curcumin pigment was further subjected to a KMO test, where the value had to be > 0.5 , followed by the Barlett's test of sphericity ($p < 0.05$) and Pearson correlation matrix, where the value had to be ≥ 0.01 (Atiqah et al., 2023). The KMO test was conducted to evaluate the adequacy of the dataset at an α of 0.05 to ensure the dataset was adequate for the MDA. The average KMO value was ranked as detailed by Ismail et al. (2021a) as Table 5.8.

Table 5.8 KMO value

KMO < 0.5	inadequate
$0.5 < \text{KMO} < 0.7$	mediocre
$0.7 < \text{KMO} < 0.8$	good
$0.8 < \text{KMO} < 0.9$	very good
KMO > 0.9	excellent

The 378 datasets produced a KMO score of 0.711 (Table 5.9), thus confirming that the dataset was in a good category and was adequate for the PCA test. Barlett's test of sphericity was carried out to ensure that the R-matrix was not equal to the identity matrix. The p for the ΔE^* stability data of the curcumin pigment exhibited highly significant differences at 0.0001.

Table 5.9 Kaiser-Meyer-Olkin and Bartlett's test for the curcumin pigment chromaticity stability

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.711
Bartlett's Test of Sphericity	Chi-square (Observed value)	6256.939
	DF	45
	<i>p</i> -value (Two-tailed)	< 0.0001

5.3.2 Correlation Coefficient Matrix (r^2)

Table 5.10 and 5.11 revealed strong positive and negative correlations between two variables in the ΔE^* stability test for the curcumin pigments. A very strong positive correlation was observed for C^* , s , b^* , L^* , and H° ($r^2 = 0.993$ to 0.922), whereas the other variables such as ΔE and t ($r^2 = 0.724$), as well as b^* and H° and C^* ($r^2 = 0.551$ to 0.517), had high and moderate positive correlations, respectively. The tables also showed the relationship between 2 groups of ΔE versus C^* , b^* , s and a^* versus H° , L^* , which produced a strong significant negative correlation ($r^2 = -0.724$ to -0.875). It can be concluded that the higher curcumin concentrations ranging from 1 to 3 mg/mL did not necessarily correlate with the ΔE^* stability of the curcumin. In general, the variables for the ΔE^* stability variables of the curcumin pigment could be grouped into four clusters, with two clusters having positive correlations and another two clusters having negative correlations.

Table 5.10 The r^2 of the curcumin pigment's ΔE^* stability

Variables	L	a	b	C	H	ΔE	s	pH	Conc.	t
L	1									
A	-0.724	1								
B	0.465	-0.518	1							
C	0.432	-0.517	0.984	1						
H	0.922	-0.832	0.551	0.517	1					
ΔE	-0.479	0.392	-0.875	-0.838	-0.520	1				
S	0.344	-0.448	0.976	0.993	0.445	-0.827	1			
pH	-0.652	0.494	-0.457	-0.422	-0.668	0.494	-0.386	1		
Conc.	-0.274	0.400	-0.084	-0.109	-0.277	0.134	-0.078	0.000	1	
T	-0.077	0.090	-0.627	-0.628	-0.110	0.724	-0.644	0.151	0.000	1
<i>Values in bold are different from 0 with a significance level $\alpha=0.01$</i>										

Table 5.11 evaluated the r^2 and its strength between two variables in the ΔE^* stability test for the curcumin pigment. The r^2 is measured on a scale from -1 to +1, as described by Guilford (1973).

Table 5.11 The strength of the r^2 between the two variables affecting the curcumin pigment's ΔE^* stability

Variables	r^2	Size of Correlation	Interpretation
C x s b x C b x s L x H	0.993 0.984 0.976 0.922	0.90 to 1.00	Very high positive correlation
ΔE x t	0.724	0.70 to 0.90	High positive correlation
b x H C x H	0.551 0.517	0.50 to 0.70	Moderate positive correlation
a x pH ΔE x pH L x b H x s L x C a x Conc. a x ΔE L x s	0.494 0.494 0.465 0.445 0.432 0.400 0.392 0.344	0.30 to 0.50	Low positive correlation
L x a ΔE x s a x H C x ΔE b x ΔE	-0.724 -0.827 -0.832 -0.838 -0.875	-0.70 to -0.90	High negative correlation
a x C a x b H x ΔE b x t C x t s x t L x pH H x pH	-0.517 -0.518 -0.520 -0.627 -0.628 -0.644 -0.652 -0.668	-0.50 to -0.70	Moderate negative correlation
s x pH C x pH a x s b x pH L x ΔE	-0.386 -0.422 -0.448 -0.457 -0.479	-0.30 to -0.50	Low negative correlation
pH x t ΔE x Conc. a x t pH x Conc. Conc. x t L x t s x Conc. b x Conc. C x Conc. H x t L x Conc. H x Conc.	0.151 0.134 0.090 0.000 0.000 -0.077 -0.078 -0.084 -0.109 -0.110 -0.274 -0.277	0.00 to 0.30 (0.00 to -0.30)	Little if any correlation
Values in bold are different from 0 with a significance level $\alpha=0.01$			

5.3.3 Principal Component Analysis (PCA)

The next step involved the analysis of the dataset using PCA to identify the variables with a high factor loading to indicate significant principal components. An FL of more than 0.75 was considered as strong, between 0.50 and 0.75 as moderate, between 0.30 and 0.49 as weak, and below 0.30 as negligible (Rani et al., 2017). Eigenvalues (EV) are used to determine the principal components with more cumulative dataset variations. An EV of 1 or more is considered as significant for the PCA (Le et al., 2017; Zubir et al., 2016a; Yang et al., 2020a). The 10 variables taken from the ΔE^* stability test for the curcumin pigment were subjected to a principal component analysis (PCA) to study which variables significantly contributed to the stability of the curcumin pigment, as detailed by Siudek and Frankowski (2018) and Vuong et al. (2020). The PCA exposed two principal components (PCs) entailing the 10 variables, which represented a cumulative variability of 76.99%. The EVs were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 5.6, and for PC2 was 2.0, as shown in Table 5.12. In principle, variables far away from the axes, F1 and F2, had a strong FL. Referring to Table 5.12, b^* , C^* , H° , ΔE and s had strong FL values ($FL > 0.75$), while L^* , a^* , pH, and time had moderate FL values ($0.500 < FL < 0.749$), and only the curcumin concentrations had weak FL values ($FL < 0.499$).

Table 5.12 Factor loadings and eigenvalues of the PCA correlation matrix for curcumin pigment ΔE stability

Variables	PC1	PC2
L	0.711	0.594
a	-0.712	-0.525
b	0.929	-0.300
C	0.914	-0.319
H	0.781	0.562
ΔE	-0.879	0.300
s	0.878	-0.397
pH	-0.646	-0.340
CONC	-0.228	-0.381
TIME	-0.571	0.629
Eigenvalue	5.658	2.041
Variability (%)	56.581	20.412
Cumulative (%)	56.581	76.993

The principal component, PC1 accounted for 56.58% of the total variance, which comprised strong FL values for b, C, H, ΔE and s, whereas for PC2, which accounted for 20.41% of the total variance for the data, no single strong variable was identified. The PCA enabled the factors to be identified and provided insights into the stability of the curcumin pigment. Therefore, the FL was utilised to determine the strength of the association between the variables in the ΔE^* stability test for the curcumin pigment. The degree of correspondence or the potential link between the variables is further explained in Figure 5.14.

Figure 5.14 summarised that all variables were either positively or negatively correlated to each other which can be grouped into four. Group 1 consists of L and H, group 2 have C, b, and s, Group 3 is pH, concentration, and a, and Group 4 is ΔE and time. From Figure 5.14, it can be summarised that all the variables were either positively or negatively correlated to each other, and could be categorised under four groups, as given below. All the variables were positively correlated as long as they were grouped close together such

as the variables in Group 1, Group 2, and Group 3. Groups 1 and 3 as well as Groups 2 and 4 were negatively correlated as they were oppositely positioned. The others were not correlated. The clusters positioned within a^* , b^* , C^* , s , ΔE , and time were all samples at a concentration of 2 mg/mL, which was confirmed to be the most potential concentration for the carotenoid pigment stability.

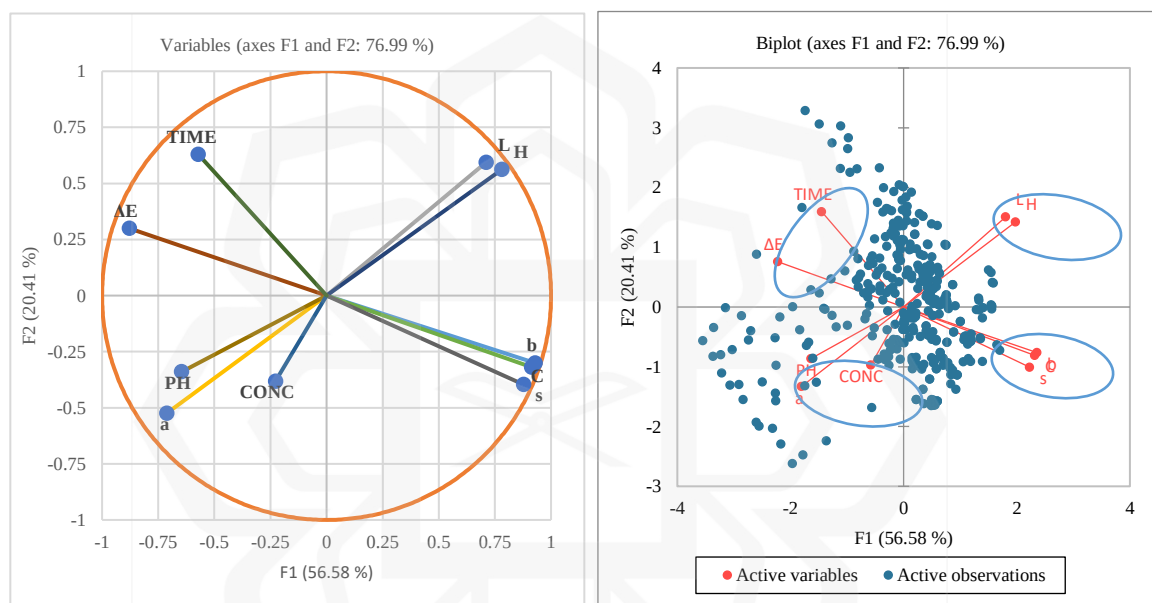


Figure 5.14 Variable plots and correlation biplots of the variables in the curcumin pigment's stability test.

It could be concluded that the higher curcumin concentrations ranging from 1 to 3 mg/mL did not necessarily correlate with the ΔE^* stability of the curcumin. In general, the variables for the ΔE^* stability of the curcumin pigment could be grouped into four clusters, with two clusters having positive correlations and another two having negative correlations, as indicated in Figure 5.14.

5.4 SUMMARY OF RESULTS

1. Generally, the higher the curcumin concentration, the lower the duration of exposure, and the darker the conditions, the lower the degradation percentage.
2. The degradation of the curcumin pigment was reduced by treatment with NaCl at a higher concentration of 3 mg/mL in dark conditions.
3. The curcumin pigment was more stable in highly acidic conditions or at a pH of less than 7.
4. A pH of more than 7 and a curcumin concentration of more than 1 mg/mL caused a decrease in the colour degradation and stability of the curcumin pigment even in dark conditions.
5. The curcumin pigment was more stable in UV-B at higher concentrations as compared to UV-A.
6. The curcumin concentration at 3 mg/mL showed better stability towards heat treatment.
7. A significant correlation was observed between the C*, s, intensity, L*, and H° of the curcumin pigment and the duration of exposure to dark and light conditions.
8. The pigment of curcumin extracted from temu lawak is a potential source of natural colourant for the halal market and industry and also in coating technology.

CHAPTER SIX

CHROMATICITY STABILITY OF PLA-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS

6.1 INTRODUCTION

Polymers are substances with a long chain of repetitive smaller units bonded together and can be classified into two categories based on their origins: petrochemical-based or natural-based (Williams and Hillmyer, 2008) and have a wide range of applications from plastics as packaging materials. However, these convenient petrochemical-based materials that are non-biodegradable and designed for immediate disposal have caused a lot of pollution problems ever since they became dominant in the marketplace around 1940 (Jambeck et al., 2015). Fortunately, poly (lactic acid) or known as PLA (Figure 6.1), is a green polymer from renewable resources (manufactured by the polymerisation of lactic acid monomers derived from the fermentation of starch feedstocks) which were biodegradable and compostable (bioplastic). However, it has lower strength, brittle, flammable and high permeability to most gases and solvents as compared to metal and ceramics, which limits its application in the industry (Shirai et al., 2013b). Therefore, the ultimate objective of this chapter is to evaluate the effects of salinity, alkalinity and acidity, UV irradiation and heat on PLA-curcumin chromaticity stability extracted from *C. xanthorrhiza* Roxb. as a potential natural colourant source for coating ingredient.

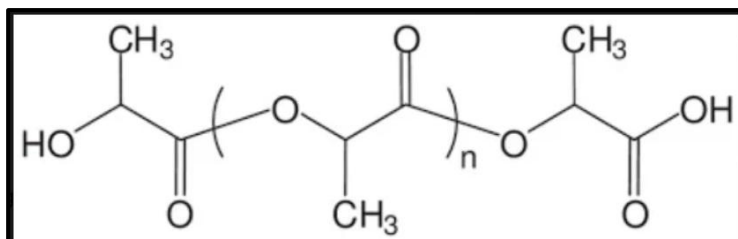


Figure 6.1 Chemical structure of polylactic acid

6.2 RESULTS AND DISCUSSIONS

6.2.1 Effect of light, pigment concentration and time of exposure on PLA-curcumin stability

The stability test on PLA-curcumin was aiming to observe the effects of weathering including sodium chloride (seawater salinity), pH (acidity and alkalinity), sunlight exposure (UV rays), and heat (temperature) towards this integration as potential coating material, as well as to study the factors of degradation. The CIE L*a*b* colour difference (ΔE^*_{LAB}) is used to assessed the stabilisation of PLA-curcumin coating as described by Mokrzyck and Tatol, (2011).

Colour difference or chromaticity relative measurements of PLA-curcumin at concentration of 5 g/L, 10 g/L, and 20 g/L, time of exposure from week 0 until week 4, and in response to light and dark condition were presented in Figure 6.2. The results of chromaticity established that ΔE^* value > 5.0 for all three concentrations after 4 weeks of exposure in response to light and dark condition indicated clearly visible of two different colours. Higher PLA-curcumin concentration (10 and 20 g/L) under light condition shows higher colour degradation compared to dark conditions but both conditions with time exposure and concentration were confirmed to contribute to the colour differences. There

is also a strong relationship between the saturation, intensity, and concentration of the PLA-curcumin yellow colour with time exposure in response to light and dark conditions. Marked differences were observed for PLA-curcumin yellow intensity (b^*) and colour saturation (s) degradation value for all three concentrations and time of exposure developing under light and dark (Figure 6.2). PLA-curcumin at concentration of 5 g/L in response to light condition were observed to have substantially lower degradation value of s and b^* compared to higher concentration (10 and 20 g/L) which found to have relatively higher degradation of s and b^* value. However, the yellow colours of PLA-curcumin at all concentrations only have minimum degradation in response to dark conditions throughout 4 weeks exposure.

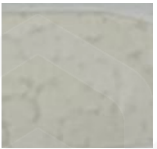
Colour stability of PLA-curcumin pigment that has been tested for a month are exhibited by the chromaticity, yellow intensity and saturation graphs as shown in Figure 6.2. The reading was taken weekly until the fourth week of exposure, the chromaticity of 5, 10, and 20 g/L PLA-curcumin pigments show that the PLA-curcumin concentration of 20 g/L in response to light condition were observed to have relatively highest degradation of s (51.6%) and b^* (82.4%) value whereas PLA-curcumin pigment with the lowest concentration of 5 g/L in response to dark condition were observed to have substantially lowest degradation percentage of s (0.7%) and b^* (9.5%) (Table 6.1). Chromaticity for PLA-curcumin pigment with 5, 10 and 20 G/L established that ΔE^* value > 5.0 for all three concentrations for both conditions (light and dark) in which under the light condition, the PLA-curcumin pigment colour lost the original before test colour (Table 6.2). Previous research indicated that degradation was significantly greater in the presence of light than it was in the absence of light (Suresh et al., 2013a), and the evidence is consistent with this study. According to some research (Lucas-Gonzales et al., 2023; Roy and Rhim, 2020c), one of the ways that light can speed up food oxidation processes and change the packaged product's visual attractiveness. In comparison to light circumstances, a study indicated that PLA-curcumin composites have superior colour stability in dark situations. It is also proved from the previous study that, compared to exposure to light, colour degradation might reach 89.92%, but in the dark, it was much less to 40% (Britton et al., 1996). This suggests that

keeping the composite dark preserves the colour more vividly and steadily (Britton et al., 1996). The study also found that when PLA-curcumin composites were kept in the dark, they remained stable under both basic and acidic conditions. This makes them appropriate for a variety of applications where little to no light exposure occurs (Britton et al., 1996). Moreover, microencapsulation of unstable natural pigment is a frequently used method to stabilise and protect bioactive compounds in microscopic particles and developing a shield to the external environment. It provides protection against degradation reactions and prevents the loss in term of colour, and bioactive compounds (Neves et al., 2019).

Table 6.1 Effects of 4 weeks in light and dark condition on colour degradation of PLA-curcumin

Degradation percentage (%)					
CIE L*a*b*	Condition	Curcumin concentration (g/L)			
		5	10	20	
Colour saturation (s)	Light	3.7	10.7	51.6	
	Dark	0.7	9.9	3.5	
Yellow intensity (b*)	Light	44.0	100	82.4	
	Dark	9.5	23.5	8.0	
Chromaticity (ΔE^*_{Lab})	Light	> 5.0			
	Dark	> 5.0			
ΔE^*_{Lab} value	Indication				
< 1.0	No colour difference is visible				
< 2.0	colour difference is only slightly visible				
< 3.5	noticeable colour difference				
< 5.0	clear difference in colour is noticed				
> 5.0	indicate clearly two different colours				

Table 6.2 Effects of 4 weeks in light and dark condition on colour degradation of PLA-curcumin

LIGHT				DARK			
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L	
WEEK 0							
	b* 61.50	b* 73.44	b* 88.88	b* 63.73	b* 72.48	b* 87.22	
WEEK 4							
	b* 0.38	b* -0.75	b* 15.66	b* 57.65	b* 71.42	b* 83.58	

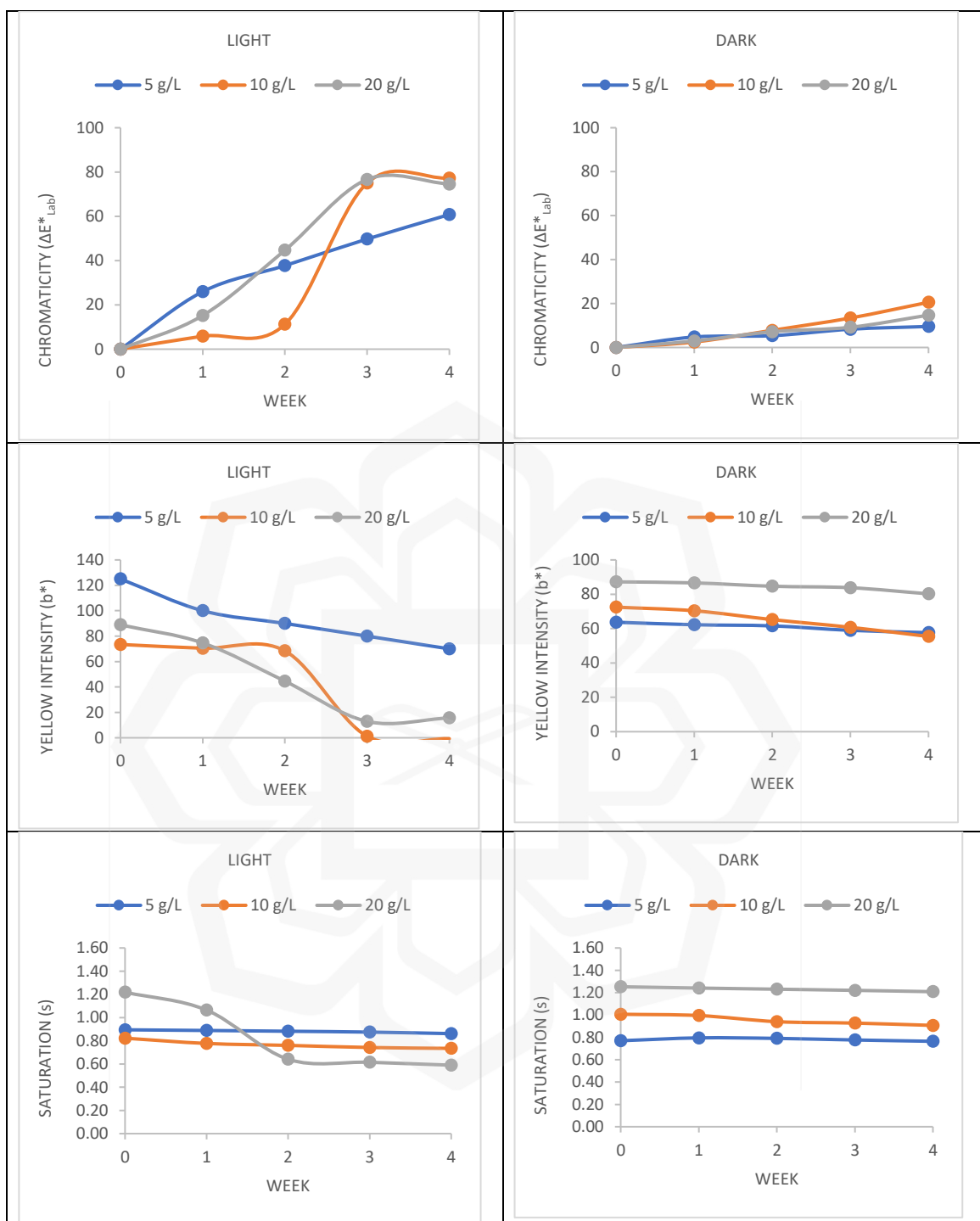


Figure 6.2 CIE L*a*b* colour difference or Chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PLA-curcumin in light and dark condition for different exposure time

6.2.2 Effect of NaCl

Colour stability is calculated by the chromaticity and colour saturation graphs of PLA-curcumin pigment, which are added with 5, 10, 20, and 30 g/L NaCl and stored in light and dark conditions. It was taken for 4 weeks, and the results are shown in Figures 6.3 to 6.5 respectively. Colour intensity and brightness are responsible for chromaticity (C^*) values of the PLA-curcumin pigment, for the saturation (s), is known as the vividness of an area visualised, where it is given by the ratio of chromaticity to lightness. The degradation percentage (%) analysis of colour saturation (s) and yellow intensity (b^*) for different concentrations of PLA-curcumin in light and dark conditions after 4 weeks treated with different concentrations of salinity is shown in Table 6.3. PLA-curcumin concentrations of 5 g/L in response to light were observed to have the highest degradation of s (95.7%) and b^* (96.9%) as compared to PLA-curcumin pigment with the lowest salinity concentration (5 g/L) which is in 20 g/L of curcumin concentration with the lowest percentage degradation (%) of s 32.7%. For dark conditions, it was revealed that, the highest degradation of colour saturation was observed in 5 g/L which was treated in 30 g/L of NaCl concentrations with the value of s (14.2%) and b^* (19.8%).

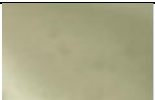
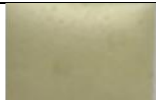
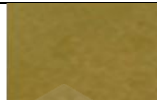
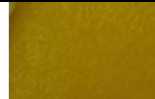
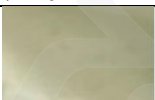
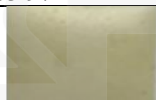
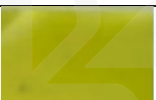
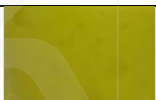
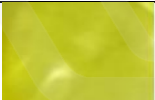
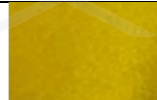
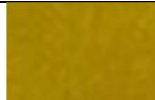
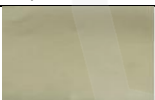
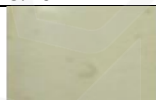
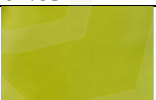
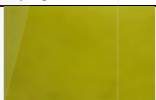
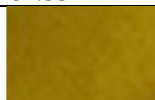
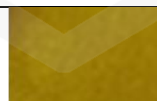
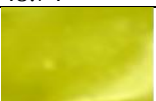
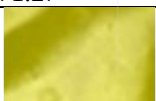
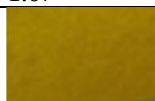
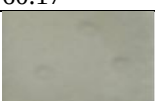
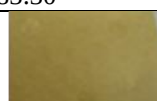
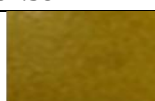
Chromaticity for curcumin pigment after being treated with 5, 10, 20 and 30 g/L NaCl established that ΔE^* value > 5.0 for all three concentrations. The most significant colour changed from yellow to almost colourless occurred to PLA-curcumin at 5 g/L and 10 g/L treated with NaCl under light conditions (Table 6.4). The graphs also show that adding NaCl does affect the colour stability. The results indicate that the presence of NaCl has slightly significant effect on the stability of the PLA-curcumin. However, the presence of light has induced the photodegradation of the coating. Many different types of polymers can be used, including bioplastics, biopolymers, and commercial plastics. Biopolymers are one of these and are used to draw attention due to their reproducibility, biodegradability, and excellent functional properties (Rouf and Kokini, 2018b). From the previous study states that, the kind of material used in contact with food, the migrant's characteristics, and

the food all have an impact on chemical migration (Muncke, 2014a). Chemical exposure over an extended period is demonstrated by food contact materials (Geueke et al., 2014). Thus, incorporation of curcumin into PLA induces the degradation of the coating as well. This agrees with the study done by Suresh et al. (2013a), in which in the presence of light the degradation was much higher as compared to in the absence of light. While Elloumi et al. (2020) confirmed that higher salinity level may cause to lower the carotenoid content which coincide with this study that found the colour degradation of curcumin pigment obviously occurs at higher NaCl concentrations.

Table 6.3 Effects of NaCl exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin

Degradation percentage (%)									
CIE L*a*b*	Curcumin (g/L)	Light				Dark			
		NaCl concentration (g/L)							
		5	10	20	30	5	10	20	30
Colour saturation (s)	5	90.5	89.1	91.0	95.7	16.5	7.5	15.2	9.8
	10	77.1	68.6	83.4	74.1	2.9	6.4	16.4	7.4
	20	32.7	47.7	83.4	63.9	14.0	2.6	9.6	14.2
Yellow intensity (b*)	5	91.2	89.7	91.7	96.9	24.0	15.7	22.2	19.8
	10	81.2	78.5	86.8	79.9	28.1	16.2	10.9	13.5
	20	56.9	54.9	86.8	70.4	12.7	17.9	28.2	13.2
Chromaticity (ΔE^*_{Lab})	5	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0
	10	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0
	20	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0
ΔE^*_{Lab}	Indication								
< 1.0	No colour difference is visible								
< 2.0	colour difference is only slightly visible								
< 3.5	noticeable colour difference								
< 5.0	clear difference in colour is noticed								
> 5.0	indicate clearly two different colours								

Table 6.4 Effects of NaCl exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin

WEEK	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0 5 g/L NaCl	 b* 73.12	 b* 76.70	 b* 77.37	 b* 70.80	 b* 76.98	 b* 76.94
WEEK 4 5 g/L NaCl	 b* 6.43	 b* 16.31	 b* 39.06	 b* 60.47	 b* 69.67	 b* 67.13
WEEK 0 10 g/L NaCl	 b* 71.29	 b* 63.97	 b* 79.15	 b* 73.42	 b* 67.94	 b* 75.41
WEEK 4 10 g/L NaCl	 b* 7.36	 b* 18.17	 b* 40.00	 b* 61.93	 b* 66.97	 b* 74.88
WEEK 0 20 g/L NaCl	 b* 71.71	 b* 76.70	 b* 77.05	 b* 62.63	 b* 77.40	 b* 81.33
WEEK 4 20 g/L NaCl	 b* 5.95	 b* 11.43	 b* 42.86	 b* 48.74	 b* 71.27	 b* 71.07
WEEK 0 30 g/L NaCl	 b* 60.17	 b* 75.87	 b* 83.30	 b* 70.26	 b* 78.13	 b* 81.58
WEEK 4 30 g/L NaCl	 b* 2.20	 b* 15.28	 b* 24.65	 b* 58.67	 b* 67.56	 b* 75.47

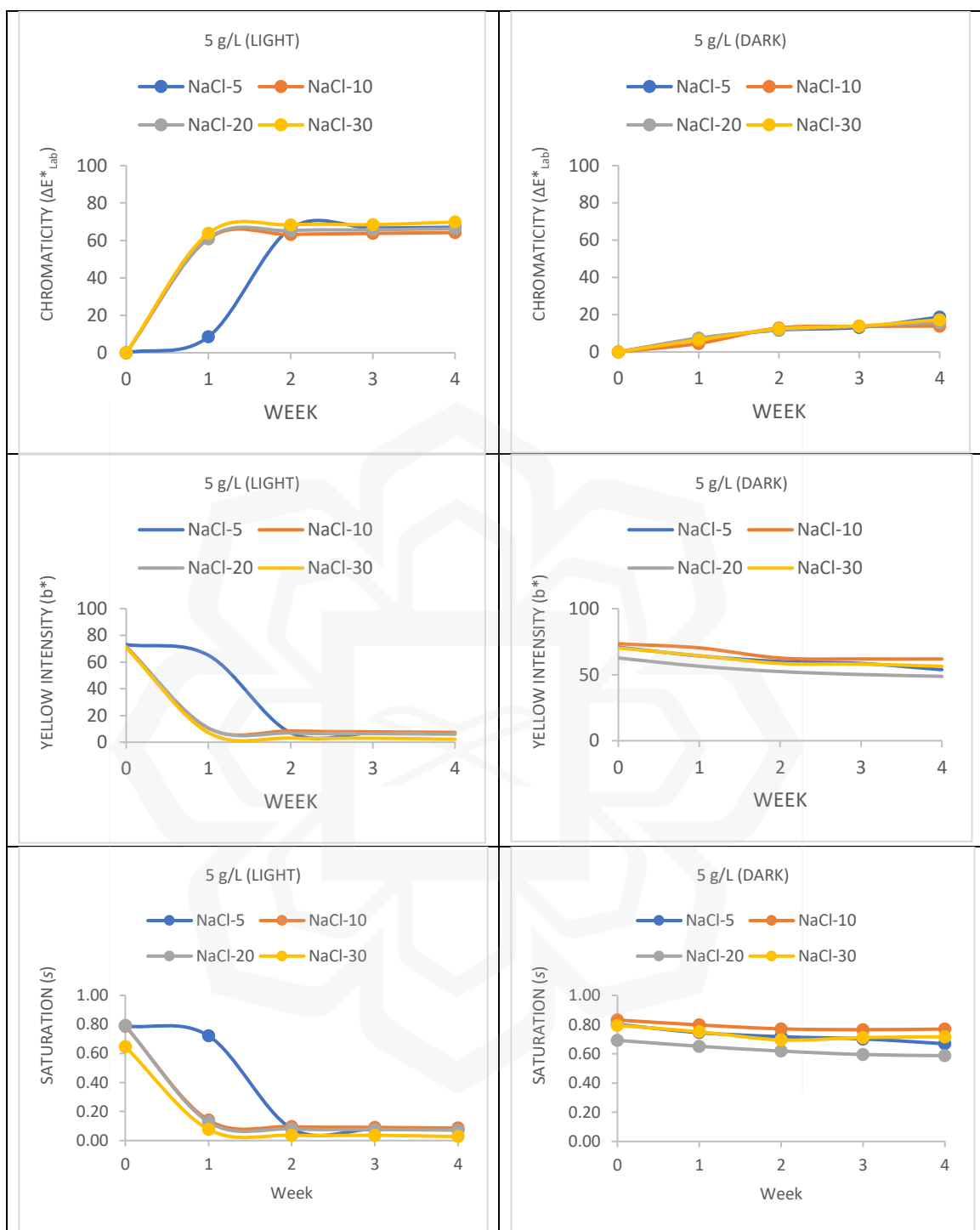


Figure 6.3 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L PLA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

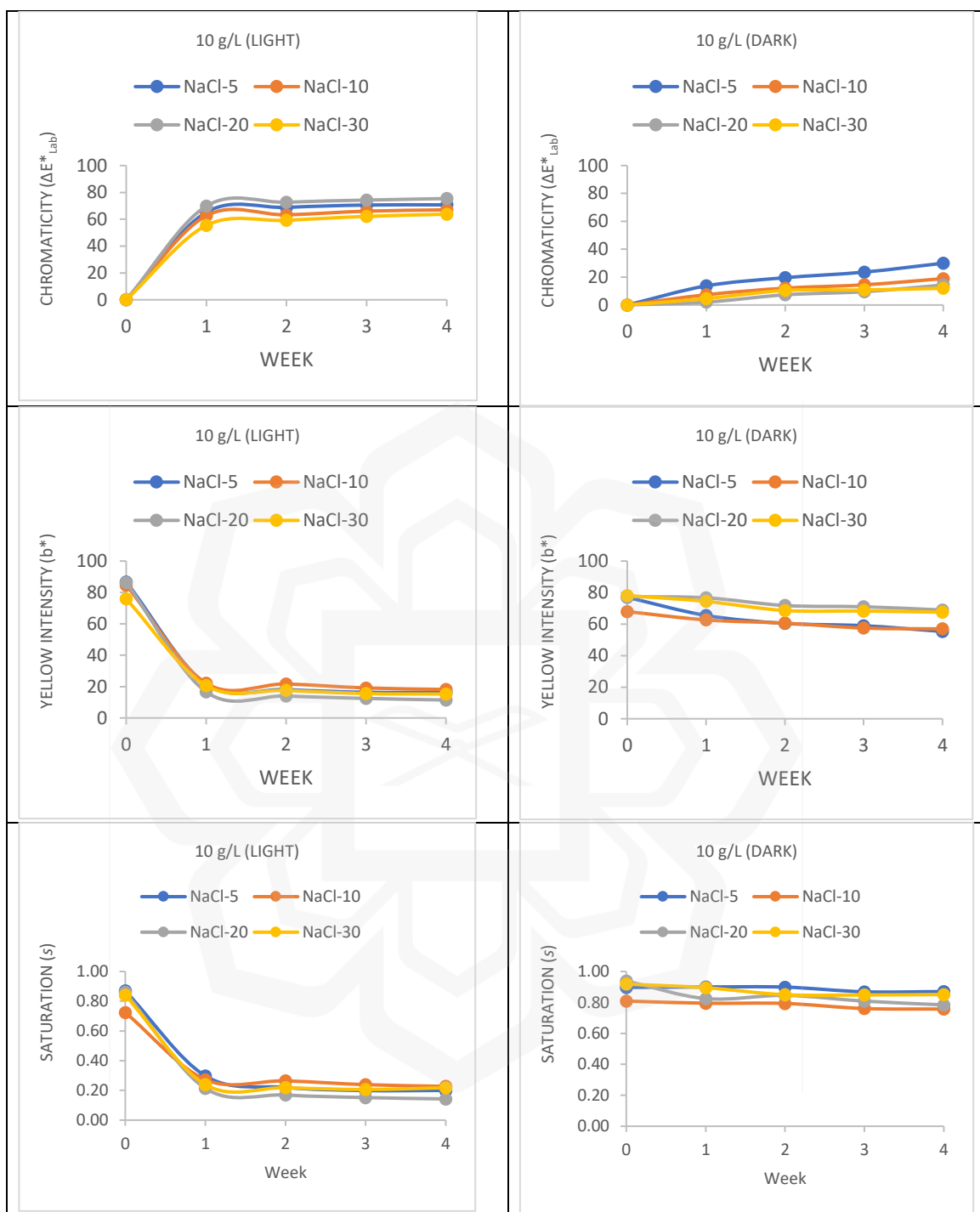


Figure 6.4 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PLA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

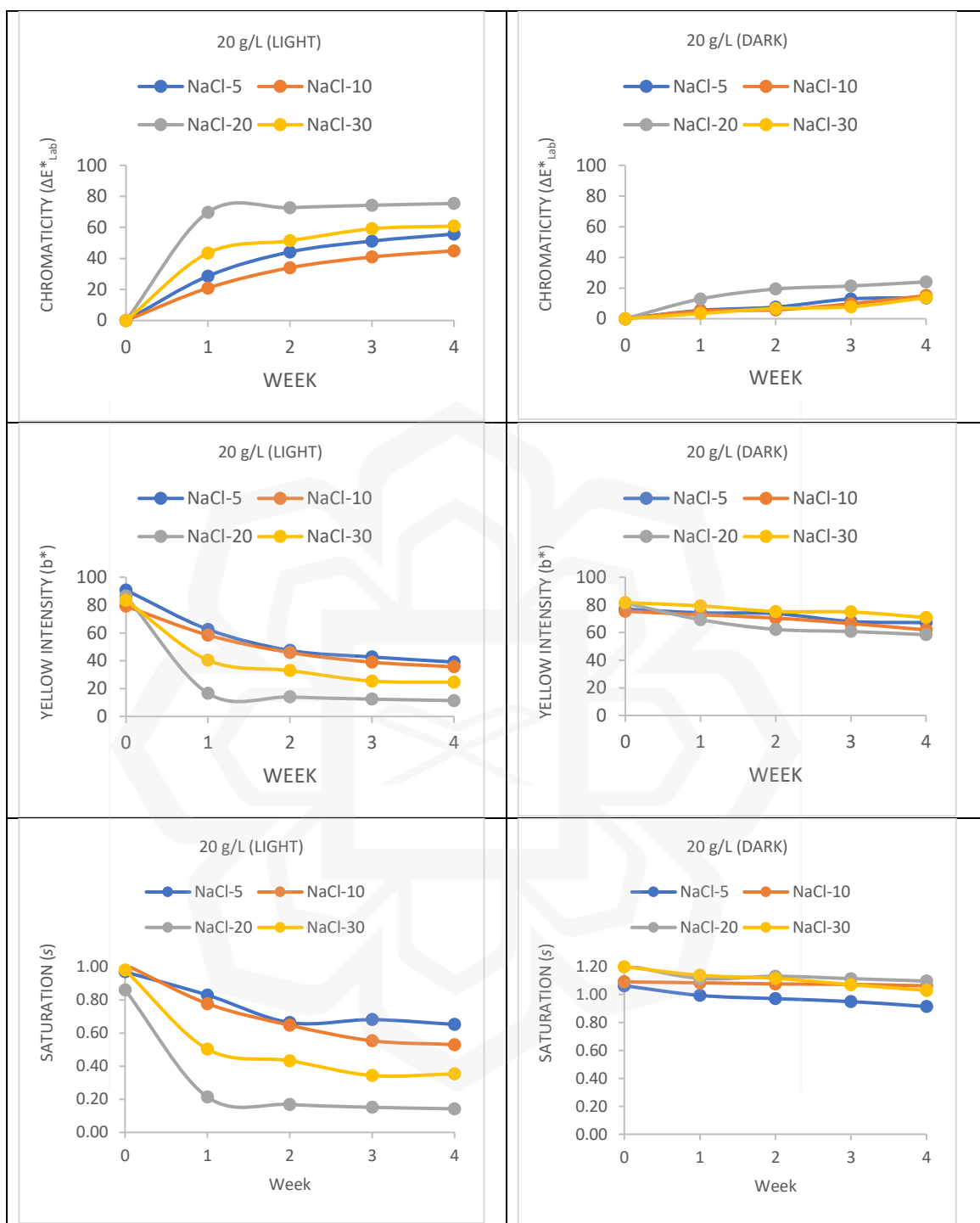


Figure 6.5 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PLA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

6.2.3 Effect of pH

Colour stability is demonstrated by the chromaticity and colour saturation, which is showed the ratio of chromaticity to lightness. It is showed from the graphs of PLA-curcumin pigment, that is treated with different value of pH 3, 5, 7, 9 and 11 that are being stored in dark and light condition in 4 weeks (Figure 6.6 to 6.8). PLA-curcumin concentration of 5 g/L in response to light condition were observed to have highest degradation percentage of s (91.0%) and b^* (91.0%) at pH 7 whereas PLA-curcumin pigment with lowest concentration of 20 g/L was found to have lowest degradation of s (15.5%) and b^* (27.4%) value at pH 11. In dark condition, the percentage of s and b^* were developed by the value of (31.7%) and (33.2%) that were tested in 5 g/L at pH 9 (Table 6.5). It is showed that, the degradation percentage is still lower when it is tested in dark condition as compared to light condition. As for 10 g/L of PLA-curcumin concentration, it was estimated that, the degradation percentage of s (0.0%) and b^* (13.9%) value at pH 3, which showed the lowest percentage of degradation. It is revealed that chromaticity ΔE^* value > 5.0 for all three concentrations of PLA-curcumin pigment after treated with pH 3, 5, 7, 9 and 11 with the obvious colour different occurred at PLA-curcumin of 5g/L and 10 g/L that turn from yellowish into greyish colour under light condition (Table 6.6).

According to Nouredin, El-Shishtawy, and Al-Footy (2019), curcumin's pH colour changes to red when the conditions are altered to an alkaline state. So, to create intelligent packaging systems, curcumin's features can function as the best possible pH-dependent colour-changing pigment. In this research, the results revealed the ability of pH to influence the activities of curcumin and PLA-curcumin. Overall, the dark condition was preferable and more stable for maintaining curcumin brightness, resulting in a slower degradation rate. This is due to the reason of, by comparing to one stored in dark conditions, PLA-curcumin stored under light conditions exhibits a faster rate of degradation. The colour stability of PLA-curcumin has been found to be unaffected by pH, however, according to experimental findings from model systems, exposure to light might hasten the fading of colours and the

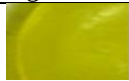
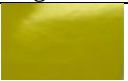
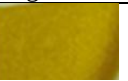
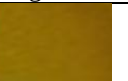
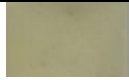
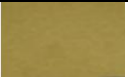
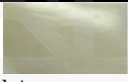
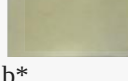
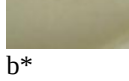
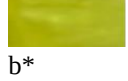
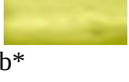
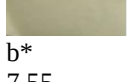
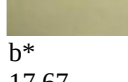
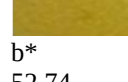
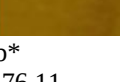
loss of curcumin due to photodegradation (Mirzaee et al., 2014; Pricez and Buescher, 1996). When curcumin was added to biopolymer films made of carrageenan, polylactic acid, gelatine, and gum/poly (vinyl alcohol), the colour of the films changed in response to the pH (Aliabbasi, Fathi, and Emam-Djomeh, 2021a). The phenoxide anion formed when curcumin was tested under basic conditions caused the colour change by demonstrating the reaction between phenolic hydroxyl groups and the hydroxyl ions (Liu et al., 2018a). Because of their biodegradability, non-toxicity, biocompatibility, and abundance, various types of biodegradable polymers have been developed from a variety of natural resources, such as proteins, polysaccharides, and lipids, and have been used to produce natural colours that have been blended with colour indicators to create colour indicator smart packaging film (Kumar, Mukherjee, and Dutta, 2020).

Curcumin may also be thought of as immobilised in a film matrix, which demonstrated a pronounced pH-responsive colour change needed for the applications of intelligent food packaging. Due to the alteration in its dominant structure, which is studied under various pH conditions, curcumin can change colour, which can be used to monitor food quality in real-time (Priyadarshi et al., 2021). Since curcumin has pH-dependent colour change properties and functional properties that can be used in food packaging applications, it has attracted the attention of numerous scientists in recent years. This is because curcumin may help increase the food's quality and shelf life. It is well known that temperature affects the mechanisms of adsorption/desorption permeability, solubility, and diffusion phenomena (Robertson, 2006; Siracusa et al., 2015). Curcumin can be used as a pH-responsive colour changing agent when mixed with biopolymer films like polylactic acid, carrageenan, gelatine, gum/poly (vinyl alcohol) films (Fathi, Emam-Djomeh, and Aliabbasi, 2021). This is the consequence of curcumin's colour shifting under basic conditions because hydroxyl ions and phenolic hydroxyl groups react to generate phenoxide anion. (Liu et al., 2018a).

Table 6.5 Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin

Degradation percentage (%)											
CIE L*a*b*	Curcumin (g/L)	Light					Dark				
		pH									
		3	5	7	9	11	3	5	7	9	11
Colour saturation (s)	5	44.3	90.0	91.0	89.4	89.7	5.2	5.9	12.7	31.7	12.4
	10	79.3	86.2	86.2	94.5	75.1	0.0	10.7	5.0	13.0	13.4
	20	59.6	65.5	36.9	94.5	15.5	3.9	9.4	13.7	6.7	6.9
Yellow intensity (b*)	5	35.7	90.8	91.0	90.4	90.1	17.2	19.4	22.9	33.2	20.9
	10	80.9	87.0	87.0	95.0	78.1	13.9	21.2	12.8	15.9	17.8
	20	64.7	69.0	40.7	95.0	27.4	14.5	17.5	13.9	11.3	16.1
Chromaticity (ΔE* _{Lab})	5	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0
	10	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0
	20	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0
ΔE* _{Lab}	Indication										
< 1.0	No colour difference is visible										
< 2.0	colour difference is only slightly visible										
< 3.5	noticeable colour difference										
< 5.0	clear difference in colour is noticed										
> 5.0	indicate clearly two different colours										

Table 6.6 Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PLA-curcumin

WEEK	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0 pH 3	 b* 78.33	 b* 78.50	 b* 78.90	 b* 69.62	 b* 77.37	 b* 82.59
WEEK 4 pH 3	 b* 50.40	 b* 15.00	 b* 27.85	 b* 57.64	 b* 66.90	 b* 74.28
WEEK 0 pH 5	 b* 68.22	 b* 75.33	 b* 84.97	 b* 44.11	 b* 76.81	 b* 84.93
WEEK 4 pH 5	 b* 6.30	 b* 9.81	 b* 26.31	 b* 35.56	 b* 62.21	 b* 75.08
WEEK 0 pH 7	 b* 58.01	 b* 75.33	 b* 76.32	 b* 72.76	 b* 70.42	 b* 85.02
WEEK 4 pH 7	 b* 5.19	 b* 9.81	 b* 45.24	 b* 56.10	 b* 61.43	 b* 73.22
WEEK 0 pH 9	 b* 79.38	 b* 68.12	 b* 68.12	 b* 75.66	 b* 72.67	 b* 77.74
WEEK 4 pH 9	 b* 7.64	 b* 3.41	 b* 3.41	 b* 50.55	 b* 61.11	 b* 68.94
WEEK 0 pH 11	 b* 76.34	 b* 80.68	 b* 70.62	 b* 62.58	 b* 73.42	 b* 81.56
WEEK 4 pH 11	 b* 7.55	 b* 17.67	 b* 52.74	 b* 49.51	 b* 63.04	 b* 76.11

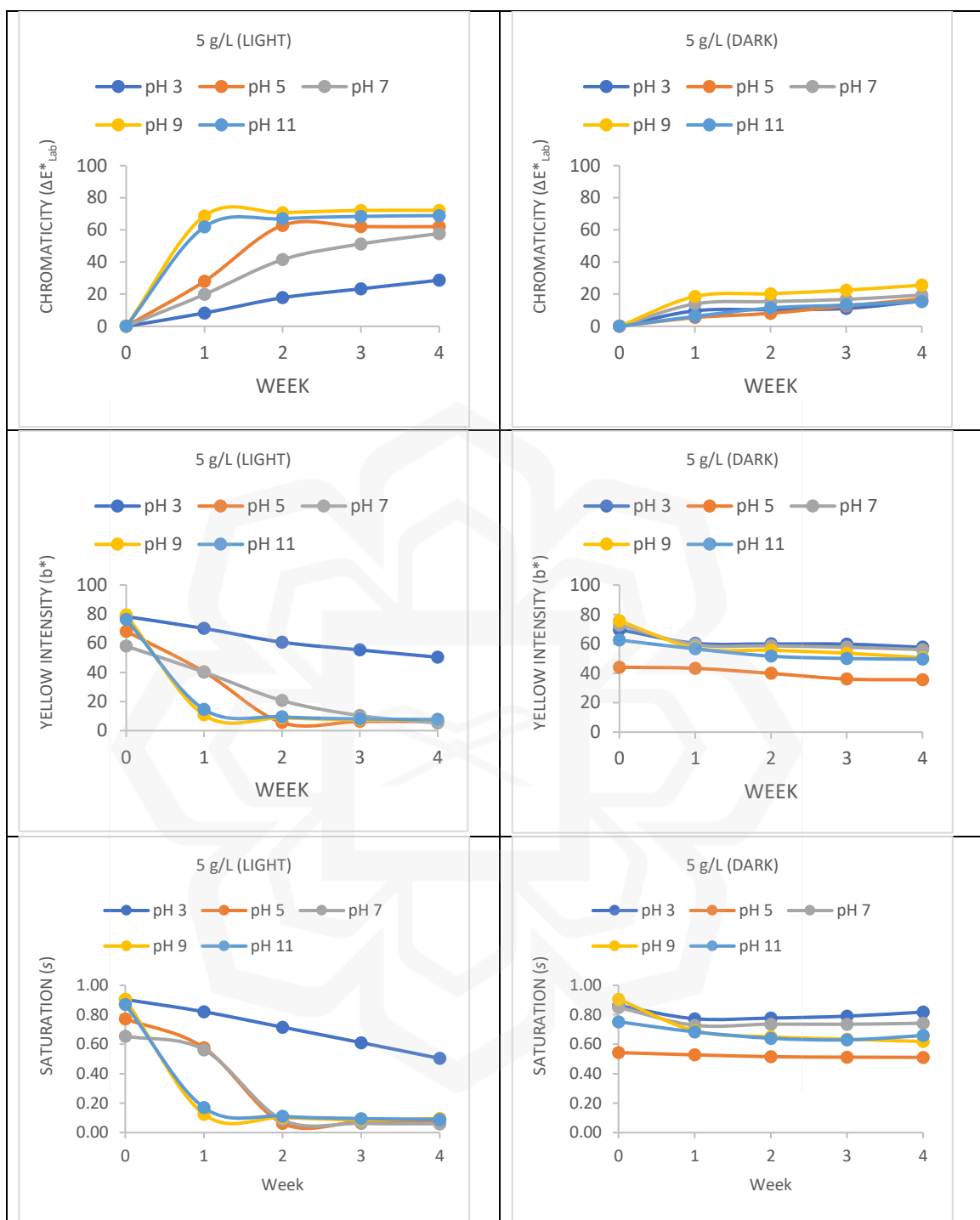


Figure 6.6 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values (%) for 5 g/L PLA-curcumin at different pH in light and dark condition for different exposure time

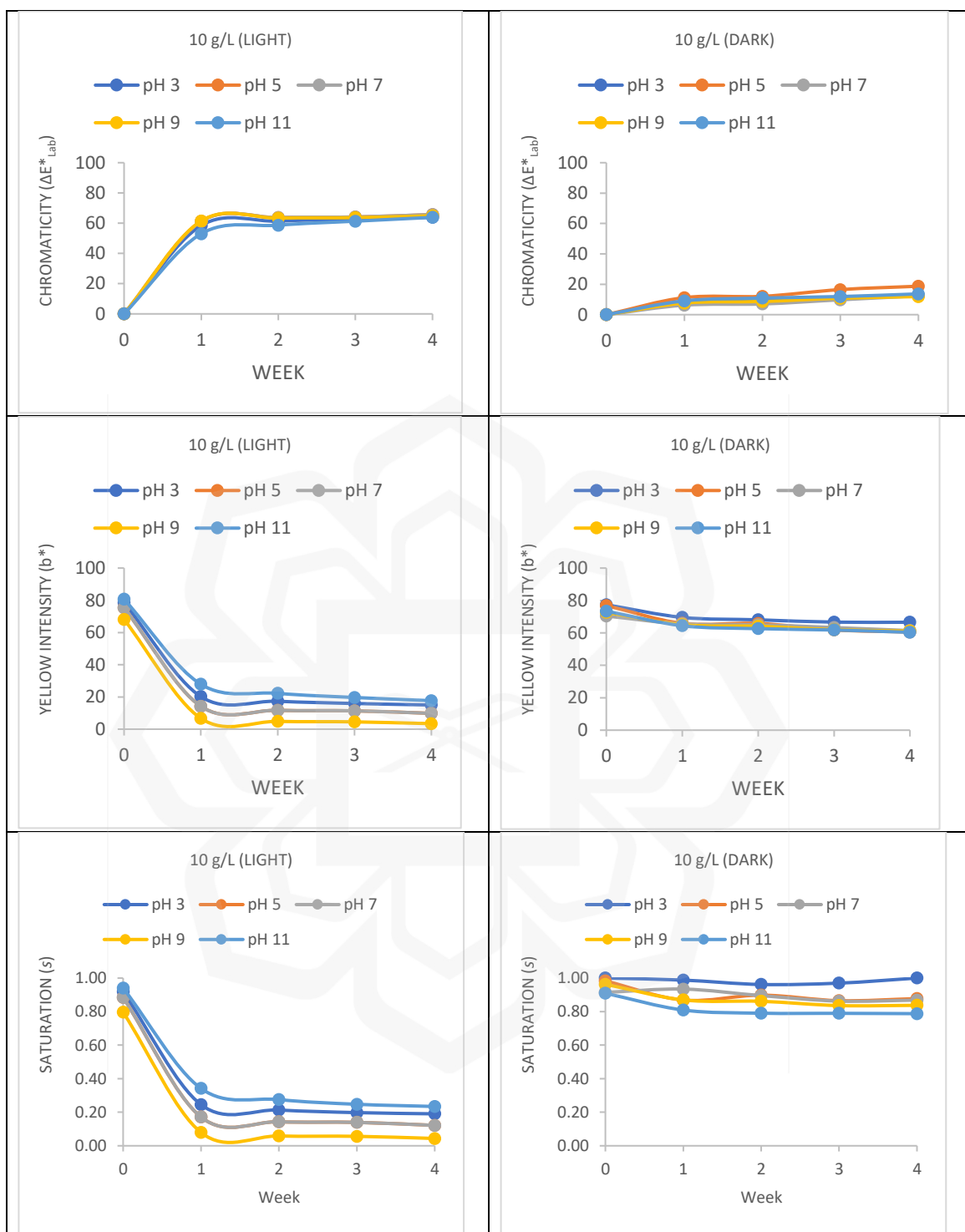


Figure 6.7 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PLA-curcumin at different pH in light and dark condition for different exposure time

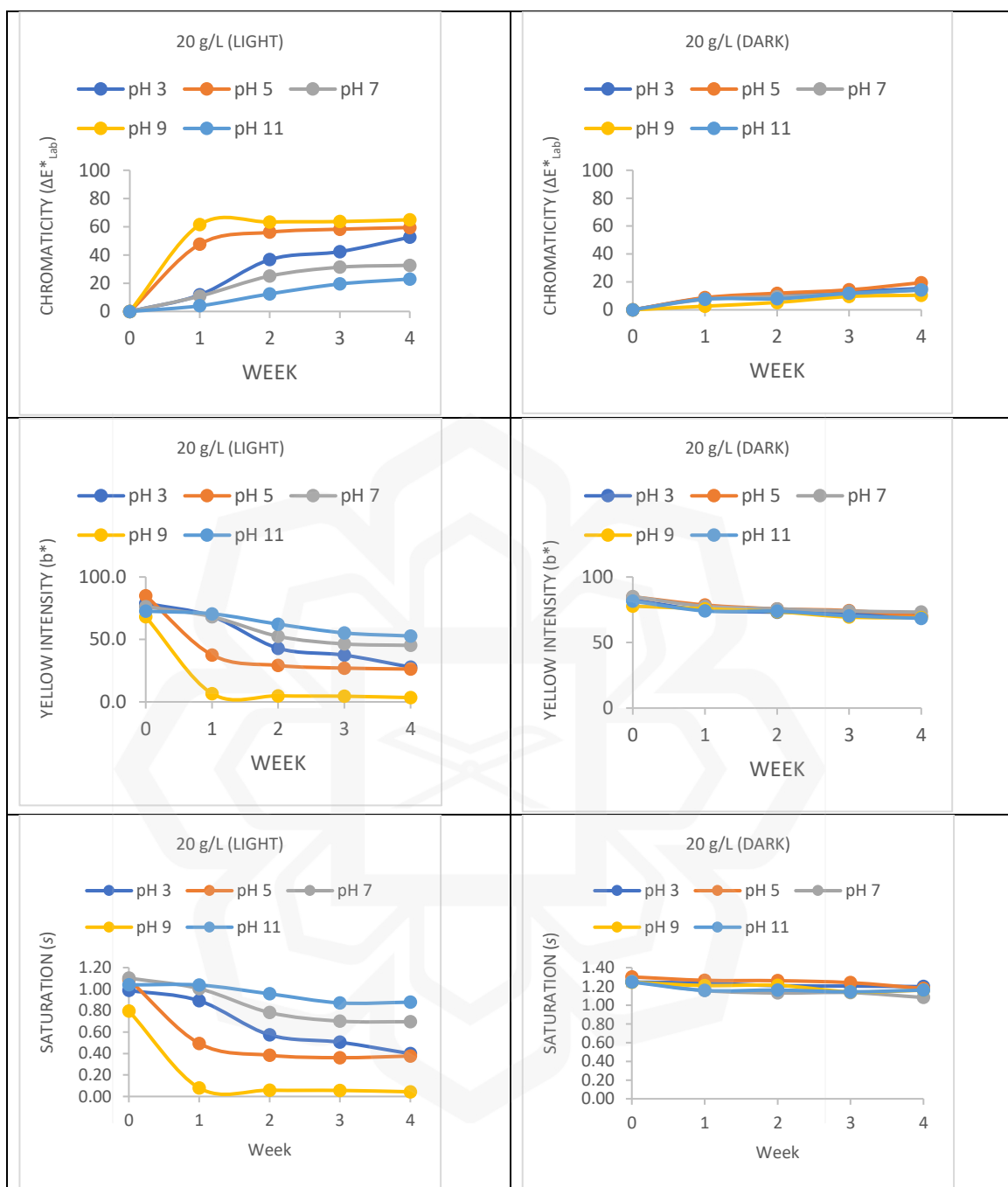


Figure 6.8 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PLA-curcumin at different pH in light and dark condition for different exposure time

6.2.4 Effect of UV irradiation

Colour stability of PLA-curcumin pigment that is irradiated under UVB (312 nm) for eight hours are exhibited by the chromaticity and colour saturation graphs as shown in Figure 6.9. The exposure of PLA-curcumin at concentration of 5 g/L and 10 g/L under UVB irradiations for eight hours resulted slightly constant degradation with period of exposure. Whereas PLA-curcumin at 20 g/L shows high stability towards the UV irradiation. After eight hours of being irradiated under UVB light, the chromaticity of 5, 10, and 20 g/L PLA-curcumin pigments show the highest percentage degradation of s (98.8%) and b^* (98.9%) at 5 g/L, and the degradation percentage (%) showed the lowest of s (5.2%) and b^* (8.6%) at 20 g/L of PLA-curcumin concentration (Table 6.7). Overall, it can be seen that, the percentage of colour saturation (s) and yellow intensity (b^*) were showed the highest degradation for 5 and 10 g/L of PLA-curcumin pigment. The chromaticity (ΔE^*) corresponds to all the concentration tested for curcumin (5, 10 and 20 g/L) with the value of > 5.0 , which indicate clearly two different colours. The most significant colour changes can be seen at PLA-curcumin of 5 g/L and 10 g/L that turn from yellow to grey while at 20 g/L the colour only has small changes from dark brown to yellowish brown (Table 6.8).

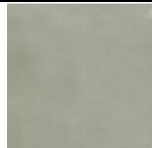
For instance, the exposure of composites to ultraviolet light causes photoirradiation, which compromises their mechanical and physical properties due to the breakage of organic polymer chemical bonds (Siakeng et al., 2018). This is due to the highest concentrations pigmented can maintain the colour degradation towards the ability of exposing under the UV rays. The results of UV irradiation showed that PLA containing curcumin was very susceptible to colour change. Naturally occurring dyes have the potential to be used as indicator substances that change colour in response to different stimuli, as demonstrated by the test, which employed treatment types appropriate for the experiment. The films entirely biodegrade, are safe for the environment, and pose no health risks to people. The colour shade (ΔE) indicated that the natural colourant extract contained polyphenolic chemicals, which contributed to higher values in the films. It showed from the previous study, when

twin-screw extrusion is combined with values of 1, 3, 5, 7, 10, 15, and 20 weight %, the composite films made of PLA exhibit greater oxygen, water vapour barrier, ultraviolet radiation, and hydrophobic properties than the PLA polymer in its pristine form (Reddy and Kim, 2018). As comparing from the previous study, by employing solution casting with 0.25, 0.5, 1.0, and 1.5 weight %, curcumin is employed to aid in improving the oxidation activity and hydrophobicity of PLA film (Roy and Rhim, 2020d). It also supported from Reddy and Kim (2018), which reported on the production of polylactic acid (PLA)-based functional films that were combined with curcumin using a twin-screw extruder. They indicated that adding curcumin can improve the hydrophobicity and UV light barrier qualities of the PLA sheet. Using a solution casting technique, Roy and Rhim (2020d) created a PLA-based film reinforced with curcumin. They saw that the PLA/curcumin film had better UV light barrier qualities, but that the film's mechanical and thermal qualities were unaffected.

Table 6.7 Effects of 8 hours of UVB irradiation on colour degradation for different concentrations of PLA-curcumin

UV-B Degradation percentage (%)			
CIE L*a*b*	Curcumin concentration (g/L)		
	5	10	20
s	98.8	95.3	5.2
b*	98.9	94.8	8.6
ΔE^*_{Lab}	> 5.0		
ΔE^*_{Lab} value	Indication		
< 1.0	No colour difference is visible		
< 2.0	colour difference is only slightly visible		
< 3.5	noticeable colour difference		
< 5.0	clear difference in colour is noticed		
> 5.0	indicate clearly two different colours		

Table 6.8 PLA-curcumin's CIELAB colour based values after 8 hours of UVB exposure

0 HOUR OF EXPOSURE			8 HOURS OF EXPOSURE		
5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
					
b*	b*	b*	b*	b*	b*
58.13	77.38	69.55	0.66	4.03	74.88

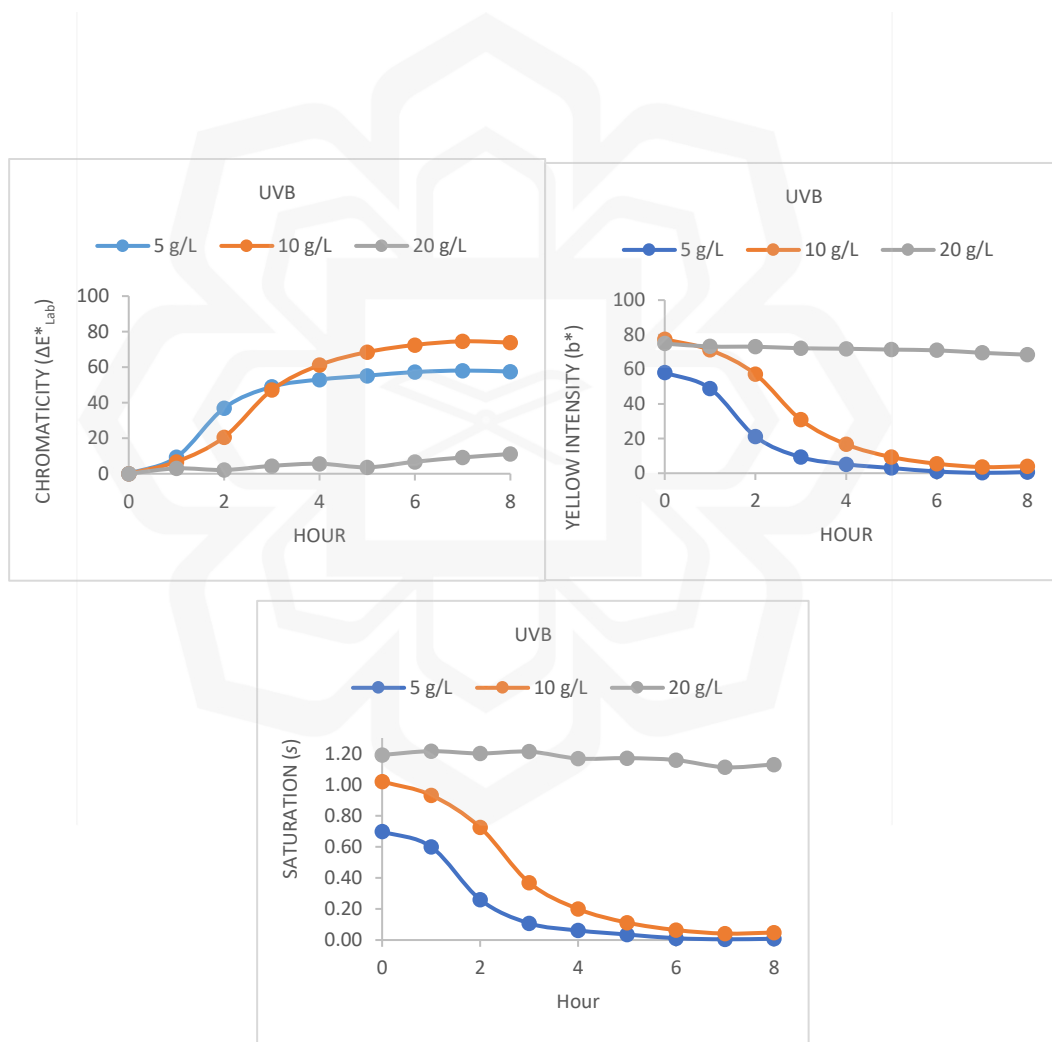


Figure 6.9 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PLA-curcumin within 8 hours of UVB treatment

6.2.5 Effect of Heating

Colour stability is presented by the chromaticity and colour saturation graphs of PLA-curcumin pigment, which are incubated in various temperatures, for 10, 20, 30, 40, 50, 60, and 70 °C in three hours of exposure as shown in Figure 6.10. The highest PLA-curcumin concentration (20 g/L) showed the highest *s* and *b** degradation percentage of 28.7% and 30.3% at 20 °C, followed by heating at 60 °C (*s*-19.3% and *b** 42.9%). Besides, the medium PLA-curcumin concentration (10 g/L) estimated the lowest *s* and *b** degradation percentage of 0.3% and 6.9% at 50 °C (Table 6.9). Chromaticity for PLA-curcumin pigment after treated under different temperatures, established that, ΔE^* value > 5.0 for all the different temperatures tested for 20 g/L of PLA-curcumin concentration. At this concentration, the colour was changed to darker yellowish brown with the increase of temperature (Table 6.10).

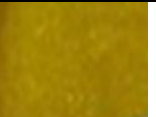
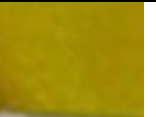
A previous study has proven that curcumin can degrade due to heat processing, in addition to the effects of light exposure (Chen et al., 2014). Therefore, it depends on the temperature, which may have an impact on how the gas passes through the material. The rate at which food respiration or material permeability increases or decreases at a given temperature relies on the polymeric layer that is submerged at that temperature. In other words, it is imperative to comprehend the fundamentals of PLAs' gas barrier performance under various temperature tests when storing food. It is said that food packaging serves as a material that comes into direct touch with food and that over 6500 chemicals are utilised in the production of products that encounter food (Muncke, 2016). Everything relies on the materials used, which allow pollutants to migrate into food and harm or endanger consumers (Muncke, 2016; Geueke et al., 2014; Muncke, 2014b). They are all different sorts of materials. According to research conducted by Imam et al. (2012), Scarfato et al. (2015), Dahman (2014), Darie et al. (2016), and Panseri et al. (2018), the Food and Drug Administration (FDA) has approved the use of LA monomer as a food ingredient.

The natural pigments' antioxidant properties are what lead to the increased oxidation temperature. Greater stability and enhanced resistance to oxidation can be achieved by PLA materials including food colouring at higher oxidation temperatures. The dyes impart on the polymers a stunning and consistent colour. Bright yellow colour was imparted to PLA polymer by curcumin. PLA yellow samples were seen to have discoloured visually. This indicates that PLA is a green polymer of interest with low toxicity as a bonus. Given the minimal migration of LA from PLA packaging containers to food, PLA are safe to use as food packaging materials. Because of the way PLA monomers are employed and arranged, PLA has a greater temperature tolerance, making it a suitable material for packing hot food. PLA is a semi-crystalline or amorphous polymer with a glass transition temperature (T_g) of about 55 °C and a melting temperature (TMT) of 180 °C, respectively. The mechanical and physical qualities were enhanced using natural colourants. Consequently, a range of packaging applications can make use of these biodegradable packaging materials.

Table 6.9 Effects of 3 hours exposure to different temperatures on PLA-curcumin colour degradation

Degradation percentage (%)				
		Curcumin concentration (g/L)		
CIE L*a*b*	°C	5	10	20
Colour saturation (s)	10	0.7	2.8	2.1
	20	2.9	8.6	8.7
	30	8.4	4.1	9.4
	40	1.7	6.5	7.8
	50	5.7	0.3	10.2
	60	8.7	6.1	19.3
	70	2.0	5.3	10.9
Yellow intensity (b*)	10	4.7	6.9	6.8
	20	4.2	7.7	30.3
	30	5.2	16.2	6.6
	40	8.9	13.3	16.0
	50	8.9	6.9	20.5
	60	11.7	9.9	42.9
	70	12.7	23.1	32.6
ΔE^*	10	> 5.0	< 5.0	> 5.0
	20	< 5.0	> 5.0	> 5.0
	30	< 5.0	> 5.0	> 5.0
	40	> 5.0	> 5.0	> 5.0
	50	> 5.0	> 5.0	> 5.0
	60	> 5.0	> 5.0	> 5.0
	70	> 5.0	> 5.0	> 5.0
ΔE^* value	Indication			
< 1.0	No colour difference is visible			
< 2.0	colour difference is only slightly visible			
< 3.5	noticeable colour difference			
< 5.0	clear difference in colour is noticed			
> 5.0	indicate clearly two different colours			

Table 6.10 Effects of temperatures exposure for 4 weeks on colour degradation of PLA-curcumin

TEMP	0 HOUR OF EXPOSURE			3 HOURS OF EXPOSURE		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
10 °C	 b* 74.23	 b* 73.44	 b* 88.88	 b* 70.73	 b* 77.17	 b* 82.82
20 °C	 b* 74.23	 b* 73.44	 b* 88.88	 b* 71.08	 b* 77.30	 b* 61.98
30 °C	 b* 74.23	 b* 73.44	 b* 88.88	 b* 77.50	 b* 84.16	 b* 83.06
40 °C	 b* 74.23	 b* 73.44	 b* 88.88	 b* 67.66	 b* 76.55	 b* 74.69
50 °C	 b* 74.23	 b* 73.44	 b* 88.88	 b* 67.60	 b* 78.88	 b* 70.67
60 °C	 b* 74.23	 b* 73.44	 b* 88.88	 b* 64.79	 b* 66.15	 b* 50.79
70 °C	 b* 74.23	 b* 73.44	 b* 88.88	 b* 64.79	 b* 56.50	 b* 59.86

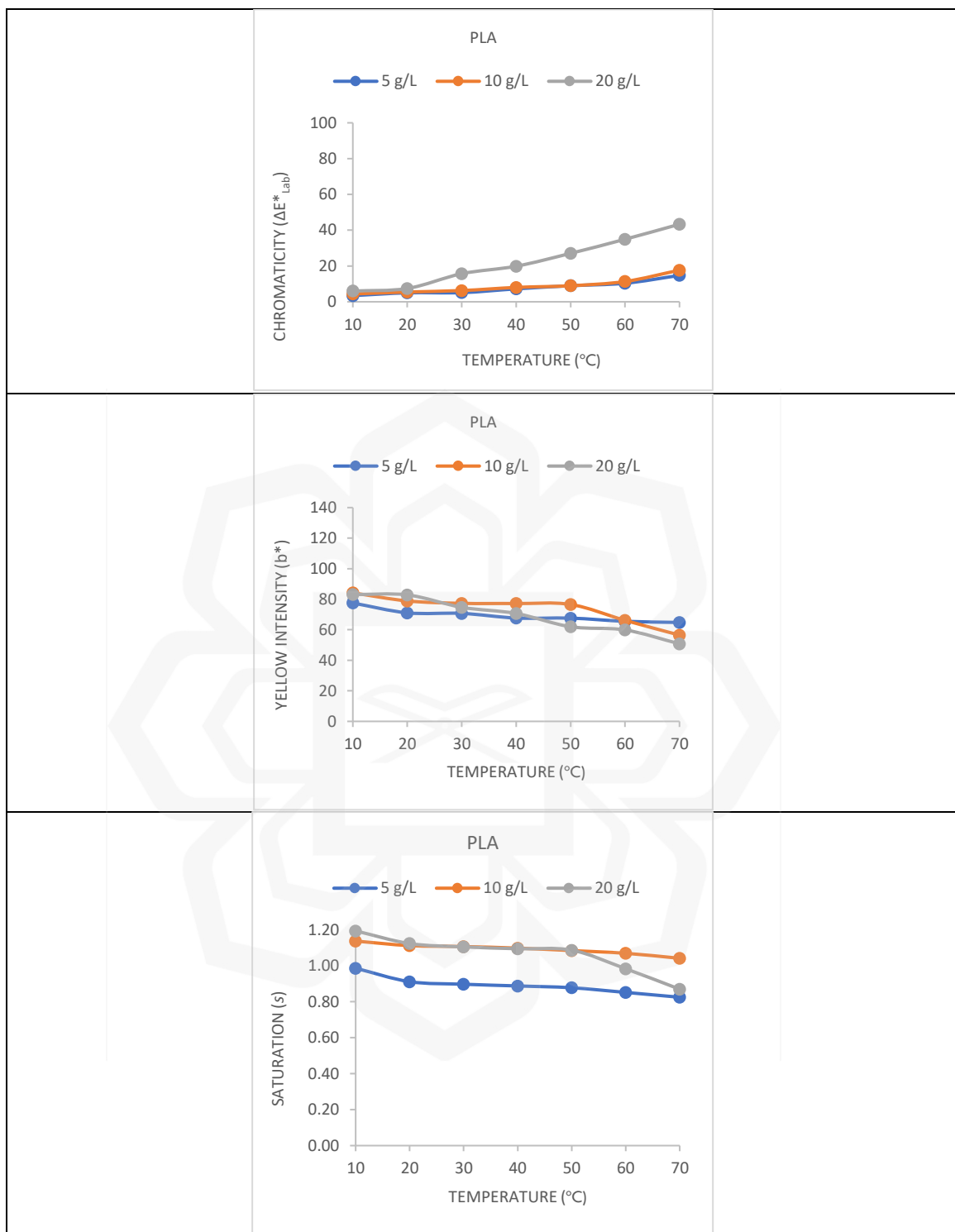


Figure 6.10 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PLA-curcumin when treated at different temperature for 3 hours

6.3 MULTIVARIATE DATA ANALYSIS

6.3.1 Sampling Adequacy for Multivariate analysis

The results from 10 variables were analysed using multivariate data analysis to determine the significant variables contributing to PLA-curcumin chromaticity stability. Data transformation is a component of the initial preparation of data prior to statistical analysis. The entire data set was normalised through the use of variable transformation. Figure 6.11 revealed the box and whisker plot to show the comparison in distributions of variables graphically to identify outliers. Outliers detected at L, a, and H in this boxplot signify unusual values representing the overall data distribution. In this plot, the outliers remained as the data is significant to show the highest values for each variable. The median score of H (hue) is the highest, and s (saturation) is the lowest.

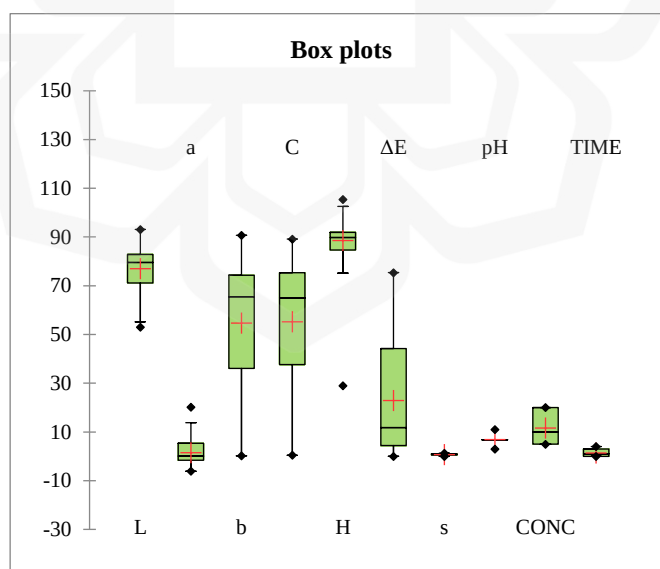


Figure 6.11 Box-and-whisker plots of PLA-curcumin pigment chromaticity stability variables at different concentrations and time of exposure.

Sampling adequacy for PLA-curcumin pigment chromaticity stability were tested by using Kaiser-Meyer-Olkin (KMO). For the KMO test, the total KMO value is 0.717, which showed the data set is sufficient for multivariate analysis. It means that, the data set that employed in this PCA are enough for those purposes of PCA. As for the Bartlett's sphericity test, the p-value is <0.0001, and the alpha value is 0.05 which indicate the p-value is lower than 0.05, then it should be reject the H_0 , and accept H_a (Ismail et al., 2021b). The p-value for the PLA-curcumin pigment chromaticity stability data exhibited highly significant differences at 0.0001 (Table 6.11).

Table 6.11 Kaiser-Meyer-Olkin and Bartlett's test for PLA-curcumin pigment chromaticity stability

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.717
Bartlett's Test of Sphericity	Chi-square (Observed value)	5700.07
	DF	45
	p-value (Two-tailed)	< 0.0001

6.3.2 Correlation Coefficient Matrix (r^2)

Table 6.12 and 6.13 showed a positive and negative correlations strength between two variables among PLA-curcumin pigment chromaticity stability test. A very strong positive correlation was observed among C versus b^* ($r^2 = 0.988$), s versus b^* ($r^2 = 0.957$), s versus C ($r^2 = 0.967$) and CONC versus a ($r^2 = 0.809$). In addition, the tables also showed the relationship between groups of ΔE versus b^* ($r^2 = -0.948$), ΔE versus C ($r^2 = -0.940$) that are produced a strong significant negative correlation. Other than that, a high negative correlation was showed among the groups of a^* versus L ($r^2 = -0.705$), H versus a^* ($r^2 = -0.711$) and s versus ΔE ($r^2 = -0.883$). In general, the PLA-curcumin pigment chromaticity

stability variables can be grouped into four clusters, which justify with two cluster with positive correlations and another two clusters with negative correlations.

Table 6.12 Correlation coefficient matrix for the PLA-curcumin pigment chromaticity stability

Variables	L	a	b	C	H	ΔE	s	pH	CONC	TIME
L	1									
a	-0.705	1								
b	-0.337	0.238	1							
C	-0.333	0.228	0.988	1						
H	0.460	-0.711	-0.074	-0.069	1					
ΔE	0.222	-0.072	-0.948	-0.940	-0.064	1				
s	-0.549	0.408	0.957	0.967	-0.196	-0.883	1			
pH	0.033	-0.001	-0.061	-0.060	0.031	0.037	-0.057	1		
CONC	-0.698	0.809	0.270	0.268	-0.521	-0.094	0.425	0.000	1	
TIME	-0.197	-0.099	-0.470	-0.488	0.024	0.476	-0.400	0.017	0.000	1

Values in bold are different from 0 with a significance level $\alpha=0.01$

Table 6.13 evaluated the correlation coefficient (r^2) or the strength of the correlation between two variables among PLA-curcumin pigment chromaticity stability test. The correlation coefficient is measured on a scale from -1 to +1 as described by Guilford (1973).

Table 6.13 Correlation coefficient (r^2) strength between two variables among PLA-curcumin pigment chromaticity stability test

Variables	r^2	Size of Correlation	Interpretation
b x C b x s s x C	0.988 0.957 0.967	0.90 to 1.00	Very high positive correlation
Conc x a	0.809	0.70 to 0.90	High positive correlation
-	-	0.50 to 0.70	Moderate positive correlation
t x ΔE H x L Conc x s s x a	0.476 0.460 0.425 0.40	0.30 to 0.50	Low positive correlation
ΔE x b ΔE x C s x ΔE H x a a x L	-0.948 -0.940 -0.883 -0.711 -0.705	-0.70 to -0.90	High negative correlation
Conc x L s x L Conc x H	-0.698 -0.549 -0.521	-0.50 to -0.70	Moderate negative correlation
t x C t x b t x s b x L C x L	-0.488 -0.470 -0.400 -0.337 -0.333	-0.30 to -0.50	Low negative correlation
Conc x b Conc x C b x a C x a ΔE x L pH x ΔE pH x L pH x H t x H pH x t pH x Conc. t x Conc. t x L s x H t x a Conc x H H x b ΔE x a H x C ΔE x H pH x b pH x C pH x a	0.270 0.268 0.238 0.228 0.222 0.037 0.033 0.031 0.024 0.017 0.000 0.000 -0.197 -0.196 -0.099 -0.094 -0.074 -0.072 -0.069 -0.064 -0.061 -0.060 -0.001	0.00 to 0.30 (0.00 to -0.30)	Little if any correlation
Values in bold are different from 0 with a significance level $\alpha=0.01$			

6.3.3 Principal Component Analysis (PCA)

The PCA exposed two principal components (PCs) entailing 10 variables that represent cumulative variability of 72.28%. The eigenvalues (EV) were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 4.642, and for PC2 was 2.586 as showed in Table 6.14. In principle, variables far away from the axes F1 and F2 had a strong factor loading (FL). Referring to the Table 6.14, b^* , C^* , ΔE and s had strong FL ($FL > 0.75$) whereas L^* , a^* , and concentration had moderate FL ($0.500 < FL < 0.749$) and H and pH had a weak FL ($FL < 0.499$).

Table 6.14 Principal components factor loading and eigenvalues of the correlation matrix for PLA-curcumin pigment chromaticity stability

Variables	PC1	PC2
L	-0.603	-0.611
a	0.564	0.740
b	0.920	-0.353
C	0.920	-0.361
H	-0.344	-0.697
ΔE	-0.828	0.504
s	0.974	-0.150
pH	-0.065	0.022
Concentration	0.561	0.684
Time	-0.442	0.424
Eigenvalue	4.642	2.586
Variability (%)	46.422	25.860
Cumulative (%)	46.422	72.282

The first principal PC1 accounted for 46.42% of the total variance, which comprised strong factor loadings of b , C , ΔE and s whereas for PC2, no strong variables were identified, comprising of 25.86% of the total variance for the data. The PCA analysis allows for identifying factors and provides insight in PLA-curcumin pigment stability. Therefore, factor loadings can be utilised to determine the strength of the association between variables in PLA-curcumin pigment chromaticity stability test. The degree of correspondence or their potential between the variables can be further explained in Figure 6.12.

Figure 6.12 summarised that all variables were either positively or negatively correlated to each other which can be grouped into four groups. Group 1 consist of L and H , group 2 with C , b , and s , group 3 is concentration and a , while group 4 is ΔE , time and pH. All variables were positively correlated as long as they were grouped close together such as group 2 and group 3 variables. Group 1 and group 3 as well as group 2 and group 4 were negatively correlated as there were at the opposite positioned. The others were no correlation. The biplot in Figure 6.12 exhibited three clusters, which at the right F1 axes represent the cluster of PLA-curcumin samples with highest concentration (20 g/L) that maintained the strongest colour after treatment in dark condition. While the clusters at the middle bottom of F1 axes represent cluster of PLA-curcumin samples at 5 g/L and 10 g/L concentration which still maintained or have minimum colour degradation after treatment also in dark condition. The third cluster positioned at the left side of F1 axes represent all the PLA-curcumin samples that loss the original colour or the highest colour degradation after treatment under light condition. The clusters positioned within a^* and concentration which is the PLA-curcumin at 20 g/L were confirmed to be the most potential concentration for carotenoid pigment stability test. It can be concluded that the higher PLA-curcumin concentrations, time and light were necessary correlate with the curcumin chromaticity stability.

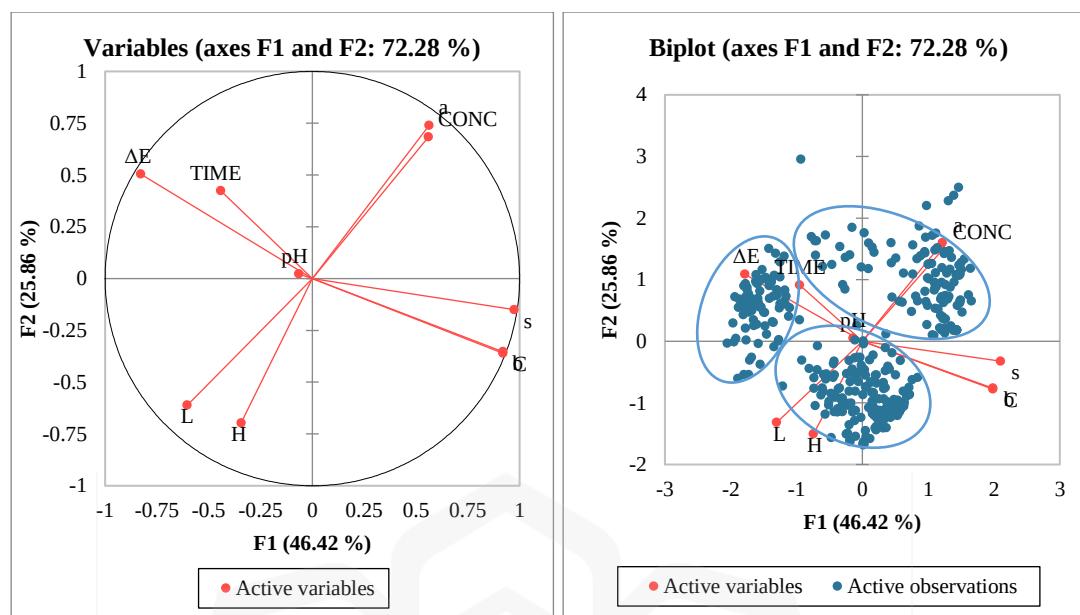


Figure 6.12 Variables plot and correlation biplot for PLA-curcumin pigment stability test variables

6.4 SUMMARY OF THE RESULTS

Several intrinsic and extrinsic factors influencing the curcumin-PLA stabilities have been investigated in this study, namely, salinity, pH, light vs dark (throughout four weeks), heat, and UV irradiation. From the results, it can be deduced that:

1. In 10 g/L of curcumin concentration, the highest degradation occurred under light conditions, whereas in dark conditions, the least degradation occurred in 5 g/L of curcumin concentration.
2. The highest degradation was at pH 9 in 10 and 20 g/L of curcumin concentrations, whereas no degradation from colour saturation (s) was shown at pH 3 in 10 g/L of curcumin concentration.
3. The highest degradation at 30 g/L of NaCl in 5 g/L of curcumin concentration was shown under light conditions.

4. Chitosan-Curcumin showed the highest degradation in 5 g/L of curcumin concentration, whereas for the heat treatment, the least degradation occurred in 5 g/L of curcumin concentration at 10 °C.
5. Light presence is the main factor that considerably effects polymer colour fluctuations and stability, according to experiments on salinity, pH, and light exposure stability. Photodegradation of the coating was induced by the curcumin-PLA's low resistance to light exposure.
6. Previous research supports the findings that degradation is more significant in the presence of light, emphasising the role of light in accelerating oxidation processes and affecting the visual attractiveness of packaged products. In conclusion, the results underscore the importance of considering light exposure in assessing the stability of curcumin pigments and their applications in various products. Mitigating light exposure can help preserve the colour stability and visual appeal of curcumin-containing materials over time.

CHAPTER SEVEN

CHROMATICITY STABILITY OF PVA-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS

7.1 INTRODUCTION

Poly(vinyl) alcohol (PVA) is known as synthetic biomaterial which is have their characteristics as non-toxic, biocompatible, water soluble, and biodegradable with excellent mechanical properties. The physical characteristics and its specific functional can be used as it is dependable on the degree of polymerization and the degree of hydrolysis. The main raw material used in the manufacture of poly(vinyl) alcohol is the vinyl acetate monomer. PVA can be explained through the polymerization process, and the polymer itself is built on a carbon-chain backbone, with a hydroxyl group (-OH) which is on every other carbon (Figure 7.1). The physicochemical properties of polymers are largely determined by the type of physical bonds between polymer chains and the chemical bonds along them. PVA is widely used in industry due to its high water absorption capability. The abundance of hydroxyl (-OH) groups within the polymer structure enables the formation of hydrogen bonds, making poly(vinyl alcohol) highly soluble in water. For this reason, PVA is specified by strong hydrophilic and hydrogen bonding character. Other than that, PVA is characterized by its biodegradability and possesses unique properties, such as excellent film-forming ability, adhesive properties, and emulsifying capabilities. PVA is classified as nontoxic, odourless, and a good resistant to oil and grease.

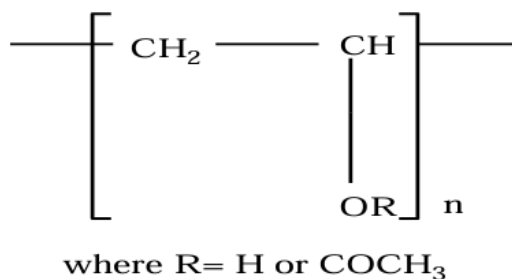


Figure 7.1 Chemical structure illustration pair

7.2 RESULTS AND DISCUSSIONS

7.2.1 Effect of light, pigment concentration and time of exposure on PVA-curcumin stability

The study of chromatic stability and the aspects of degradation on PVA-curcumin is aimed to observe the effects of four weathering factors which are sodium chloride (seawater salinity), pH (acidity and alkalinity), sunlight exposure (UV rays), and heat (temperature) towards this integration of this material as potential coating material. The CIE L*a*b* colour difference (ΔE^*_{LAB}) is used to assessed the stabilisation of PVA-curcumin coating as described by Mokrzyck and Tatol (2011).

Chromaticity measurements of PVA-curcumin pigment from 5 to 20 g/L, and the exposing time under light and dark treatment from week 0 until week 4 were recorded (Figure 7.2). It can be demonstrated that, the highest degradation percentage of s (14.3%) and b* (52%) occurred in response to light condition in 10 g/L of curcumin concentration as compared to 5 and 20 g/L of curcumin concentrations. In contrast to the dark condition, the 10 g/L curcumin concentration showed the lowest degradation percentages for s (6.6%)

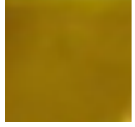
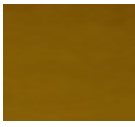
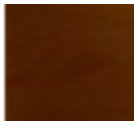
and b (11.1%) (Table 7.1). Based on these findings, curcumin is predicted to have strong photodynamic efficacy over the observed time range and has good photostability in the dark as compared to the exposure to light. Overall, the chromaticity established that ΔE^* value >5.0 for all the three concentrations that were in response to light and dark condition, that were indicated clearly visible of two different colours. Table 7.2 shows the different colour of PVA-curcumin under light and dark conditions.

Curcumin is a photosensitive substance that produces reactive oxygen species (ROS) primarily consisting of singlet oxygen species via the type II photosensitive reaction mechanism. This process allows curcumin to exert its photodynamic impact (Laneri et al., 2023a; Wang et al., 2023a; Liu et al., 2023). It has been documented that curcumin's photodynamic action can efficiently manage microbial contamination in freshwater and agricultural products (Roy et al., 2022). This study uses absorption and fluorescence spectroscopy to examine the photochemical breakdown of curcumin. It was discovered that under fluorescent light, curcumin undergoes considerable degradation, which lowers the intensity of its fluorescence. The work emphasises that fluorescence lifetime measurements can be used to track the breakdown of curcumin molecules as part of the degradation process (Kotha and Luthria, 2019a). Curcumin often shows good photostability when exposed to dark environments. It does not considerably deteriorate in the absence of light, according to this. Research has indicated that curcumin does not suffer photodegradation when exposed to light; instead, it is stable and maintains its chemical structure when stored in the dark (Kotha and Luthria, 2019b).

Table 7.1 Effects of 4 weeks exposure to light and dark conditions on PVA-curcumin colour degradation

Degradation percentage (%)						
CIE L*a*b*	Condition	Curcumin concentration (g/L)				
		5	10	20		
Colour saturation (s)	Light	13.5	14.3	12.2		
	Dark	32.4	6.6	8.7		
Yellow intensity (b*)	Light	29.2	52.0	31.7		
	Dark	28.9	11.1	77.2		
Chromaticity (ΔE^*_{Lab})	Light	> 5.0				
	Dark	> 5.0				
ΔE^*_{Lab} value	Indication					
< 1.0	No colour difference is visible					
< 2.0	colour difference is only slightly visible					
< 3.5	noticeable colour difference					
< 5.0	clear difference in colour is noticed					
> 5.0	indicate clearly two different colours					

Table 7.2 Effects of exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin

	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0						
	b* 78.63	b* 56.95	b* 22.30	b* 72.77	b* 52.85	b* 28.18
WEEK 4						
	b* 62.01	b* 47.14	b* 38.62	b* 64.67	b* 42.74	b* 16.43

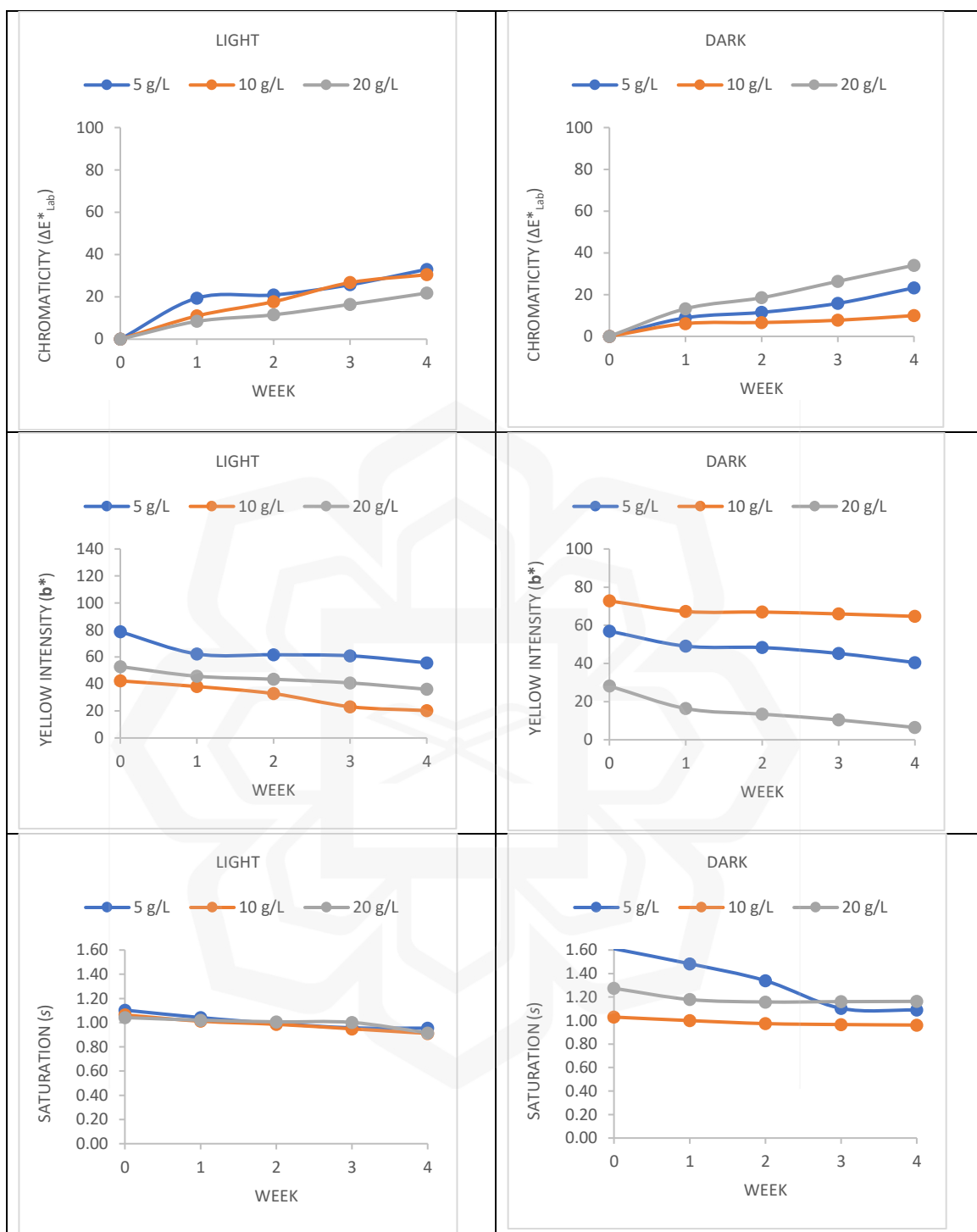


Figure 7.2 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PVA-curcumin in light and dark condition for different exposure time

7.2.2 Effect of NaCl

The results for chromaticity (ΔE^* Lab), color saturation (s), and yellow intensity (b) values for various concentrations of PVA-curcumin treated with NaCl concentrations (5, 10, 20, and 30 g/L) under light and dark conditions over 4 weeks are presented in Figures 7.3 to 7.5. The chromaticity of PVA-curcumin, after treatment with 5, 10, 20, and 30 g/L NaCl, showed a ΔE^* value greater than 5.0 for all three curcumin concentrations in response to both light and dark conditions after 4 weeks. At NaCl concentrations from 5 g/L to 20 g/L, the saturation (s) decreased from 36.9% to 21.4%, 23.6% to 11.4%, 21% to 6.8%, and 36.8% to 7.4% when exposed to light. In contrast, under dark conditions, the saturation (s) values for PVA-curcumin at 5 g/L to 30 g/L NaCl concentrations were reduced from 38% to 12.1%, 27% to 23.8%, 35.3% to 14.5%, and 48.5% to 8.4%, respectively (Table 7.3). The highest degradation of yellow intensity (b^*) occurred at 5 g/L NaCl, with degradation ranging from 47.9% to 51.6% at NaCl concentrations between 5 g/L and 20 g/L under light exposure. Under dark conditions, the highest degradation was observed from 48.1% to 67.9% at a 30 g/L NaCl concentration. It is evident that the degradation percentage for yellow intensity (b^*) was higher under dark conditions, particularly at the highest PVA-curcumin pigment concentration and the highest salinity concentration. Table 7.4 illustrates the different colors of PVA-curcumin before and after 4 weeks of treatment with varying NaCl concentrations under light and dark conditions.

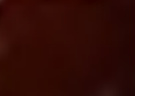
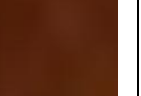
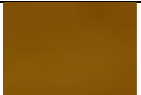
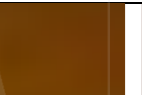
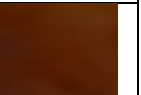
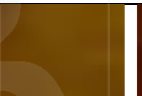
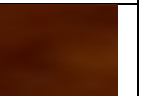
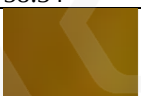
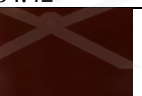
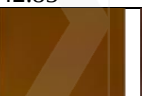
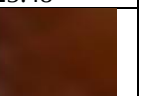
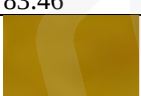
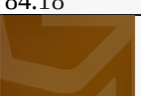
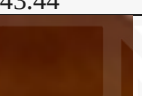
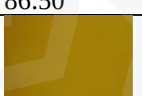
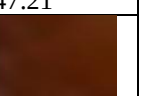
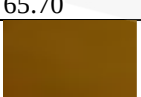
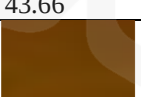
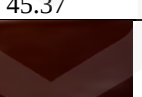
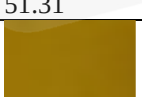
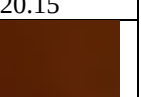
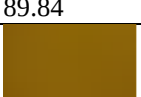
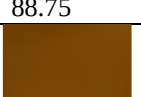
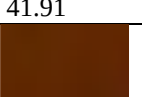
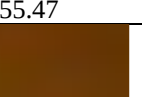
When curcumin is encapsulated in polyvinyl alcohol (PVA), salinity, or the presence of salts in a solution, can affect the solubility and stability of the compound. Elevated salt levels have the potential to modify the ionic strength of the solution, hence impacting the interplay between PVA and curcumin. This could result in precipitation or decreased stability (Bar-Sela et al., 2010; Yallapu et al., 2012). It demonstrated the curcumin film's potential as a useful sensor for the real-time observation of food degradation (Ma et al., 2017a). Active and smart packaging films have been manufactured using curcumin's functional characteristics. On polymer-based films containing curcumin, several reports

have already been published. This is accomplished by using a variety of polymers, including commercial plastics, biopolymers, and bioplastics. Of these, biopolymers are garnering the most interest because of their exceptional functional characteristics, repeatability, and biodegradability (Rouf and Kokini, 2018b).

Table 7.3 Effects of 4 weeks exposure to NaCl, light and dark conditions on colour degradation of PVA-curcumin

		Degradation percentage (%)							
CIE L*a*b*	Curcumin concentration (g/L)	Light				Dark			
		NaCl concentration (g/L)							
		5	10	20	30	5	10	20	30
Colour saturation (s)	5	36.9	23.6	21.0	36.8	38.0	27.0	35.3	48.5
	10	31.1	48.8	32.0	27.8	30.7	38.5	27.6	9.8
	20	21.4	11.4	6.8	7.4	12.1	23.8	14.5	8.4
Yellow intensity (b*)	5	47.9	34.6	33.3	45.4	52.5	42.8	40.7	48.1
	10	56.3	55.7	41.1	43.6	53.1	55.6	59.2	54.9
	20	51.6	56.3	36.2	30.3	61.9	45.1	57.3	67.9
Chromaticity (ΔE^*_{Lab})	5	> 5.0							
	10								
	20								
ΔE^*_{Lab} value	Indication								
< 1.0	No colour difference is visible								
< 2.0	colour difference is only slightly visible								
< 3.5	noticeable colour difference								
< 5.0	clear difference in colour is noticed								
> 5.0	indicate clearly two different colours								

Table 7.4 Effects of NaCl exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin

WEEK	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0 5 mg/mL NaCl	 b* 88.29	 b* 83.43	 b* 49.07	 b* 84.99	 b* 97.79	 b* 36.02
WEEK 4 5 mg/mL NaCl	 b* 60.54	 b* 56.50	 b* 33.75	 b* 44.34	 b* 45.91	 b* 13.71
WEEK 0 10 mg/mL NaCl	 b* 89.18	 b* 89.28	 b* 32.95	 b* 82.64	 b* 96.58	 b* 46.45
WEEK 4 10 mg/mL NaCl	 b* 58.34	 b* 42.93	 b* 34.42	 b* 47.27	 b* 42.85	 b* 25.48
WEEK 0 20 mg/mL NaCl	 b* 83.46	 b* 84.18	 b* 43.44	 b* 86.50	 b* 82.19	 b* 47.21
WEEK 4 20 mg/mL NaCl	 b* 65.70	 b* 43.66	 b* 45.37	 b* 51.31	 b* 33.50	 b* 20.15
WEEK 0 30 mg/mL NaCl	 b* 89.84	 b* 88.75	 b* 41.91	 b* 86.66	 b* 55.47	 b* 49.13
WEEK 4 30 mg/mL NaCl	 b* 59.02	 b* 50.06	 b* 31.21	 b* 45.02	 b* 25.00	 b* 15.75

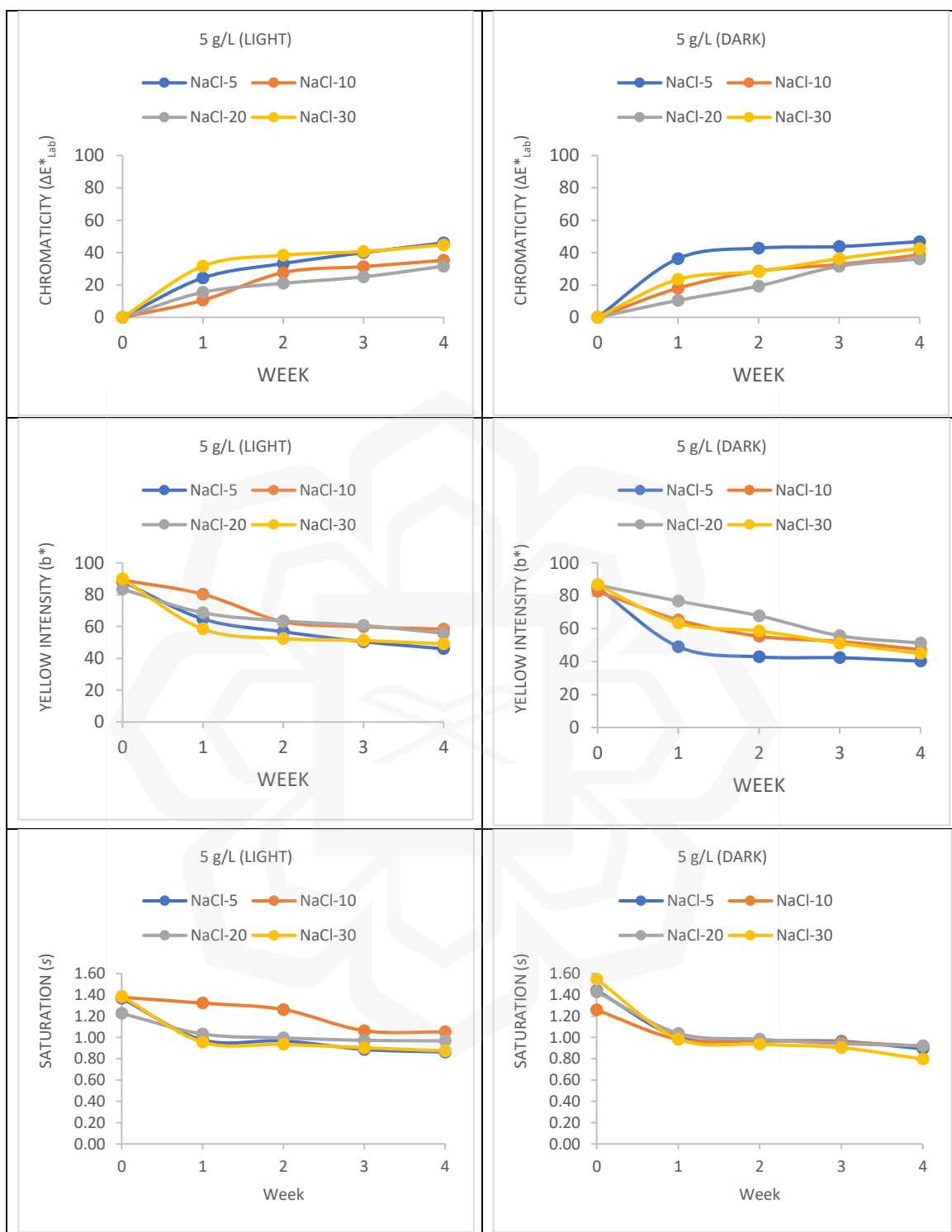


Figure 7.3 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L PVA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

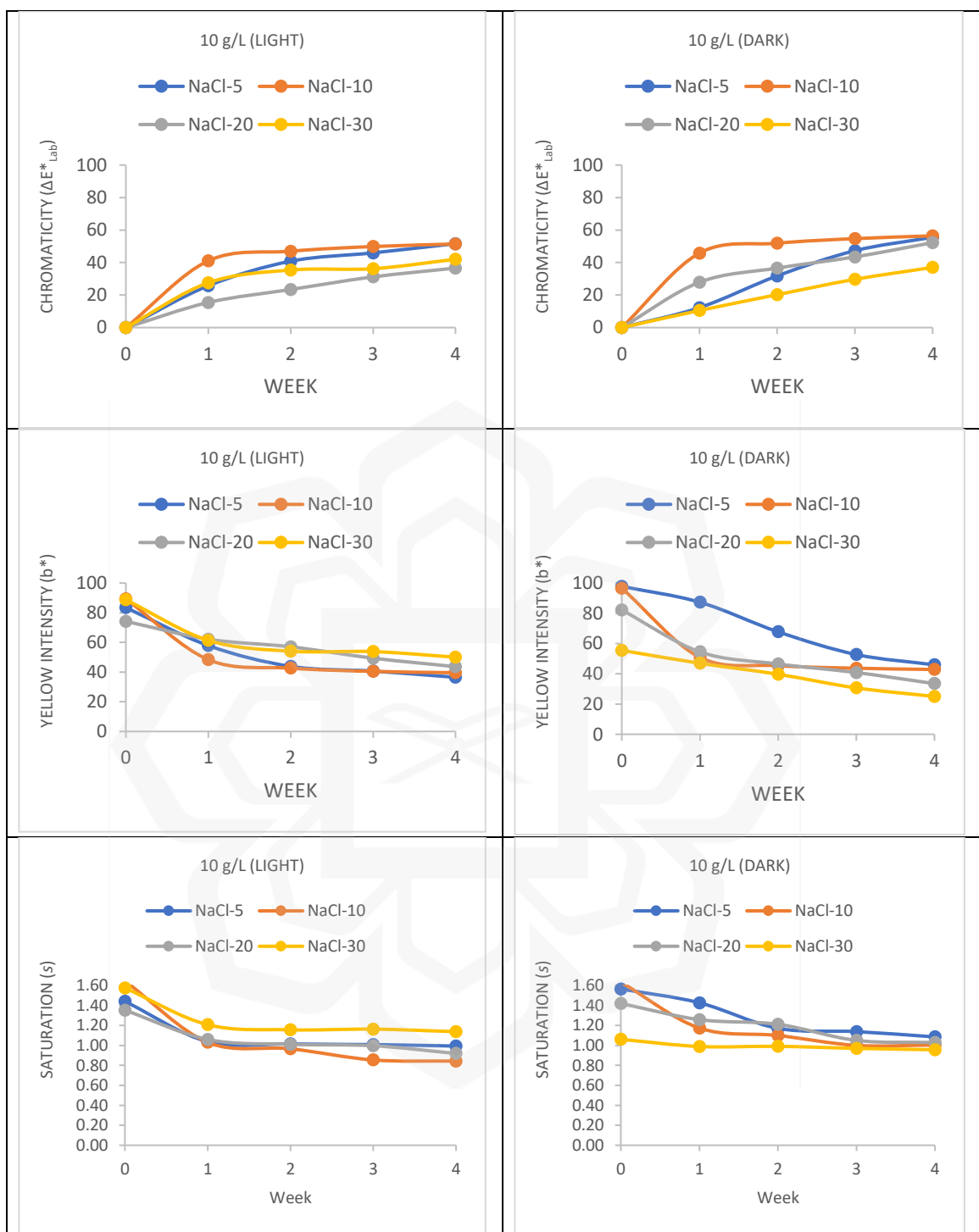


Figure 7.4 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PVA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

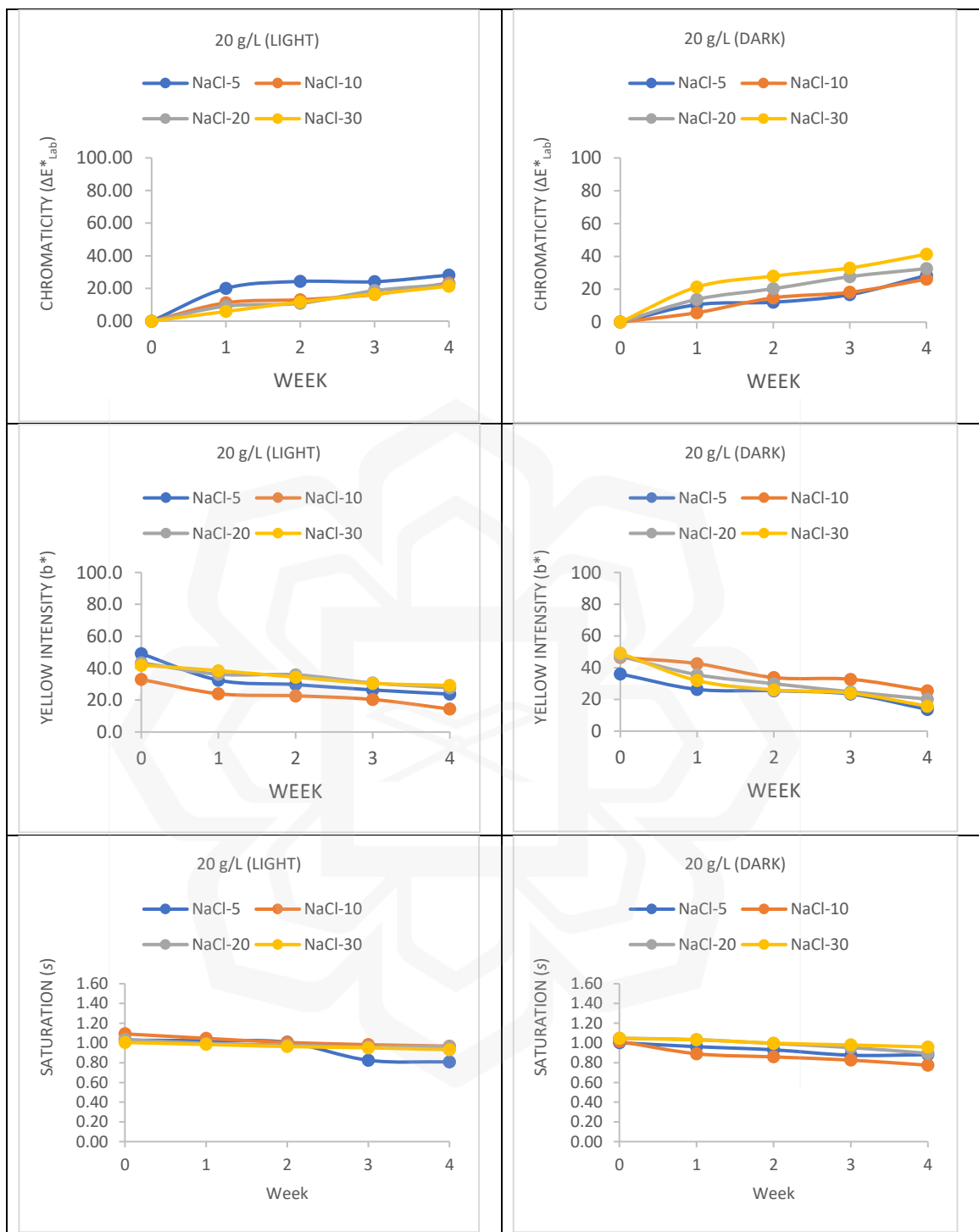


Figure 7.5 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PVA-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

7.2.3 Effect of pH

The investigation of PVA-curcumin stability at five different pH (3, 5, 7, 9, and 11) under light and dark condition for 4 weeks exposure time using the CIELab value is a method to confirm colour degradation and changes. Figure 7.6 to Figure 7.8 shows chromaticity (ΔE^*_{Lab}), colour saturation (s) and yellow intensity (b^*) values for different concentrations of PVA-curcumin treated with different pH. PVA-curcumin concentration of 20 g/L in response to light condition was observed to degrade at a faster rate with the degradation percentage of s (37.7%) and b^* (94.3%) at pH 11, followed by pH 9, with the degradation percentage of s (19.6%) and b^* (68.6%). In response to dark condition, it was identified that, the degradation percentage of s (46.2%) and b^* (82.6%) was the highest at pH 11 in PVA-curcumin concentration of 20 g/L (Table 7.5). From the analysis, it can be investigated that, the affected concentration is most likely in the highest concentration of PVA-Curcumin concentration (20 g/L). The results revealed that the chromaticity ΔE^* value was greater than 5.0 for all tested concentrations of PVA-curcumin pigment treated under both light and dark conditions. The most noticeable colour difference was observed in the PVA-curcumin pigment at 20 g/L, which exhibited the darkest reddish-brown color after 4 weeks of treatment at pH 11 (Table 7.6). When the pH was less than 8, the curcumin was yellow; at pH 8, it displayed a light-yellow colour; and at pH 9–11, it turned reddish brown. This is because curcumin undergoes reversible structural alteration at various pH values (Qianyun Ma et al., 2017). This explains why curcumin appears in the keto form in neutral and acidic environments, while the enol form appears at pH values higher than 8 (Esatbeyoglu et al., 2012).

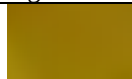
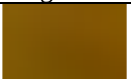
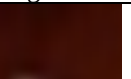
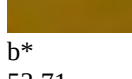
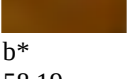
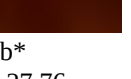
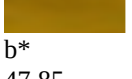
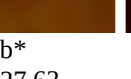
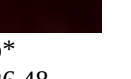
As a natural colourant, curcumin has demonstrated the ability to identify pH-sensitive indications. Curcumin's colour will shift from yellow to red due to a pH increase to 8, and then from red to brown due to a greater alkaline pH (Liu et al., 2018c). The type and concentration of the colourant will also affect the pH at which the colours shift. The hue of curcumin will shift from yellow to a brownish yellow (pH > 9). The observable

colour reaction and notable impact on the oxidation stability of packing material are indicative of the colour changes caused by food colouring agents. When added to biopolymer films such carrageenan, gelatine, polylactic acid, and gum/poly (vinyl alcohol) films, curcumin changes colour in response to pH (Aliabbasi, Fathi, and Emam-Djomeh, 2021b). According to Liu et al. (2018c), the phenoxide anion created when phenolic hydroxyl groups combine with hydroxyl ions is what causes curcumin to change colour under basic circumstances. This finding is consistent with other research showing curcumin to be extremely susceptible to chemical breakdown at physiological pH levels (Nimiya et al., 2016).

Table 7.5 Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin

		Degradation percentage (%)											
CIE L*a*b*	Curcumin concentration (g/L)		Light						Dark				
			pH										
		3	5	7	9	11	3	5	7	9	11		
Colour saturation (s)	5	10.4	7.2	11.2	10.1	5.3	12.5	5.7	4.4	8.1	9.3		
	10	17.6	8.2	8.0	16.7	13.1	8.4	2.7	15.6	8.5	8.3		
	20	6.2	8.6	5.1	19.6	37.7	18.5	6.9	11.7	9.1	46.2		
Yellow intensity (b*)	5	31.7	14.7	27.4	14.2	22.2	22.5	23.3	19.3	29.8	33.6		
	10	39.1	36.8	37.1	40.6	32.1	30.5	51.7	38.4	30.0	45.5		
	20	57.1	32.9	37.9	68.6	94.3	38.8	27.7	57.5	69.2	82.6		
Chromaticity (ΔE^*_{Lab})	5	> 5.0											
	10												
	20												
ΔE^*_{Lab} value	Indication												
< 1.0	No colour difference is visible												
< 2.0	colour difference is only slightly visible												
< 3.5	noticeable colour difference												
< 5.0	clear difference in colour is noticed												
> 5.0	indicate clearly two different colours												

Table 7.6 Effects of pH exposure for 4 weeks in light and dark condition on colour degradation of PVA-curcumin

WEEK	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0 pH 3	 b* 59.02	 b* 73.63	 b* 15.45	 b* 56.55	 b* 56.90	 b* 12.16
WEEK 4 pH 3	 b* 46.29	 b* 54.87	 b* 28.83	 b* 53.71	 b* 35.07	 b* 8.85
WEEK 0 pH 5	 b* 62.65	 b* 66.30	 b* 18.90	 b* 62.69	 b* 56.88	 b* 17.32
WEEK 4 pH 5	 b* 64.22	 b* 57.76	 b* 38.55	 b* 58.44	 b* 33.77	 b* 5.44
WEEK 0 pH 7	 b* 56.30	 b* 61.71	 b* 28.99	 b* 67.91	 b* 58.91	 b* 16.91
WEEK 4 pH 7	 b* 54.67	 b* 46.53	 b* 29.46	 b* 47.70	 b* 41.26	 b* 6.14
WEEK 0 pH 9	 b* 64.74	 b* 65.75	 b* 28.57	 b* 72.12	 b* 69.51	 b* 9.23
WEEK 4 pH 9	 b* 49.64	 b* 33.67	 b* 29.32	 b* 56.11	 b* 49.85	 b* 8.20
WEEK 0 pH 11	 b* 66.65	 b* 59.15	 b* 12.26	 b* 72.05	 b* 50.68	 b* 8.54
WEEK 4 pH 11	 b* 53.71	 b* 58.19	 b* 27.76	 b* 47.85	 b* 27.63	 b* 26.48

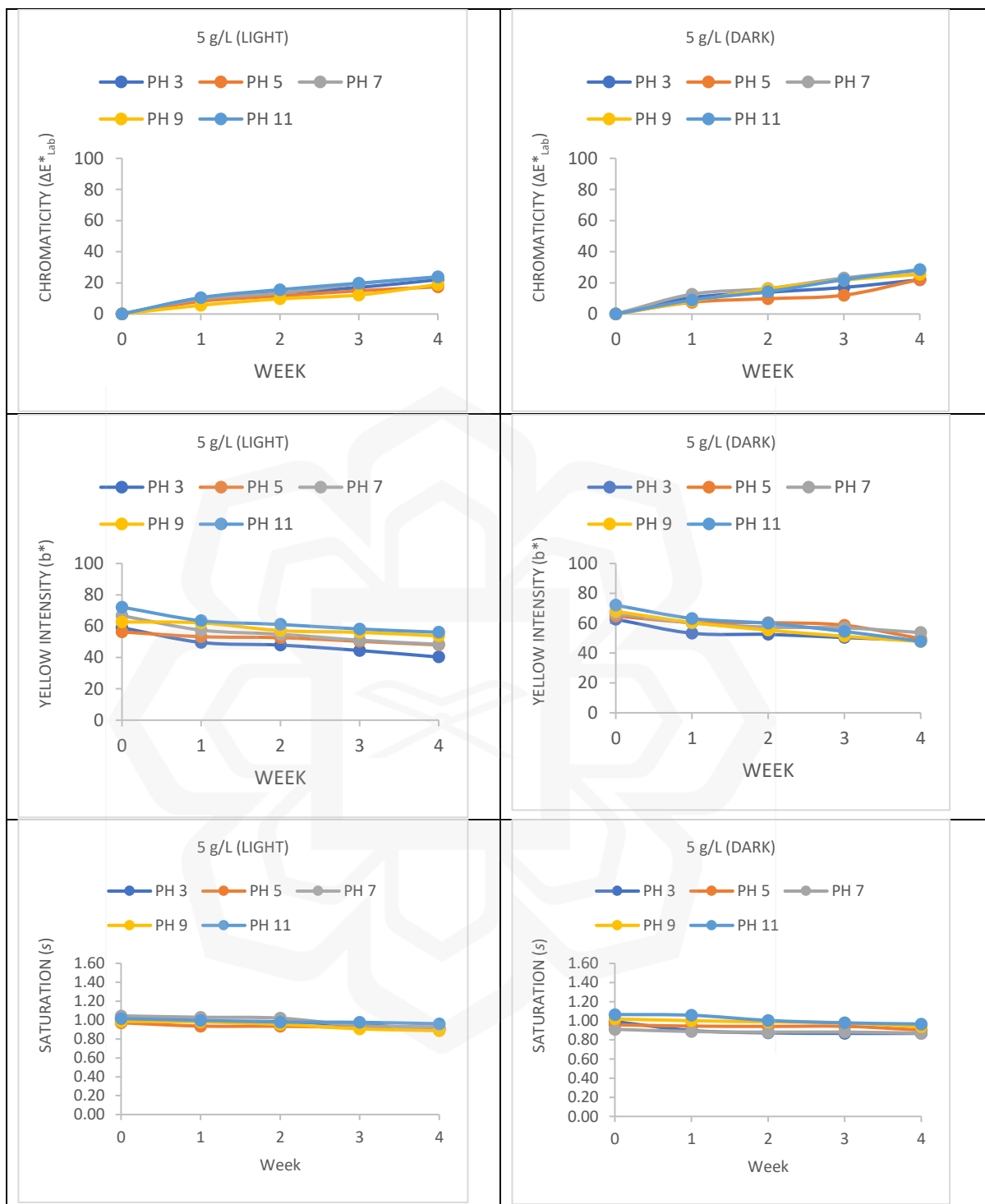


Figure 7.6 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L PVA-curcumin at different pH in light and dark condition for different exposure time

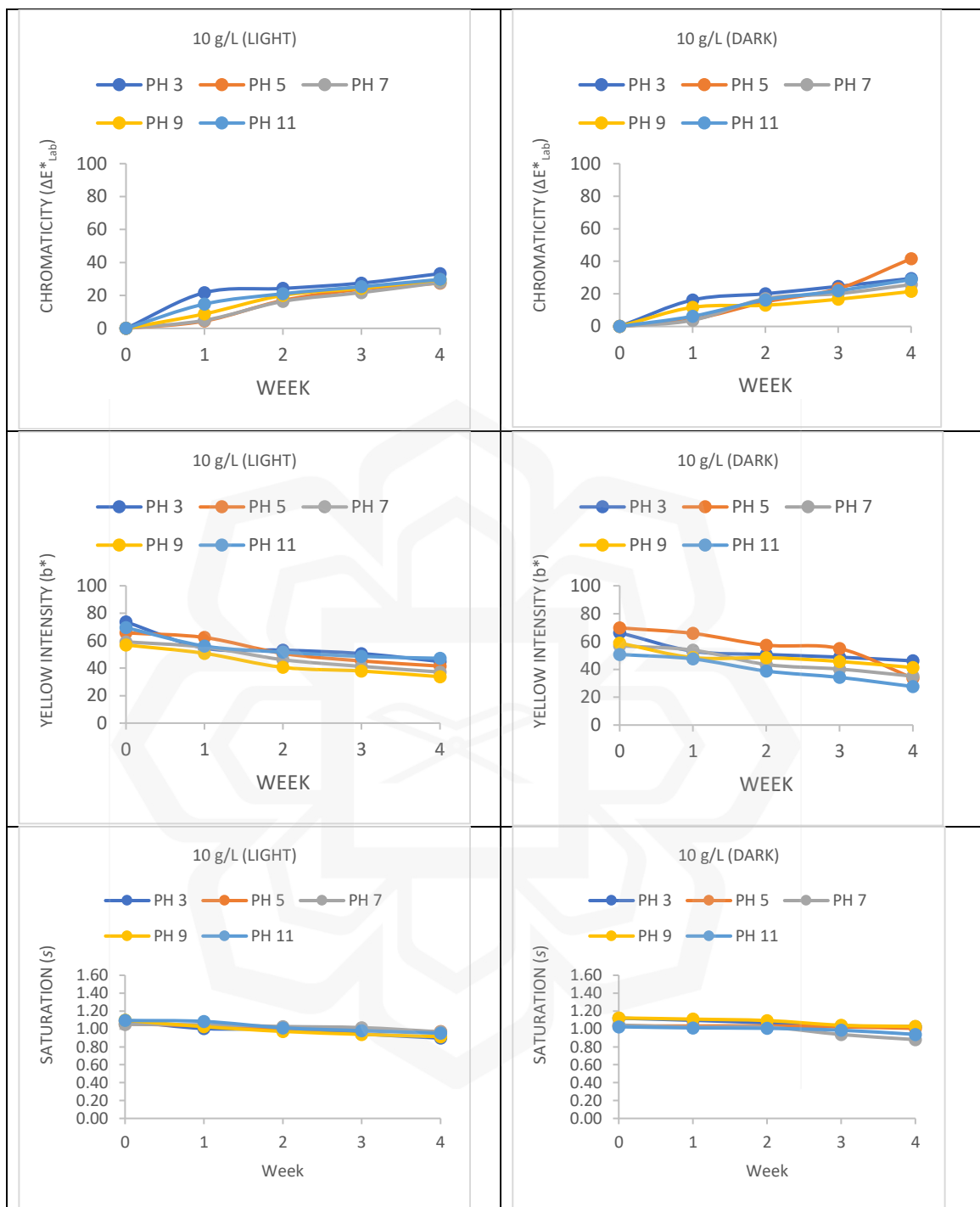


Figure 7.7 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L PVA-curcumin at different pH in light and dark condition for different exposure time

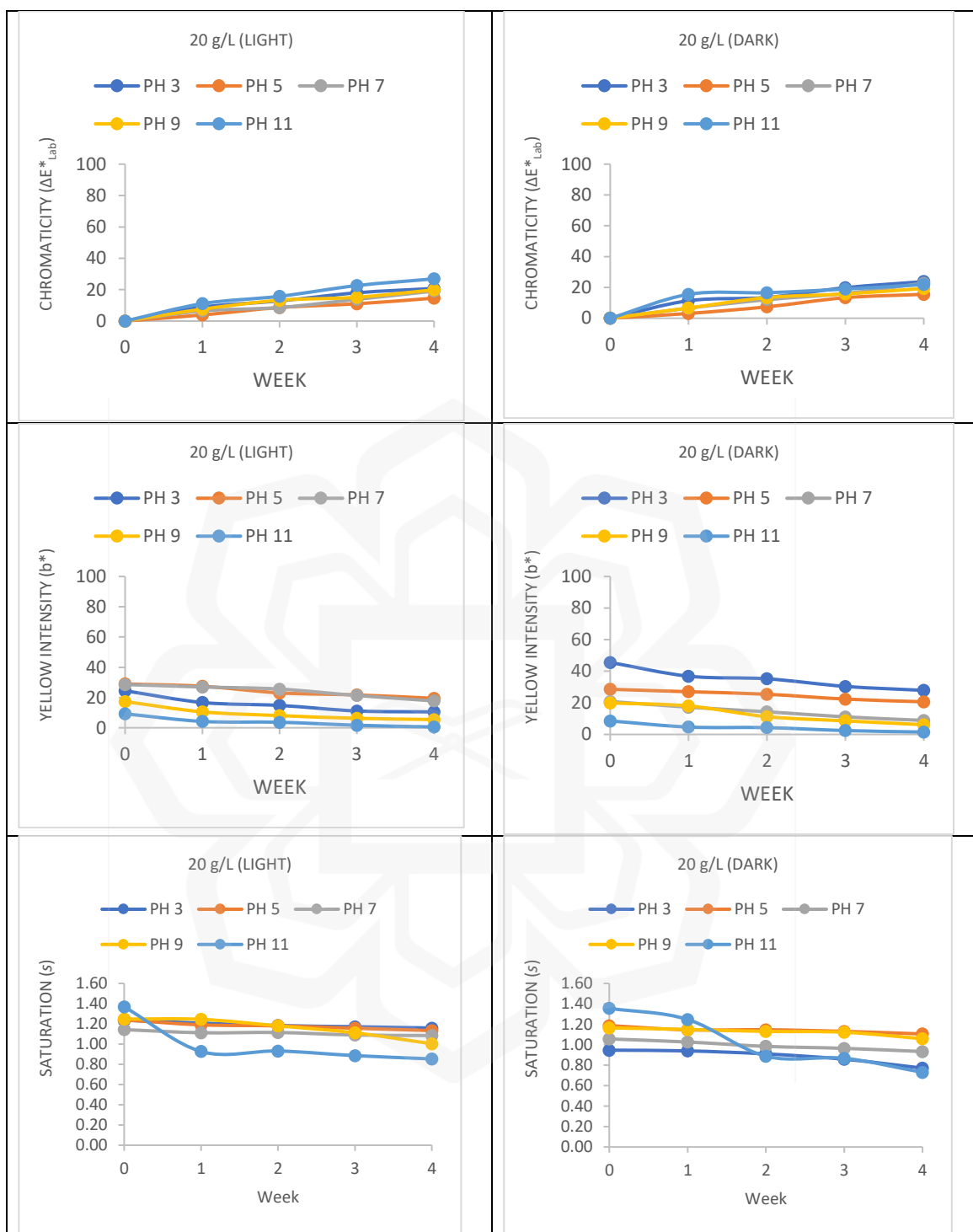


Figure 7.8 CIE L*a*b* color difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L PVA-curcumin at different pH in light and dark condition for different exposure time

7.2.4 Effect of UV Irradiation

Colour stability of curcumin pigment treated with UVB (312 nm) for eight hours are exhibited by the chromaticity (ΔE^*), colour saturation (s), and yellow intensity (b^*) value graphs as shown in Figure 7.9 and degradation percentage shown in Table 7.7. The chromaticity (ΔE^*) values at all concentrations (5, 10 and 20 g/L) were > 5.0 , that showed clearly two different colours. As referred to Table 7.8, the PVA-curcumin pigment colour was constantly decreased at all concentrations after 8 hours exposure which changed from darker to lighter yellowish-brown colour. At concentration 5 g/L to 20 g/L, its saturation and yellow intensity degraded from 28.4% to 15.6% and 19.8% to 22.3% respectively. The degradation percentage value of saturation was decreased with the increase of PVA-curcumin concentration, in contrast with the degradation percentage value of yellow intensity.

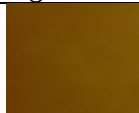
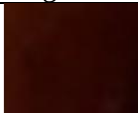
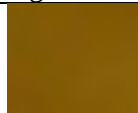
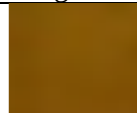
The oxidation process causes unwanted flavours and odours to emerge, which in turn shortens the packed foods' shelf life. According to Guo et al. (2014), the process of lipid oxidation may be slowed down by using a suitable formulation of biodegradable films with barrier qualities against UV light. According to their research, curcumin can be used to improve the UV light barrier qualities of the film without even lowering its transparency. By a process known as polymeric interaction, curcumin and starch molecules were able to increase the thickness of the film (Erna et al., 2022). According to Guo et al. (2014), employing an appropriate formulation of biodegradable films with UV light barrier properties may help to slow down the process of lipid oxidation. Their research indicates that curcumin can be employed to enhance the film's UV light barrier properties without sacrificing any of its transparency. Curcumin and starch molecules were able to improve the film's thickness through a mechanism known as polymeric interaction (Erna et al., 2022). Recently, Roy and Rhim (2021a) investigated the effects of curcumin and grapefruit seed extract (GSE) on polyvinyl alcohol (PVA)-based films, either separately or in combination. Curcumin may photodegrade in response to UVB exposure, losing some of

its advantageous characteristics. While PVA can offer some protection against UVB-induced deterioration, exposure to UVB radiation over an extended period of time can still have a detrimental effect on the PVA-curcumin composite's stability (Anand et al., 2007).

Table 7.7 Effects of UVB irradiation after 8 hours exposure on colour degradation for different concentrations of PVA-curcumin

UVB Degradation percentage (%)			
CIE L*a*b*	Curcumin concentration (g/L)		
	5	10	20
s	28.4	31.5	15.6
b*	19.8	19.5	22.3
ΔE^*_{Lab}	> 5.0		
ΔE^*_{Lab} value	Indication		
< 1.0	No colour difference is visible		
< 2.0	colour difference is only slightly visible		
< 3.5	noticeable colour difference		
< 5.0	clear difference in colour is noticed		
> 5.0	indicate clearly two different colours		

Table 7.8 PVA-curcumin's CIELAB colour based values after 8 hours of UVB exposure

0 HOUR OF EXPOSURE			8 HOURS OF EXPOSURE		
5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
					
b*	b*	b*	b*	b*	b*
84.50	61.05	56.10	73.50	65.14	54.03

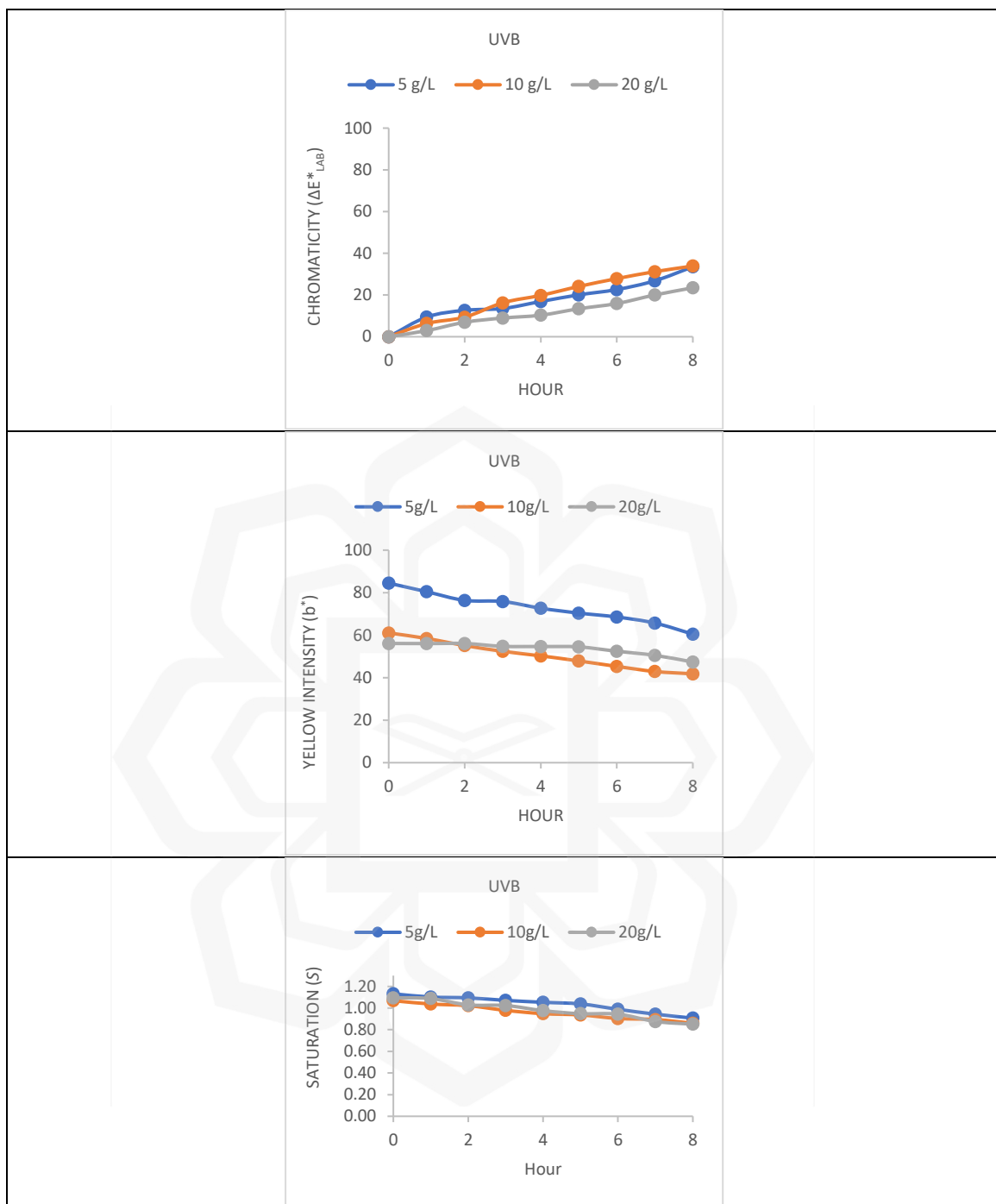


Figure 7.9 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PVA-curcumin when treated with UV radiation for 8 hours

7.2.5 Effect of Heating

The outcomes for PVA-curcumin subjected to seven different temperatures (10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C) for three hours reveal chromaticity (ΔE^* Lab), yellow intensity (b^*), and colour saturation (s) values as shown in Figure 7.10. The results from Table 7.9 demonstrated that the degradation percentage value of colour (s) was increased from 5, 10 and 20 g/L from temperature 10°C to 70 °C with the values of 7.7% to 44.9%, 37.5% to 38.8% and 32% to 76.8% respectively. The highest degradation percentage was detected in yellow intensity (b^*) from the highest concentration of PVA-curcumin pigment (20 g/L) with the values were 59.3% and 87.6% at the temperature from 10 °C to 70 °C. The results of chromaticity established that ΔE^* value > 5.0 for the 5 g/L and 20 g/L exposed at different temperatures showed clearly visible of two different colours. Table 7.10 clearly shown the different of PVA-curcumin pigment at 20 g/L which turn to darkest reddish-brown colour after 3 hours being heated. For 10 g/L, the chromaticity identified ΔE^* value < 2.0 at 10 °C indicated no colour difference is visible, whereas for the same PVA-curcumin concentration used at 50°C investigated that, < 3.5 showed noticeable colour difference.

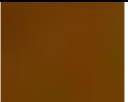
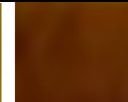
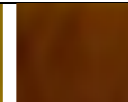
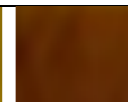
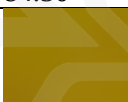
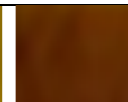
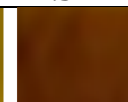
According to the findings of Tiwari et al.'s 2020 study, light, temperature, the presence of oxygen, and a host of other factors all contribute to the stability of carotenoids. However, one should take heed of its relative thermal stability (Simson et al., 2012). It has been demonstrated that heat treatments can aid in the conversion of *trans*-isomers into *cis*-isomers, which are impacted by the colour (Urbanoviciene et al., 2017). Thermal degradation is a possibility for curcumin, which could impact its stability. Nonetheless, PVA can somewhat lessen this deterioration (Esatbeyoglu et al., 2012). When paired with PVA, heat treatment can improve curcumin's solubility and stability. This is due to PVA's ability to enhance curcumin's dispersion in aqueous solutions, increasing its stability and potency (Kurien et al., 2006). The profile of curcumin release from PVA matrices can also be affected by heat treatment. For example, curcumin encapsulated in PVA has been

evolved into temperature-controlled release systems that provide a more regulated and sustained release (Rahikkala et al., 2015).

Table 7.9 Effects of temperature exposure for 3 hours on colour degradation of PVA-curcumin

Degradation percentage (%)				
	Curcumin concentration (g/L)			
CIE L*a*b*	°C	5	10	20
Colour saturation (s)	10	7.7	37.5	32.0
	20	16.5	40.1	50.1
	30	19.8	39.2	56.7
	40	23.6	4.8	58.5
	50	28.8	37.3	67.9
	60	36.2	37.3	73.6
	70	44.9	38.8	76.8
Yellow intensity (b*)	10	17.1	0.5	59.3
	20	18.8	14.0	21.5
	30	19.2	13.9	50.5
	40	18.5	11.6	53.8
	50	19.0	3.9	57.8
	60	19.0	7.7	69.0
	70	18.5	18.1	87.6
ΔE^*	10	> 5.0	< 2.0	> 5.0
	20	> 5.0	> 5.0	> 5.0
	30	> 5.0	> 5.0	> 5.0
	40	> 5.0	> 5.0	> 5.0
	50	> 5.0	< 3.5	> 5.0
	60	> 5.0	> 5.0	> 5.0
	70	> 5.0	> 5.0	> 5.0
ΔE^* value	Indication			
< 1.0	No colour difference is visible			
< 2.0	colour difference is only slightly visible			
< 3.5	noticeable colour difference			
< 5.0	clear difference in colour is noticed			
> 5.0	indicate clearly two different colours			

Table 7.10 PVA-Curcumin colours throughout 3 hours based on CIELab values at different concentrations (5, 10, and 20 g/L) incubated in different temperature

TEMP	0 HOUR OF EXPOSURE			3 HOURS OF EXPOSURE		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
10 °C	 b* 84.50	 b* 61.05	 b* 56.10	 b* 70.04	 b* 60.74	 b* 22.85
20 °C	 b* 84.50	 b* 61.05	 b* 56.10	 b* 72.30	 b* 70.87	 b* 44.02
30 °C	 b* 84.50	 b* 61.05	 b* 56.10	 b* 73.60	 b* 63.70	 b* 27.79
40 °C	 b* 84.50	 b* 61.05	 b* 56.10	 b* 66.56	 b* 66.54	 b* 25.92
50 °C	 b* 84.50	 b* 61.05	 b* 56.10	 b* 66.79	 b* 58.68	 b* 23.70
60 °C	 b* 84.50	 b* 61.05	 b* 56.10	 b* 66.46	 b* 6.33	 b* 17.37
70 °C	 b* 84.50	 b* 61.05	 b* 56.10	 b* 55.52	 b* 49.98	 b* 6.96

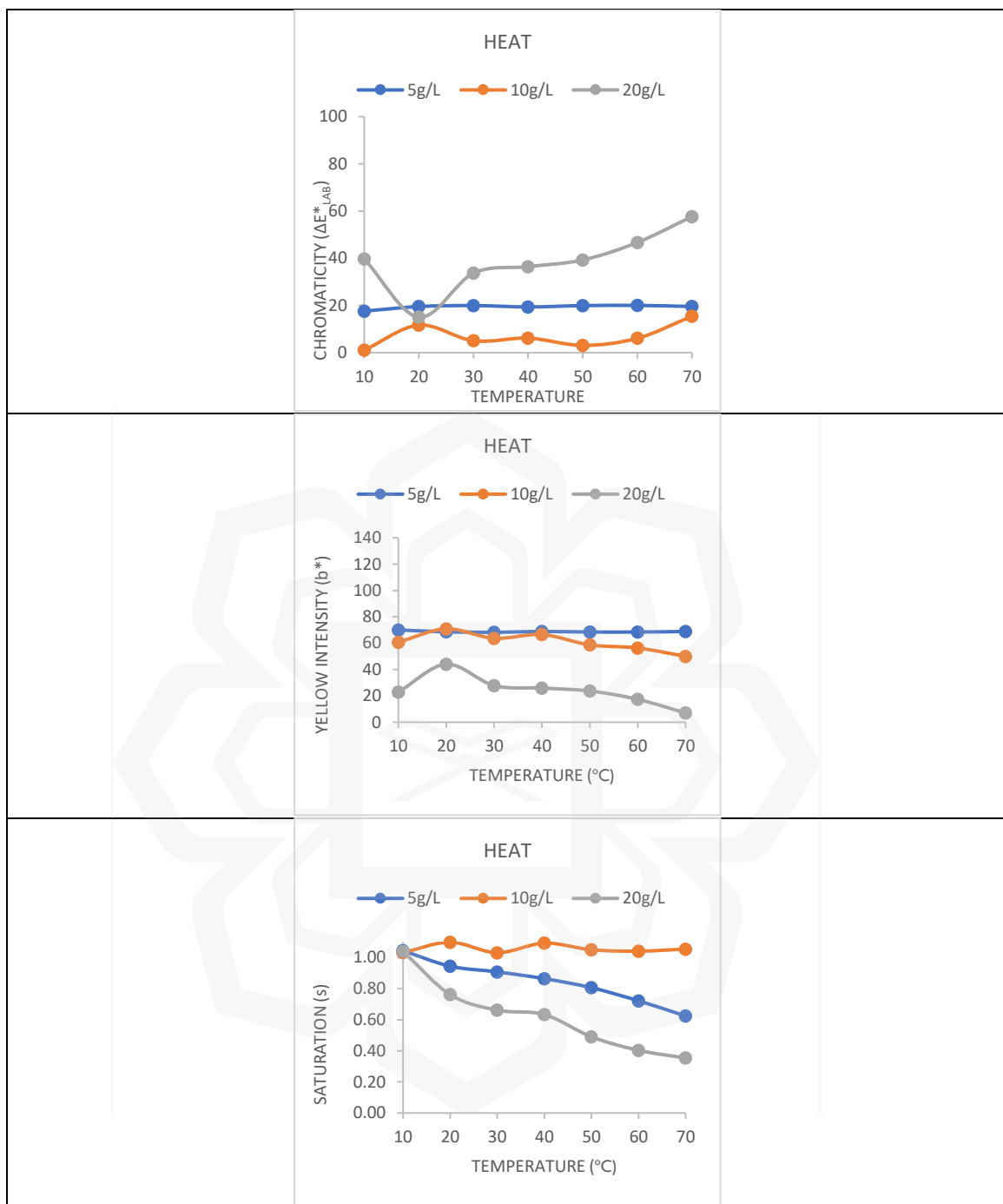


Figure 7.10 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of PVA-curcumin upon exposure to different temperature for 3 hours

7.3 MULTIVARIATE DATA ANALYSIS

7.3.1 Sampling Adequacy for Multivariate Analysis

The outcomes from 10 variables were analysed using multivariate data analysis to determine the significant variables contributing to PVA-curcumin chromaticity stability. Data transformation is a component of the initial preparation of data prior to statistical analysis. The entire data set was normalised through the use of variable transformation. Figure 7.11 revealed the box and whisker plot to show the comparison in distributions of variables graphically to identify outliers. Outliers detected at L, C, H, and ΔE in this boxplot signify unusual values representing the overall data distribution. In this plot, the outliers remained as the data is significant to show the highest values for each variable. The median score of H (hue) is the highest, and s (saturation) is the lowest.

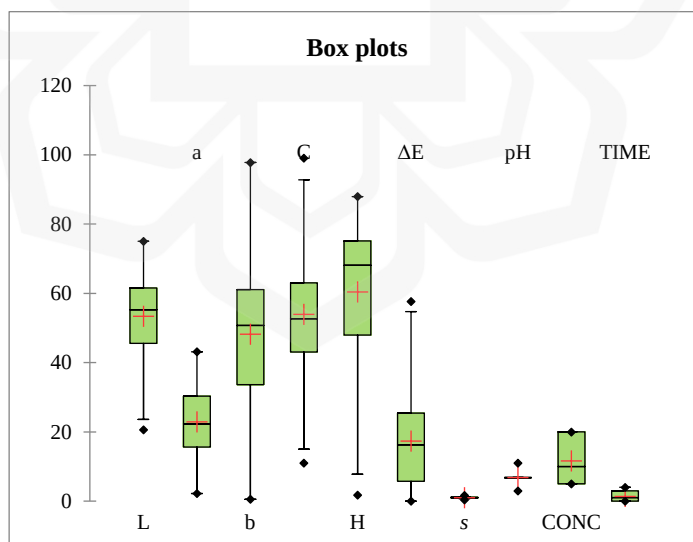


Figure 7.11 Box-and-whisker plots of PVA-curcumin pigment chromaticity stability variables at different concentrations and time of exposure

Sampling adequacy for PVA-curcumin pigment chromaticity stability data were further tested by using Kaiser-Meyer-Olkin (KMO) where the value must be larger than 0.5 followed by the Barlett's Test of Sphericity ($p < 0.05$) and Correlation Matrix Pearson where the value must be equal or greater than 0.01 (Atiqah et al., 2023). The KMO test was conducted to evaluate the dataset for adequacy at α of 0.05 to ensure the dataset is adequate for the multivariate data analysis. The KMO values for the 382 datasets produced a KMO score of 0.653 (Table 7.11) confirming the dataset is in mediocre category and adequate for PCA test. Barlett's Test of Sphericity is measured to ensure that the R-matrix does not equal the identity matrix. The p -value for the PVA-curcumin pigment chromaticity stability data exhibited highly significant differences at 0.0001.

Table 7.11 Kaiser-Meyer-Olkin and Bartlett's test for the PVA-curcumin pigment chromaticity stability

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.653
Bartlett's Test of Sphericity	Chi-square (Observed value)	4742.877
	DF	45
	p -value (Two-tailed)	< 0.0001

7.3.2 Correlation Coefficient Matrix (r^2)

Table 7.12 and 7.13 revealed a positive and negative correlations strength between two variables among PVA-curcumin pigment chromaticity stability test. A very high positive correlation was observed among L versus b^* ($r^2 = 0.935$), whereas other variables showed high positive correlation with the variables of L versus C ($r^2 = 0.792$), L versus H ($r^2 = 0.880$), a versus CONC ($r^2 = 0.743$), b^* versus C ($r^2 = 0.853$) and b^* versus H ($r^2 = 0.881$). Other than that, the variables that exhibited high negative correlation in between the variables of

L versus CONC ($r^2 = -0.744$), a versus H ($r^2 = -0.777$), b* versus COC ($r^2 = -0.742$) and H versus CONC ($r^2 = -0.820$). In general, the PVA-curcumin pigment chromaticity stability variables can be grouped into three clusters with positive correlations. Whereas the PVA-curcumin samples form three cluster as indicated in biplot (Figure 7.12).

Table 7.12 Correlation coefficient matrix for the PVA-curcumin pigment chromaticity stability

Variables	L	a	b	C	H	ΔE	s	pH	CONC	TIME
L	1									
a	-0.562	1								
b	0.935	-0.533	1							
C	0.792	-0.310	0.853	1						
H	0.880	-0.777	0.881	0.669	1					
ΔE	-0.329	-0.138	-0.335	-0.488	-0.085	1				
s	0.137	0.113	0.294	0.698	0.091	-0.448	1			
pH	-0.038	-0.087	-0.021	0.001	-0.005	-0.024	0.038	1		
CONC	-0.744	0.743	-0.742	-0.522	-0.820	-0.043	-0.013	0.000	1	
TIME	-0.463	-0.169	-0.436	-0.428	-0.168	0.545	-0.141	0.017	0.000	1

Values in bold are different from 0 with a significance level $\alpha=0.01$

Table 7.13 evaluated the correlation coefficient (r^2) or the strength of the correlation between two variables among PVA-curcumin pigment chromaticity stability test. The correlation coefficient is measured on a scale from -1 to +1 as described by Guilford (1973).

Table 7.13 The correlation coefficient (r^2) strength between two variables among PVA-curcumin pigment chromaticity stability test.

Variables	r^2	Size of Correlation	Interpretation
b x L	0.935	0.90 to 1.00	Very high positive correlation
b x H	0.881	0.70 to 0.90	High positive correlation
L x H	0.880		
b x C	0.853		
L x C	0.792		
Conc x a	0.743		
C x s	0.698	0.50 to 0.70	Moderate positive correlation
C x H	0.669		
ΔE x t	0.545		
-	-	0.30 to 0.50	Low positive correlation
Conc x H	-0.820	-0.70 to -0.90	High negative correlation
H x a	-0.777		
Conc x L	-0.744		
Conc x b	-0.742		
a x L	-0.562	-0.50 to -0.70	Moderate negative correlation
b x a	-0.533		
Conc x C	-0.522		
ΔE x C	-0.488	-0.30 to -0.50	Low negative correlation
t x L	-0.463		
ΔE x s	-0.448		
t x b	-0.436		
t x C	-0.428		
ΔE x b	-0.335		
ΔE x L	-0.329		
C x a	-0.310		
s x b	0.294	0.00 to 0.30 (0.00 to -0.30)	Little if any correlation
s x L	0.137		
s x a	0.113		
s x H	0.091		
pH x s	0.038		
t x pH	0.017		
Conc x pH	0.000		
t x Conc	0.000		
t x a	-0.169		
t x H	-0.168		
t x s	-0.141		
ΔE x a	-0.138		
pH x a	-0.087		
ΔE x H	-0.085		
Conc x ΔE	-0.043		
pH x L	-0.038		
pH x ΔE	-0.024		
pH x b	-0.021		
Conc x s	-0.013		
pH x H	-0.005		

Values in bold are different from 0 with a significance level $\alpha=0.01$

7.3.3 Principal Component Analysis (PCA)

To analyse the dataset using PCA, it is possible to identify variables with high factor loadings. The PCA exposed two principal components (PCs) entailing 10 variables that represent cumulative variability of 70.52%. The eigenvalues (EV) were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 4.878, and for PC2 was 2.173 as showed in Table 7.14. The first principal PC1 accounted for 48.78 % of the total variance, which comprised strong factor loadings of L*, b*, C*, H, and concentration whereas for PC2, contained strong factor loadings of ΔE , comprising of 21.73 % of the total variance for the data. Referring to Table 7.9, L, b*, C, H, ΔE , and concentration had strong FL ($FL > 0.75$) whereas a*, s, and time had moderate FL ($0.500 < FL < 0.749$) and pH had a weak FL ($FL < 0.499$).

Table 7.14 Principal components factor loading and eigenvalues of the correlation matrix for PVA-curcumin pigment chromaticity stability

Variables	PC1	PC2
L	0.953	0.018
a	-0.639	-0.629
b	0.970	-0.042
C	0.871	-0.373
H	0.916	0.316
ΔE	-0.355	0.751
s	0.325	-0.621
pH	0.002	0.019
Concentration	-0.799	-0.459
Time	-0.392	0.613
Eigenvalue	4.878	2.173
Variability (%)	48.781	21.734
Cumulative (%)	48.781	70.515

The PCA analysis allows for identifying factors and provides insight in PVA-curcumin pigment stability. Therefore, factor loadings can be utilised to determine the strength of the association between variables in PVA-curcumin pigment chromaticity stability test. In principle, variables far away from the axes F1 and F2 had a strong factor loading (FL). All variables were positively correlated if they were grouped close together. Group 1 can be grouped with the variables of (L and b), group 2 included with variables (a and CONC.), and for group 3 (time and ΔE). In general, the PVA-curcumin stability variables are grouped into three clusters which have positive correlations, while H variable has negative correlation with group 2 and s variable has negative correlations with group 3 as there were at the opposite positioned (Figure 7.12). The others were no correlation.

The biplot in Figure 7.12 exhibited three clusters, which at the top right of F1 axes represent the cluster of PVA-curcumin samples at the lowest concentration (5 g/L) which loss the highest colour than others. While the clusters at the bottom right of F1 axes represent cluster of PVA-curcumin samples at 10 g/L that has lower colour degradation. Whereas, cluster at the left side of F1 axes represent cluster of PVA-curcumin samples at 20 g/L that consist mixed range of colour degradation in which the samples with lower colour degradation located at the bottom and the higher at the top. It can be concluded that the colour changes or degradation of PVA-curcumin pigment is highly correlated with the concentration of the pigment, light and dark conditions, and period of exposure towards weathering effects.

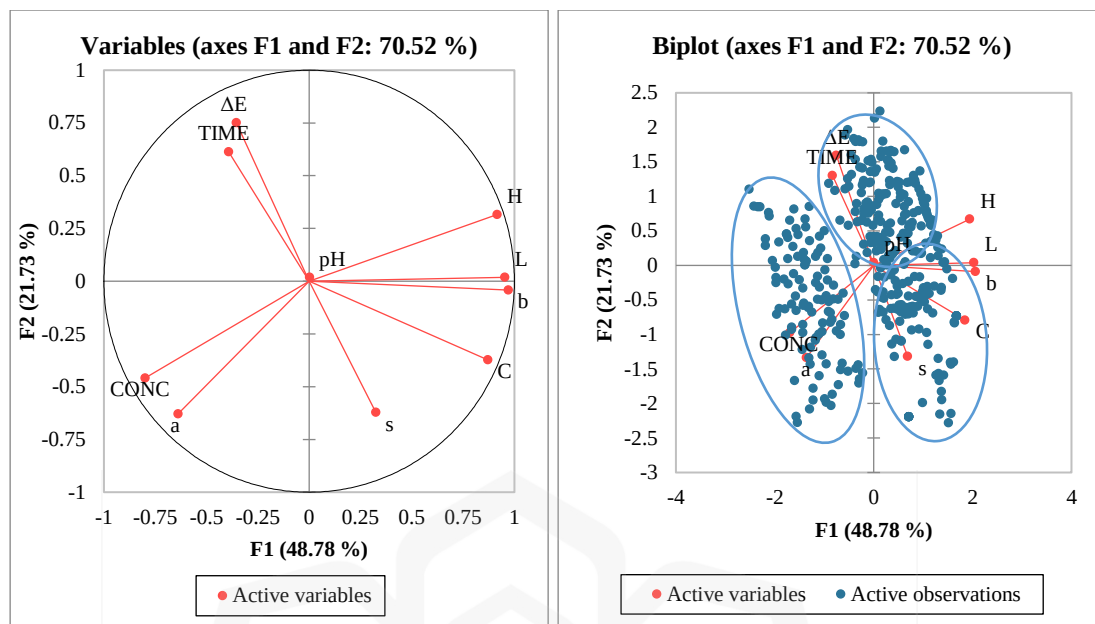
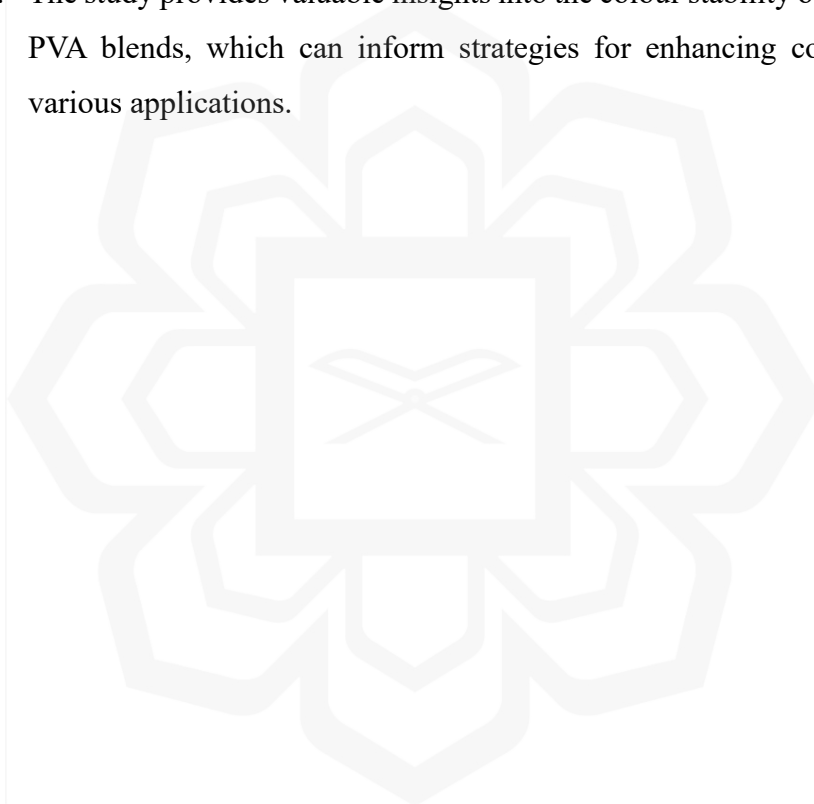


Figure 7.12 Variables plot and correlation biplot for PVA-curcumin pigment stability test variables

7.4 SUMMARY OF THE RESULTS

1. The highest degradation in 10 g/L of curcumin concentration in a light condition, whereas in dark condition, the least degradation also occurred in 10 g/L of curcumin concentration in dark condition.
2. The study reveals the highest degradation at 5 g/L of NaCl concentration in 5 g/L of PVA-curcumin concentration in light condition, whereas the least *s* value, at 30 g/L of NaCl concentration in 10 and 20 g/L of PVA-curcumin concentration.
3. The study demonstrates the degradation of PVA-curcumin was highest at pH 11 in 20 g/L in light condition, whereas in dark condition, the least degradation was at pH 7 in 5 g/L of curcumin concentration.

4. The study indicates that PVA-curcumin pigment was having the highest degradation at 10 g/L in UV-B as compared to the other concentrations. The study highlights the colour stability of curcumin pigment under UVB exposure and its potential application in improving the barrier qualities of biodegradable films for food packaging.
5. The study demonstrates the highest degradation in 20 g/L at 70 °C which showed the importance of environmental factors, such as temperature, in influencing the stability of PVA-curcumin.
6. The study provides valuable insights into the colour stability of crude curcumin-PVA blends, which can inform strategies for enhancing colour retention in various applications.



CHAPTER EIGHT

CHROMATICITY STABILITY OF CHITOSAN-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS

8.1 INTRODUCTION

Chitosan, a biopolymer derived from chitin found in the exoskeletons of crustaceans, has garnered significant attention in recent years for its potential applications in food packaging. As the demand for sustainable and eco-friendly materials continues to rise, chitosan emerges as a promising alternative to conventional synthetic polymers, primarily due to its biodegradability, non-toxicity, and excellent film-forming properties (Kumar et al., 2019b). The unique chemical structure of chitosan, which contains amino and hydroxyl groups, imparts antimicrobial properties, making it particularly effective in extending the shelf life of food products by inhibiting the growth of spoilage microorganisms (Kumari et al., 2015b). Moreover, chitosan films exhibit favourable mechanical and barrier properties, offering protection against moisture, oxygen, and volatile compounds, which are crucial for maintaining food quality (Kausar, 2017a). This multifunctional nature positions chitosan not only as a means of food preservation but also as an active packaging material that can actively interact with food products to enhance their safety and quality (Bhat et al., 2018). With increasing regulatory support and consumer preference for natural additives, the integration of chitosan into food packaging systems represents a significant step towards sustainable practices in the food industry (Rhim, 2018). In addition to its antimicrobial properties, chitosan is recognized for its film-forming ability, which allows it to create protective barriers around food items. This is particularly important for perishable goods, where exposure to air and moisture can lead to spoilage. The incorporation of natural antioxidants, such as curcumin, into chitosan-based films can further enhance their functional properties by providing additional protection against

oxidative degradation (Rouf and Kokini, 2018c). The increasing regulatory support for natural additives, along with rising consumer awareness of health and environmental issues, has led to a greater emphasis on the development of chitosan-based packaging solutions. As research continues to evolve, exploring the various modifications and applications of chitosan in food packaging will be essential for addressing contemporary challenges related to food waste and safety. With its potential to revolutionise the food packaging sector, chitosan stands at the forefront of efforts aimed at creating more sustainable and functional packaging materials for the future.

8.2 RESULTS AND DISCUSSIONS

8.2.1 Effect of light, pigment concentration and time of exposure on chitosan-curcumin stability

The stability test on chitosan-curcumin was also targeting to perceive the effects of weathering including sodium chloride (seawater salinity), pH (acidity and alkalinity), sunlight exposure (UV rays), and heat (temperature) towards this integration as potential coating material, as well as to study the aspects of degradation. The CIE L*a*b* colour difference (ΔE^*_{LAB}) is used to assess the stabilisation of chitosan-curcumin coating as described by Mokrzyck and Tatol, (2011).

The value of chromaticity or colour difference (ΔE^*_{LAB}), saturation (s) and yellow intensity (b*) of chitosan-curcumin at concentration of 5 g/L, 10 g/L, and 20 g/L, time of exposure for 4 weeks, and in response to light and dark condition were presented in Figure 8.1. The results of chromaticity for chitosan-curcumin pigmented after treated with light and dark conditions, established that ΔE^* value > 5.0 for all three concentrations (5, 10 and 20 g/L) after 4 weeks indicated clearly visible of two different colours. Chitosan-curcumin

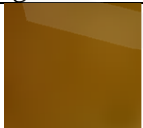
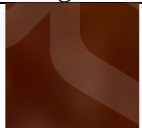
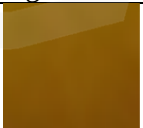
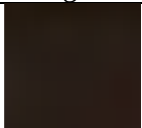
at concentration of 10 g/L under light condition shows the highest colour degradation while in dark, the similar concentration also marked to highly degraded during week 4 (Table 8.2). While at 5 g/L and 20 g/L, chitosan-curcumin shows minimal degradation in both conditions with slightly higher in light compared to dark condition within 4 weeks. There is also a strong relationship between the saturation, intensity, and concentration of the chitosan-curcumin yellow colour with time exposure in response to light and dark condition (Table 8.1). The relative degradation percentage of s and b^* values were the highest at 10 g/L of chitosan-curcumin after the exposure from week 1 until week 4 when developing in light. The values showed for saturation (s -12.5%) and (b^* -45.5%). Chitosan-curcumin concentration of 5 g/L in response to dark condition were observed to have substantially lowest degradation percentage of s (1.2%) and b^* (2.4%). Curcumin can also deteriorate when exposed to light, which over time will reduce its stability and effectiveness (Ahmed et al., 2022). According to a prior study, the addition of turmeric to chitosan films resulted in significantly higher b^* values when compared to the pure film. This could be attributed to the inherent yellowish hue of turmeric (Musso et al., 2017).

The study by Roy and Rhim (2021b) claims that darker, more yellowish films are being noticed because of the effect of colour on chitosan transparent film. Due to the action of curcumin, the primary ingredient in turmeric, which forms curcuminoids, which are polyphenolic chemicals, turmeric has a vivid yellow hue (Roy and Rhim, 2020c). It also displayed the same colour, which produced of the curcumin extracted as this study's results even though the used of the sources are the different. Curcumin might deteriorate when exposed to light because of its well-known photosensitivity. Its stability and efficacy as an antibacterial and antioxidant agent are preserved when it is kept dark (Abdullah et al., 2020b). By inhibiting oxidation and microbiological growth, these materials can help prolong the shelf life of food goods. The functional qualities of curcumin are preserved in a dark environment, increasing the effectiveness of the package (Fabra et al., 2018).

Table 8.1 Effects of 4 week in light and dark condition on colour degradation of chitosan-curcumin

Degradation percentage (%)				
CIE L*a*b*	Condition	Curcumin concentration (g/L)		
		5	10	20
Colour saturation (s)	Light	11.1	12.5	2.0
	Dark	1.2	8.7	79.8
Yellow intensity (b*)	Light	16.4	45.5	13.4
	Dark	2.4	39.0	13.5
Chromaticity (ΔE^*_{Lab})	Light	> 5.0		
	Dark	> 5.0		
ΔE^*_{Lab} value	Indication			
< 1.0	No colour difference is visible			
< 2.0	colour difference is only slightly visible			
< 3.5	noticeable colour difference			
< 5.0	clear difference in colour is noticed			
> 5.0	indicate clearly two different colours			

Table 8.2 Effects of 4 week in light and dark condition on colour degradation of chitosan-curcumin

	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0						
	b* 63.57	b* 68.28	b* 35.14	b* 63.57	b* 68.28	b* 35.14
WEEK 4						
	b* 53.15	b* 37.22	b* 30.45	b* 64.46	b* 41.62	b* 30.38

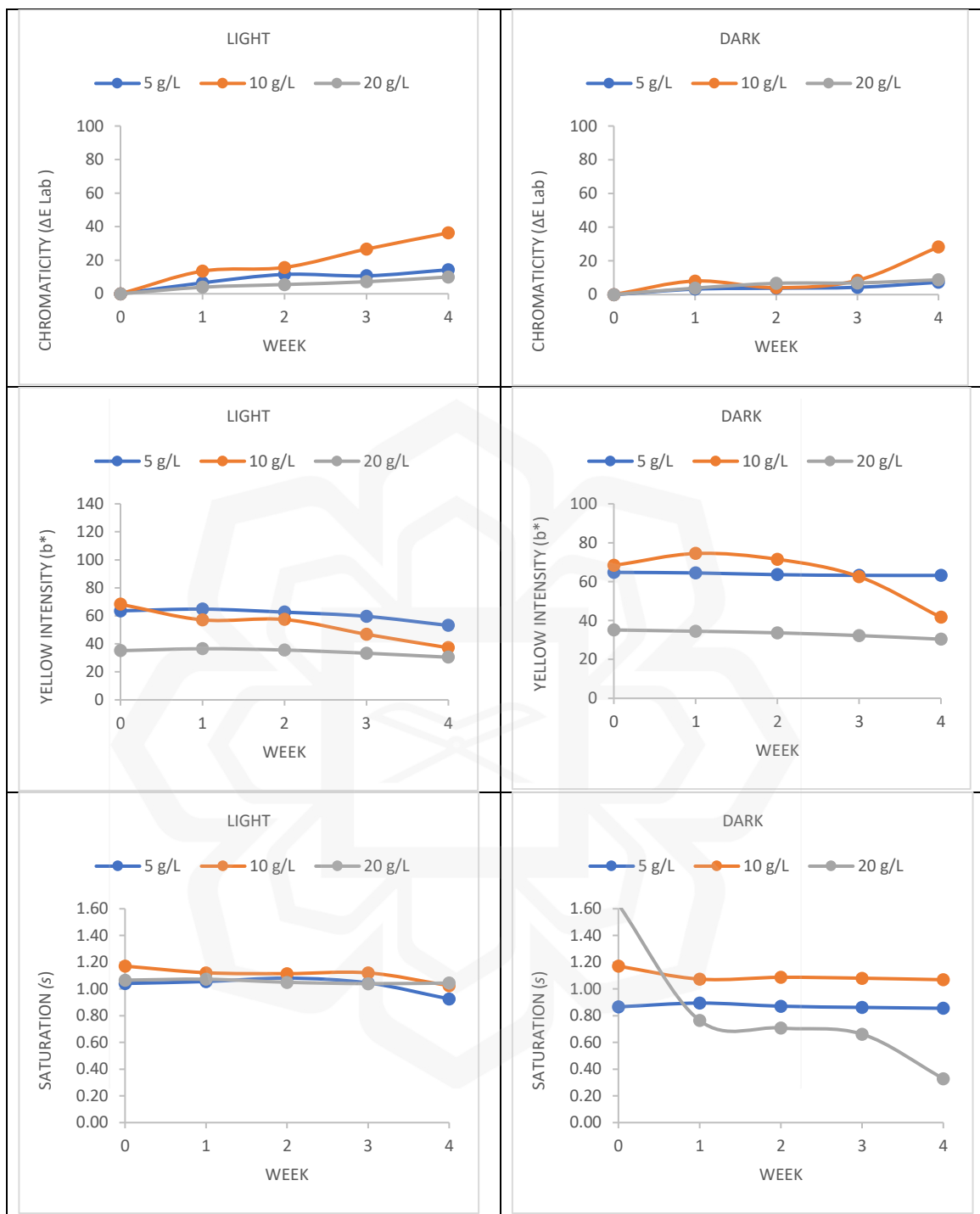


Figure 8.1 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of Chitosan-curcumin in light and dark condition for different exposure time

8.2.2 Effect of NaCl

The effect of different concentrations of NaCl (5, 10, 20, and 30 g/L) towards chitosan-curcumin pigment colour changes was shown by investigating the relationship between chromaticity (ΔE^*_{Lab}), colour saturation (s) and yellow intensity (b^*) values for different concentrations of chitosan-curcumin in light and dark condition and at different exposure time. The trends show that chitosan-curcumin at lower concentrations (5 g/L) treated with 5 g/L NaCl are highly changed under light conditions as shown in Table 8.4 where chitosan-curcumin at 5 g/L changed from yellow to brown after 4 weeks treated with 5 g/L NaCl. Unexpectedly, the colour degradation trends for chitosan-curcumin at 10 g/L and 20 g/L are highly degraded when treated with 10 and 30 g/L NaCl under dark conditions after 4 weeks (Figures 8.2 to 8.4).

Chitosan-curcumin concentration of 5 g/L in response to light condition were observed to have the highest degradation percentage of s (34.3%) and b^* (55.2%) at concentration of 5 g/L of NaCl, followed by the concentration of 20 g/L of NaCl (s -12.6% and b^* -34.8%). On the other hand, the chitosan-curcumin concentration of 10 g/L were observed to have the highest degradation of s (16.6%) and b^* (21.8%) at the concentration of 10 g/L of NaCl. Chitosan-curcumin concentration of 20 g/L in response to dark condition were observed to have the highest degradation percentage for (s -50.5% and b^* -88.2%) at 30 g/L concentration of NaCl. The degree of chitosan-curcumin s and b^* degradation between 5 and 20 g/L of NaCl concentrations is between s -19.3%, b^* -46.3% and s -15.4%, b^* -37.2% accordingly. Chromaticity for chitosan-curcumin pigment after treated with 5, 10, 20 and 30 g/L NaCl showed that ΔE^* value > 5.0 for all the three concentrations of curcumin (5, 10 and 20 g/L) (Table 8.3).

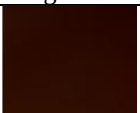
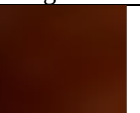
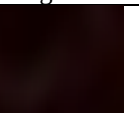
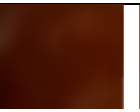
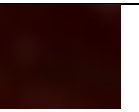
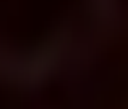
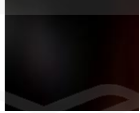
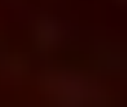
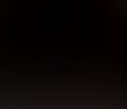
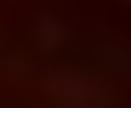
The impact of curcumin on the pectin/chitosan blended binary composite film was recently investigated by Xie et al. (2021). The use of curcumin in chitosan-based active

packaging films was reported by Liu et al. (2016). Salinity is one of several variables that can affect how stable carotenoids are. Plants and some microbes naturally contain pigments called carotenoids, which are essential for photosynthesis and defence against oxidative damage. The stability and concentration of carotenoids can be greatly affected by salinity. This combination lowers oxidative stress and microbiological development in food packaging, preserving the product's quality and safety (Abdullah et al., 2020a). Nevertheless, there is not much evidence to support the direct impact of chitosan-curcumin on the salinity of the packing environment. The main emphasis has been on their capacity to increase food products' safety and shelf life by offering antibacterial and antioxidant properties (Abdullah et al., 2020a; Ahmad et al., 2017).

Table 8.3 Effects of NaCl exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin

Degradation percentage (%)									
CIE L*a*b*	Curcumin concentration (g/L)	Light				Dark			
		NaCl concentration (g/L)							
		5	10	20	30	5	10	20	30
Colour saturation (s)	5	34.3	8.6	12.6	4.7	19.3	27.0	15.4	10.8
	10	18.8	16.6	1.5	10.9	4.1	12.8	1.8	4.0
	20	4.5	5.8	7.6	13.6	8.5	13.4	9.2	50.5
Yellow intensity (b*)	5	55.2	15.8	34.8	13.4	46.3	28.2	37.2	10.5
	10	37.9	21.8	16.5	30.8	14.1	44.8	18.2	48.1
	20	13.7	20.0	12.3	28.4	6.7	68.5	0.9	88.2
Chromaticity (ΔE* _{Lab})	5	> 5.0							
	10								
	20								
ΔE* _{Lab} value	Indication								
< 1.0	No colour difference is visible								
< 2.0	colour difference is only slightly visible								
< 3.5	noticeable colour difference								
< 5.0	clear difference in colour is noticed								
> 5.0	indicate clearly two different colours								

Table 8.4 Effects of NaCl exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin

WEEK	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0 5 mg/mL NaCl	 b* 70.88	 b* 55.50	 b* 65.32	 b* 60.29	 b* 45.50	 b* 45.43
WEEK 4 5 mg/mL NaCl	 b* 31.74	 b* 34.47	 b* 56.35	 b* 50.77	 b* 35.60	 b* 36.35
WEEK 0 10 mg/mL NaCl	 b* 61.62	 b* 53.19	 b* 55.24	 b* 60.90	 b* 52.86	 b* 35.24
WEEK 4 10 mg/mL NaCl	 b* 40.20	 b* 44.42	 b* 58.80	 b* 52.75	 b* 36.56	 b* 25.25
WEEK 0 20 mg/mL NaCl	 b* 64.10	 b* 54.01	 b* 58.49	 b* 60.47	 b* 54.01	 b* 29.49
WEEK 4 20 mg/mL NaCl	 b* 34.43	 b* 46.39	 b* 54.60	 b* 43.44	 b* 29.80	 b* 9.28
WEEK 0 30 mg/mL NaCl	 b* 63.74	 b* 59.30	 b* 54.56	 b* 59.42	 b* 56.95	 b* 44.56
WEEK 4 20 mg/mL NaCl	 b* 40.00	 b* 48.51	 b* 54.61	 b* 53.18	 b* 29.57	 b* 5.24

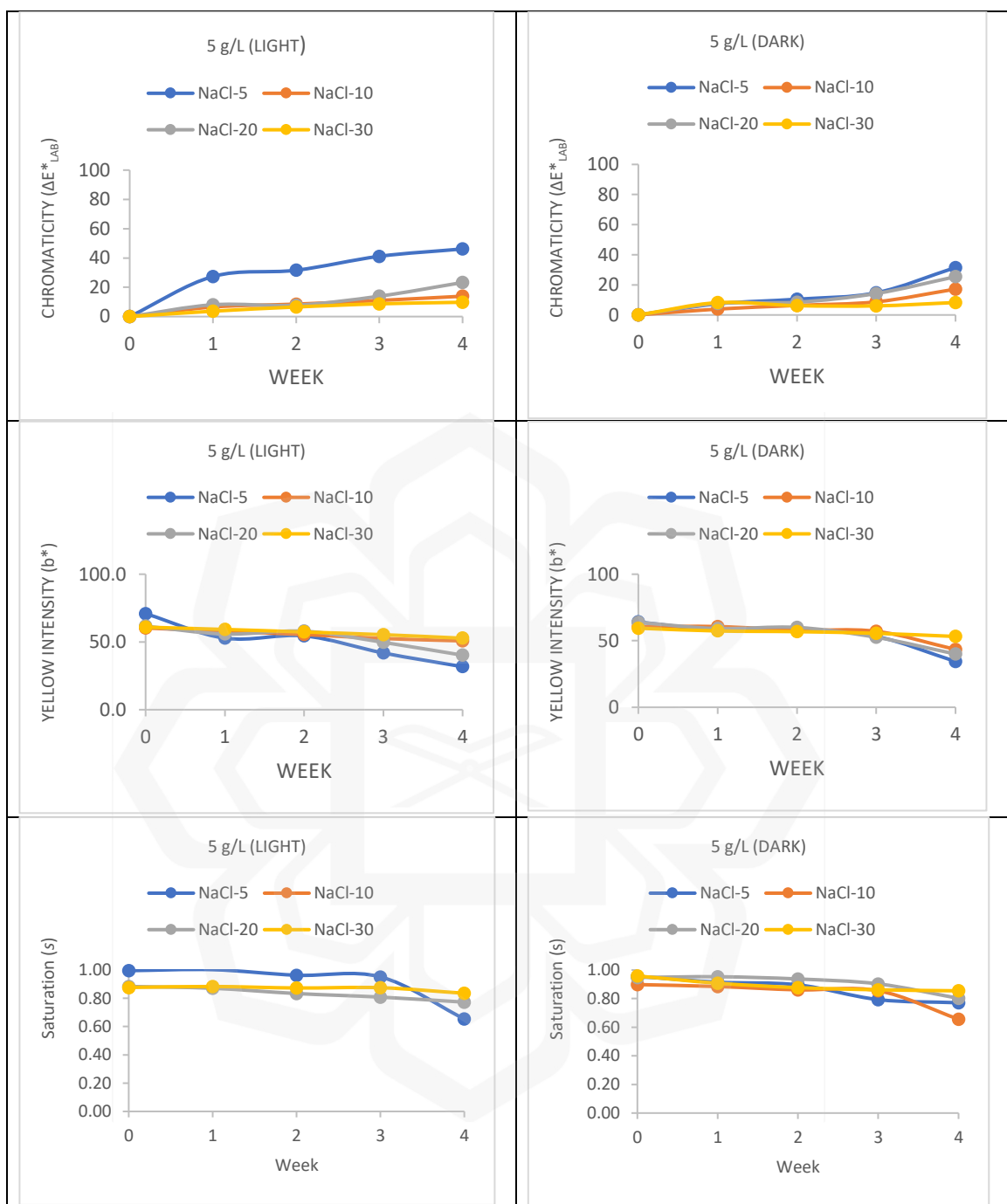


Figure 8.2 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L chitosan-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

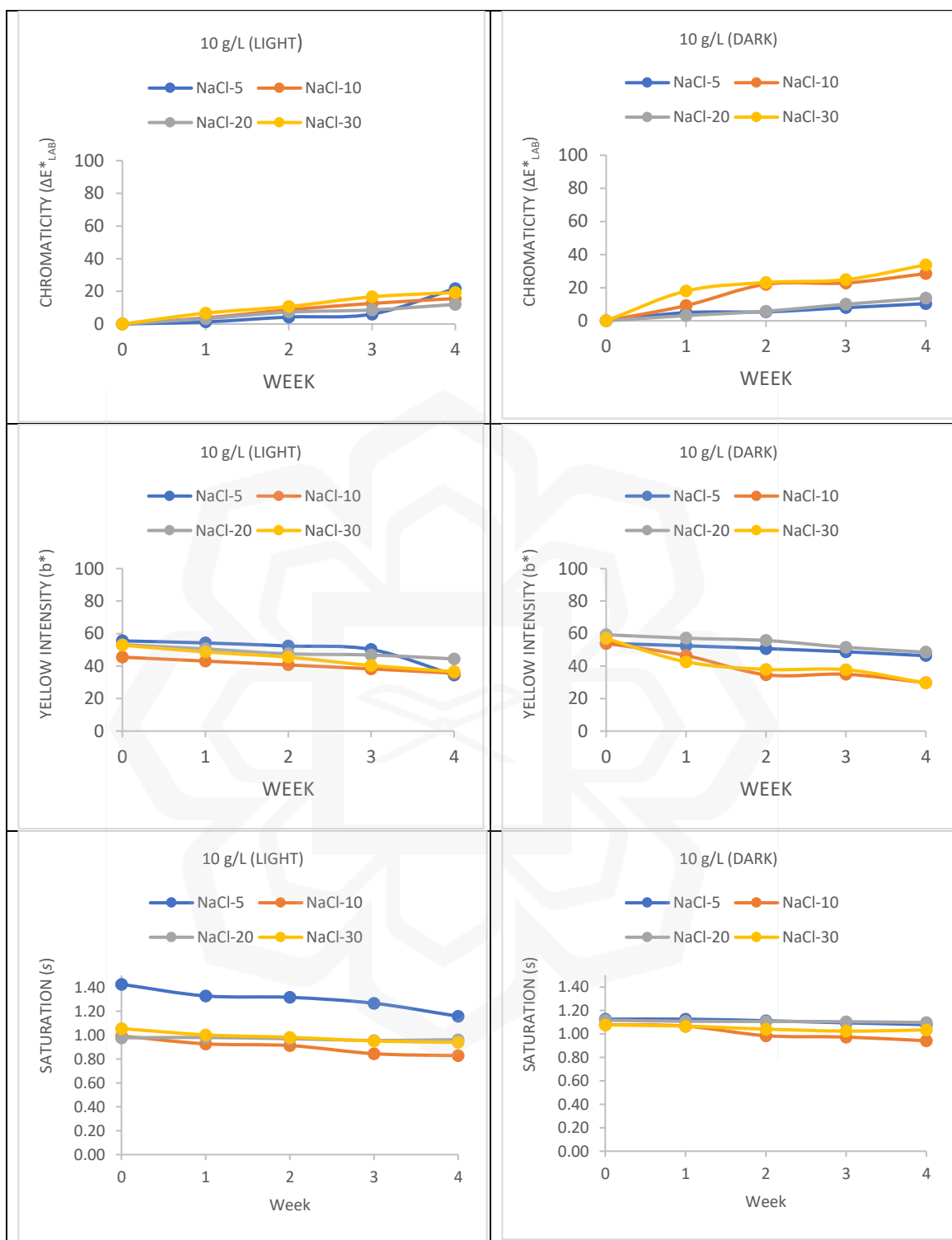


Figure 8.3 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L chitosan-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

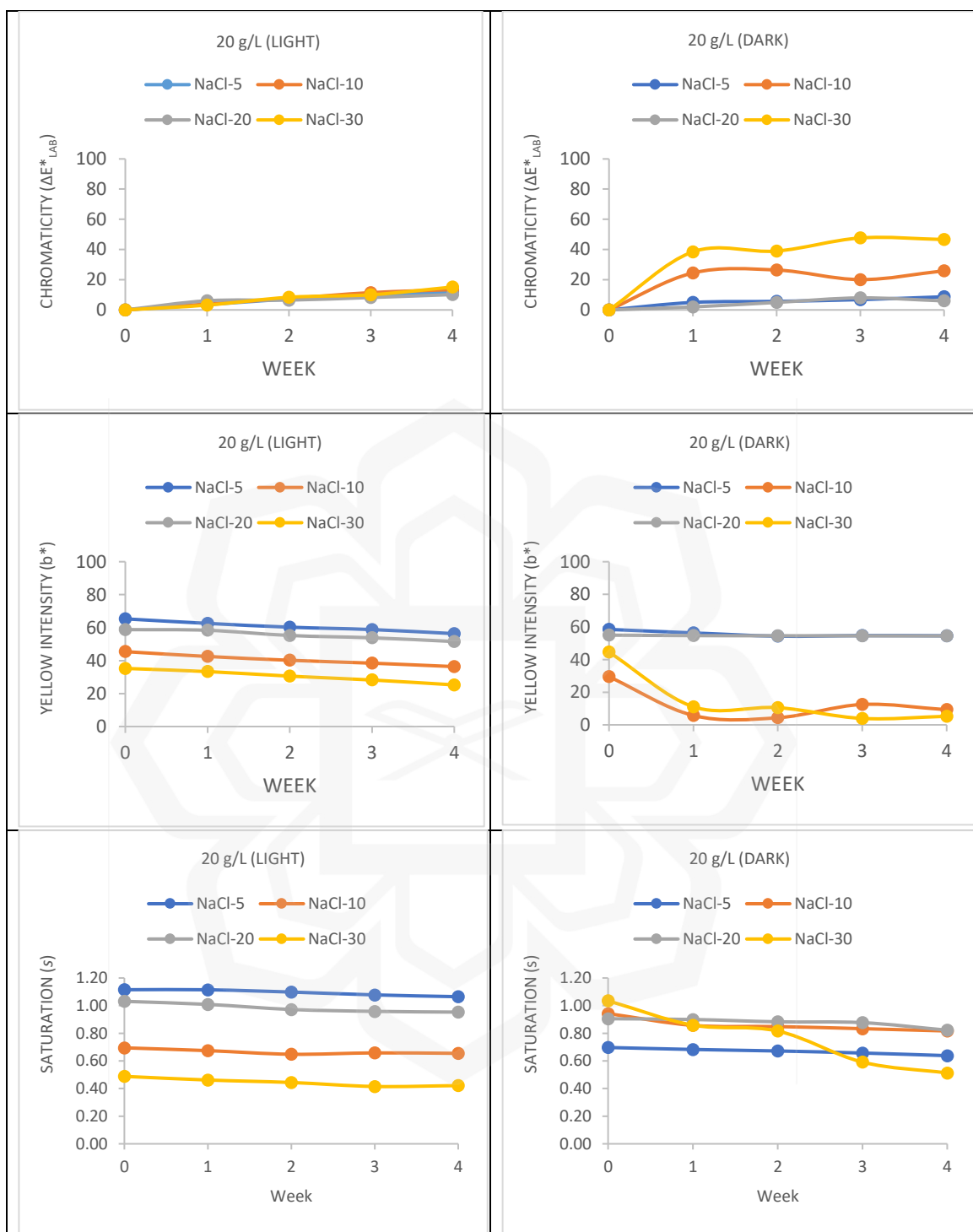


Figure 8.4 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L chitosan-curcumin at different concentrations of NaCl in light and dark condition for different exposure time

8.2.3 Effect of pH

The assessment of chitosan-curcumin stability in five different pH (3, 5, 7, 9, and 11) under light and dark condition for different exposure time conducted using the CIELab to confirm colour degradation and changes. Figures 8.5 to 8.7 showed chromaticity (ΔE^*_{Lab}), colour saturation (s) and yellow intensity (b^*) values for different concentrations of chitosan-curcumin treated with different pH. The results indicated in Table 8.5 revealed that the chromaticity ΔE^* value > 5.0 for all three concentrations curcumin (5, 10 and 20 g/L) after treated with pH solution 3, 5, 7, 9, and 11 in light and dark condition after 4 weeks. The values for saturation (s) and yellow intensity (b^*) was increased ranging from 18.6% to 50.6% and 50.6% to 83.1% for 10 g/L chitosan-curcumin concentration at pH 5, 7. The relative degradation percentage of s and b^* values were higher from 16.2% to 50.7% for 10 g/L chitosan-curcumin concentration at pH 11. Overall, the highest degradation percentage of s and b^* was detected from 10 g/L of chitosan-curcumin concentration. Chitosan-curcumin concentration of 20 g/L in response to dark condition were observed to have substantially lowest degradation percentage of s and b^* at pH 3, 5 and 9 ranging from (2.8%) to (21.2%), (3.2%) to (16.6%), (5.2%) to (18.2%) respectively. Whereas, for 10 g/L, it showed that the percentage of degradation for saturation (s) and yellow intensity (b^*) was 6.0% and 15.4%, which found less degraded. In addition, s and b^* values for chitosan-curcumin under dark condition were found less degraded as compared to light condition. However, the colour of chitosan-curcumin pigment at all concentration are visually stable after 4 weeks exposure (Table 8.6).

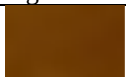
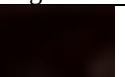
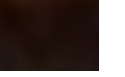
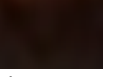
Pereira and Andrade (2017) reported that they have created a chitosan-based pH-responsive film by mixing curcumin, microcrystalline cellulose, and a eutectic combination that functioned best as plasticizing and reinforcing agents. In addition, the active packaging films made of chitosan that are employed with curcumin (Liu et al., 2016). It is possible to enhance the film's mechanical and barrier qualities by adding curcumin. The film of pectin, chitosan, and curcumin also adds to the antioxidant action. H-bonding was the mechanism

by which chitosan and curcumin interacted, according to the FTIR data. Through the experiment, they discovered that the chitosan film's mechanical properties could be enhanced by the addition of curcumin, and that the film was classified as having an effective antibacterial activity. Made up of the eutectic mixture (DES)-plasticized chitosan/curcumin film, the 1% (m/v) chitosan used can exhibit higher tensile strength and higher storage moduli with an outstanding colour change from yellow to dark red under alkaline conditions (Perreira and Andrade, 2017). Curcumin's impact on the pectin/chitosan blended with the binary composite film was recently demonstrated by a study by Xie et al. (2021).

Table 8.5 Effects of pH exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin

		Degradation percentage (%)									
CIE L*a*b*	Curcumin concentration (g/L)	Light					Dark				
		pH									
		3	5	7	9	11	3	5	7	9	11
Colour saturation (s)	5	23.7	17.0	7.6	5.6	13.5	18.6	14.3	9.5	13.9	17.2
	10	12.5	18.6	50.6	20.7	16.2	19.5	10.9	8.6	15.1	6.0
	20	8.0	14.0	14.9	3.4	8.6	2.8	3.2	12.8	5.2	9.3
Yellow intensity (b*)	5	32.0	28.0	23.1	19.9	34.4	20.2	18.4	15.0	26.8	14.2
	10	37.9	50.5	83.1	13.0	50.7	53.9	29.1	40.4	58.4	15.4
	20	17.7	24.5	62.2	13.7	24.6	21.2	16.6	37.2	18.2	13.5
Chromatic ity (ΔE^*_{Lab})	5	> 5.0									
	10										
	20										
ΔE^*_{Lab} value	Indication										
< 1.0	No colour difference is visible										
< 2.0	colour difference is only slightly visible										
< 3.5	noticeable colour difference										
< 5.0	clear difference in colour is noticed										
> 5.0	indicate clearly two different colours										

Table 8.6 Effects of pH exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin

WEEK	LIGHT			DARK		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
WEEK 0 pH 3	 b* 60.54	 b* 69.32	 b* 42.58	 b* 68.19	 b* 69.32	 b* 42.58
WEEK 4 pH 3	 b* 41.15	 b* 43.02	 b* 35.05	 b* 55.64	 b* 31.96	 b* 33.57
WEEK 0 pH 5	 b* 69.81	 b* 69.62	 b* 40.59	 b* 68.19	 b* 69.32	 b* 60.58
WEEK 4 pH 5	 b* 50.25	 b* 34.43	 b* 30.66	 b* 55.64	 b* 49.17	 b* 50.53
WEEK 0 pH 7	 b* 69.81	 b* 69.62	 b* 68.96	 b* 68.19	 b* 69.32	 b* 48.58
WEEK 4 pH 7	 b* 55.94	 b* 11.78	 b* 26.05	 b* 57.95	 b* 41.34	 b* 30.49
WEEK 0 pH 9	 b* 69.81	 b* 69.62	 b* 52.58	 b* 68.19	 b* 69.32	 b* 40.58
WEEK 4 pH 9	 b* 55.94	 b* 60.61	 b* 45.39	 b* 49.91	 b* 28.82	 b* 33.19
WEEK 0 pH 11	 b* 68.19	 b* 69.62	 b* 40.58	 b* 68.19	 b* 69.32	 b* 55.58
WEEK 4 pH 11	 b* 44.76	 b* 34.32	 b* 30.59	 b* 58.47	 b* 58.67	 b* 58.46

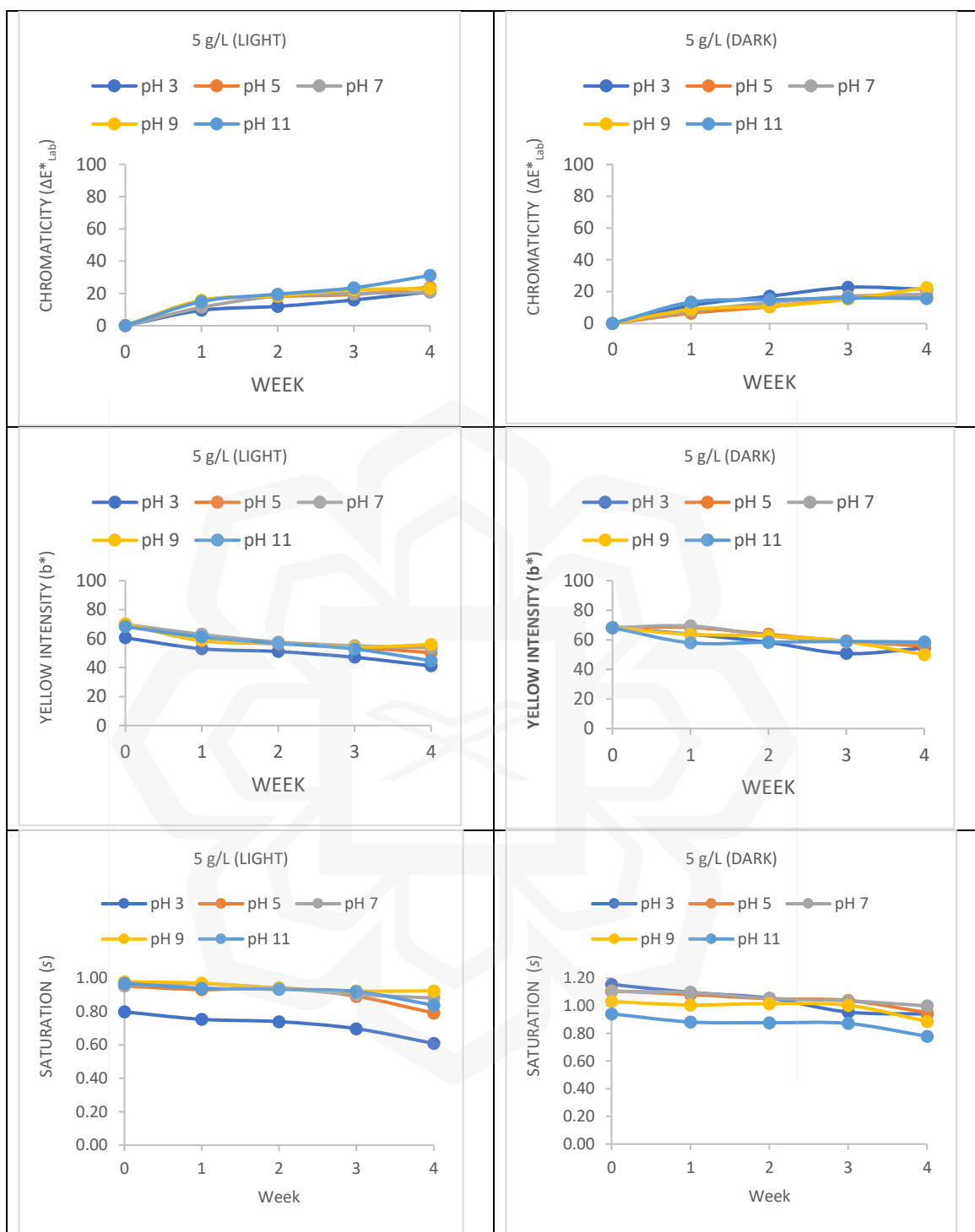


Figure 8.5 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 5 g/L chitosan-curcumin at different pH in light and dark condition for different exposure time

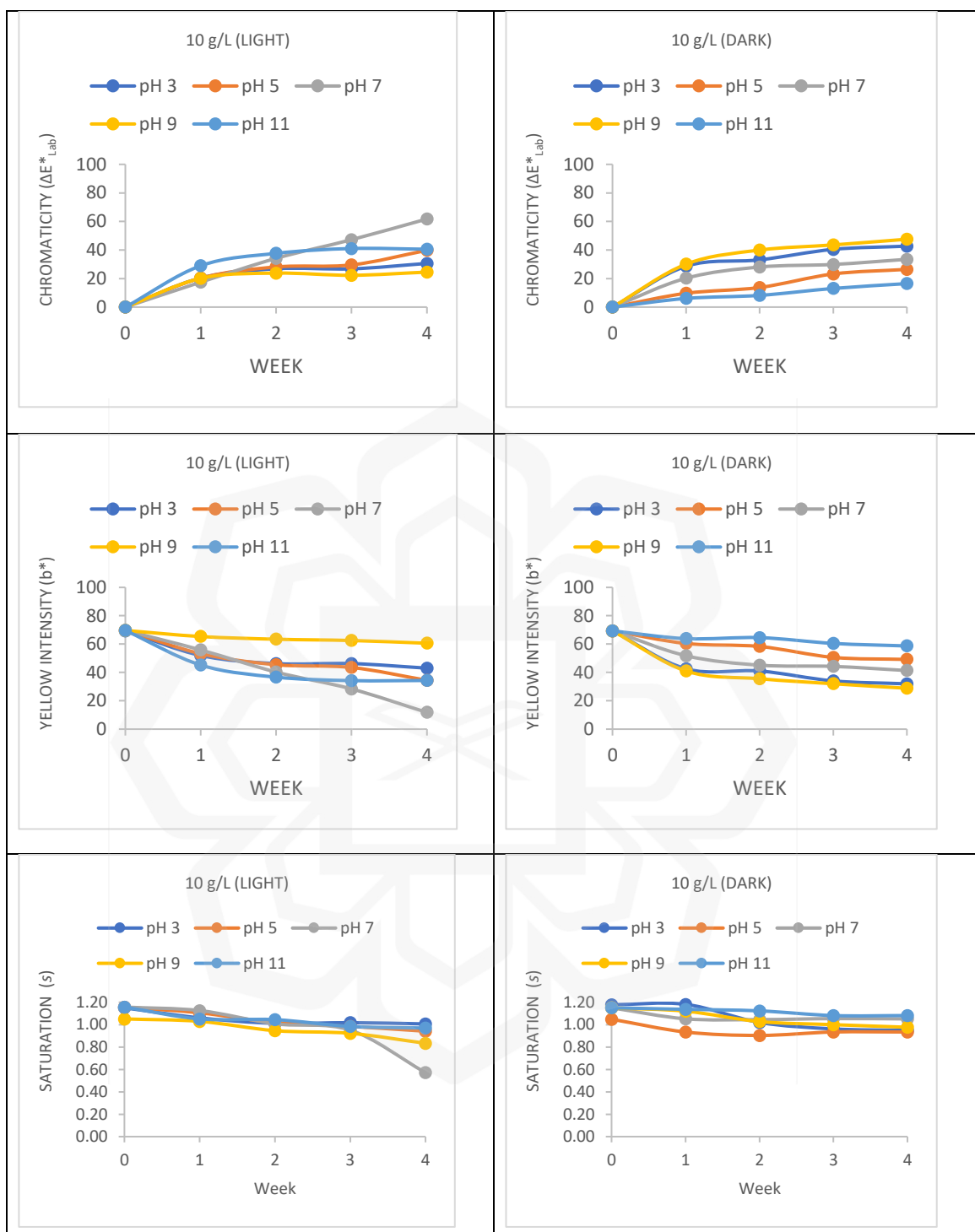


Figure 8.6 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 10 g/L chitosan-curcumin at different pH in light and dark condition for different exposure time

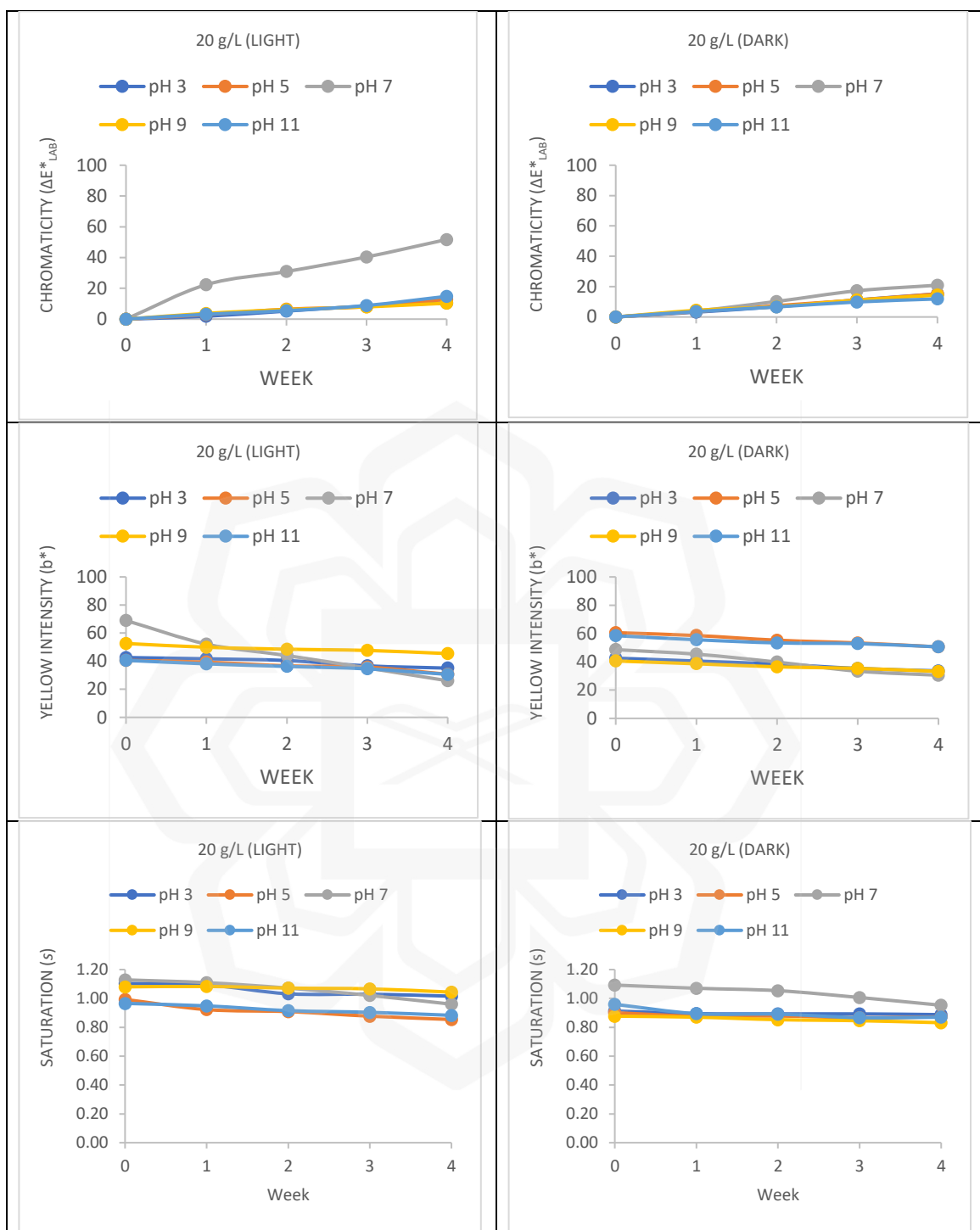


Figure 8.7 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for 20 g/L chitosan-curcumin at different pH in light and dark condition for different exposure time

8.2.4 Effect of UV Irradiation

The exposure of chitosan-curcumin at concentration of 5, 10, and 20 g/L under UVB irradiations for eight hours resulted slightly constant degradation with period of exposure (Figure 8.8). The chromaticity (ΔE^*) corresponds to colour brightness and it is based on the colour intensity. From this study, the value of ΔE^* for blended with chitosan-curcumin pigmented, which irradiated under UV-B for 8 hours was > 5.0 , which showing clearly two different colours was observed for three different chitosan-curcumin concentrations (5, 10 and 20 g/L). It can be seen as depicted in Table 8.8, chitosan-curcumin at all concentration shows slightly colour degradation after 8 hours of exposure under UVB. Also, the saturation (s) and yellow intensity (b^*) chitosan-curcumin was exposed as the higher the concentration of curcumin, the higher the degradation percentage for saturation (s) and yellow intensity (b^*). It is showed from the values for (s) and (b^*) for 20 g/L of curcumin concentration, that were found most degraded at 28.7% and 18.5% accordingly (Table 8.7).

By adding curcumin can aid to improve the chitosan film's mechanical qualities, and that it works best and most effectively when combined with antibacterial action. Its capacity to produce translucent films with UV protection that useful property for food packaging applications is one of curcumin's most promising bioactive agent properties. The findings indicate that the inclusion of curcumin in the film can enhance its ability to block UV light and act as a barrier against all three of the tested polysaccharides without compromising the film's transparency. Additionally, it demonstrated remarkable antioxidant activity and has the potential to function as the greatest UV barrier for the packaging materials.

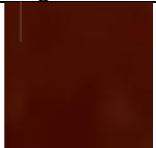
Leceta et al. (2013) stated that chitosan-based films are great and can aid in the preventative impact of chitosan films, which may help to retard the product oxidation that is generated by UV light. The same authors also investigated the impact of curcumin on different carbohydrate-based films (agar, carrageenan, and chitosan) and demonstrated that

adding curcumin enhanced the three poly saccharides' ability to block UV light while barely affecting the film's transparency (Roy and Rhim, 2020b).

Table 8.7 Effects of UVB exposure in 8 hours on colour degradation of chitosan-curcumin

UVB Degradation percentage (%)			
CIE L*a*b*	Curcumin concentration (g/L)		
	5	10	20
s	27.6	15.4	18.5
b*	17.9	25.0	28.7
ΔE^*_{Lab}	> 5.0		
ΔE^*_{Lab} value	Indication		
< 1.0	No colour difference is visible		
< 2.0	colour difference is only slightly visible		
< 3.5	noticeable colour difference		
< 5.0	clear difference in colour is noticed		
> 5.0	indicate clearly two different colours		

Table 8.8 Effects of UVB exposure in 8 hours on colour degradation of chitosan-curcumin

0 HOUR OF EXPOSURE			8 HOURS OF EXPOSURE		
5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
					
b*	b*	b*	b*	b*	b*
77.07	65.67	65.32	63.27	49.23	46.56

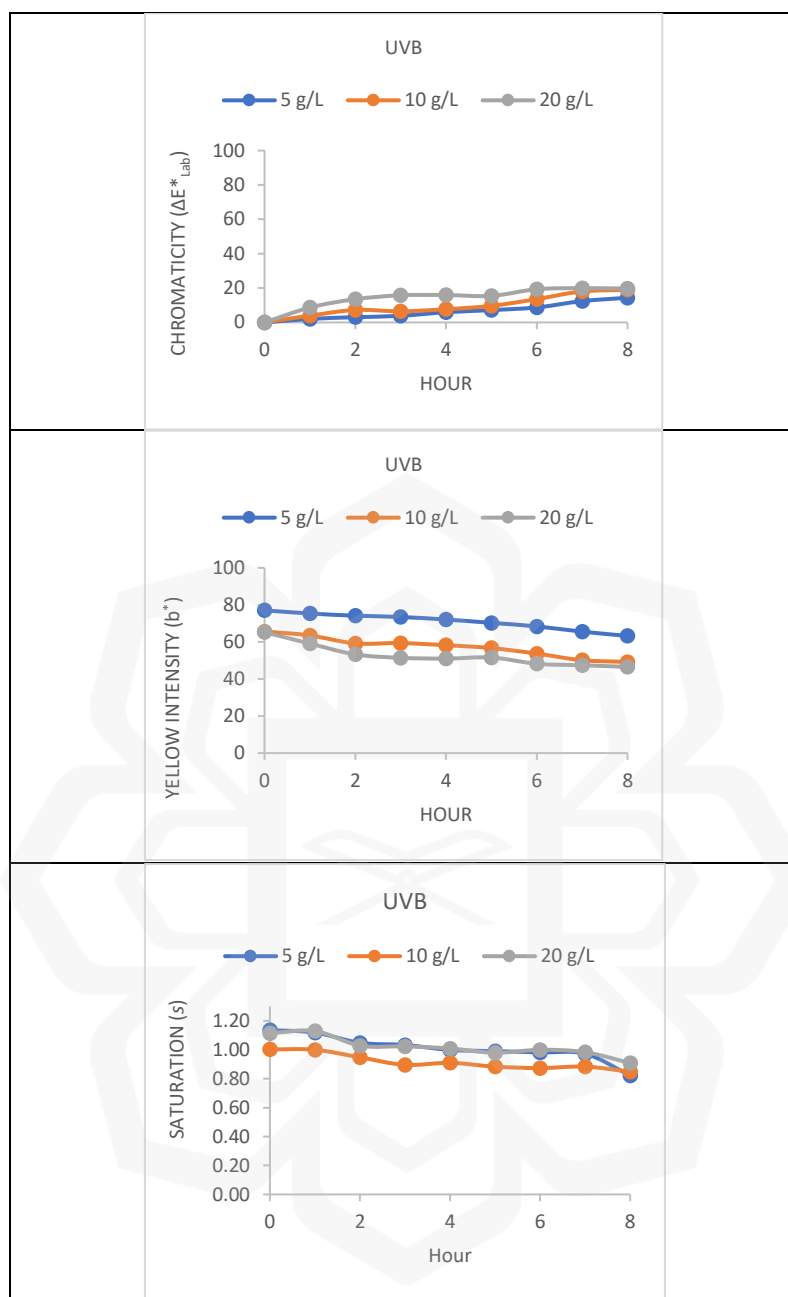


Figure 8.8 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of chitosan-curcumin after treated with UV radiation for 8 hours

8.2.5 Effect of Heating

The outcomes for chitosan-curcumin exposed to seven different temperatures (10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C) for three hours period reveal that chitosan-curcumin at all concentration shows elevated colour changes with the rise of temperature (Figure 8.9). The highest degradation exhibited in 20 g/L of chitosan-curcumin pigment for colour saturation (s) and yellow intensity (b^*) with the values of 88.5% and 89.4% at 30 °C followed by 10 g/L of chitosan-curcumin pigment, with (s) and (b^*) was 88.7% and 88.1% at 60 °C and in 5 g/L of chitosan-curcumin at 70 °C with (s) and (b^*) was 53% and 68% respectively (Table 8.9). The higher the chitosan-curcumin pigment, the higher the degradation percentage (%), even though it is exposed under low temperature, contrary with the lowest chitosan-curcumin concentration, which was seen to have a high degradation at the highest temperature tested. Chitosan-curcumin concentration of 5 g/L in response to 10 °C were observed to have the lowest degradation percentage of s (3.2%) and b^* (9.6%), whereas chitosan-curcumin pigment with the highest concentration of 20 g/L was found to have the lowest degradation of s (10.2%) and b^* (23.3%) value. All the temperature and chitosan-curcumin pigment were showed the chromaticity established that ΔE^* value > 5.0 that clearly visible of two different colours, obviously shown by chitosan-curcumin at 5 g/L that changed from yellowish to brownish colour tone (Table 8.10).

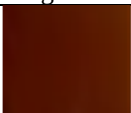
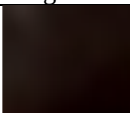
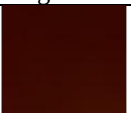
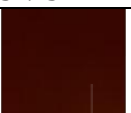
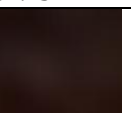
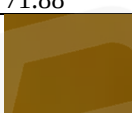
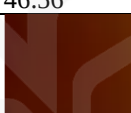
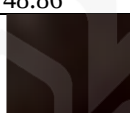
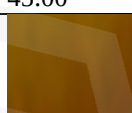
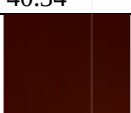
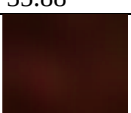
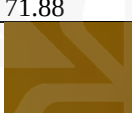
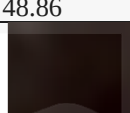
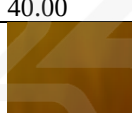
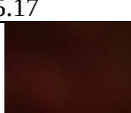
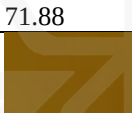
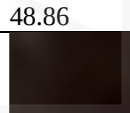
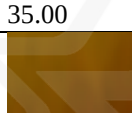
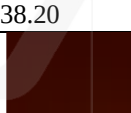
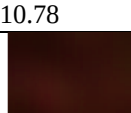
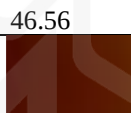
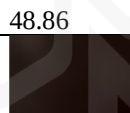
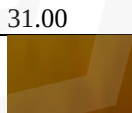
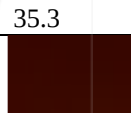
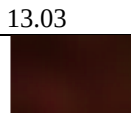
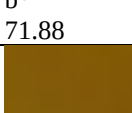
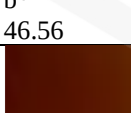
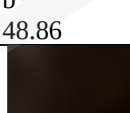
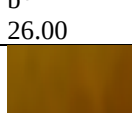
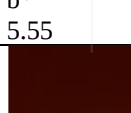
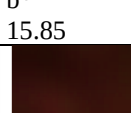
Further research may reveal that the addition of curcumin to the composite film contributes to its strong antioxidant activity, regulates curcumin release, and helps it display good heat-sealing qualities. The effects of curcumin on various carbohydrates (agar, carrageenan, and chitosan) are also being investigated by Roy and Rhim (2020b). According to the research, adding curcumin to various types of polymers has a range of effects on their physicochemical qualities. The reported studies indicate that curcumin has good effects on antibacterial, antioxidant, and antifungal characteristics. Research on chitosan-based active packaging films utilising curcumin has been conducted by Liu et al. (2016). From the study, they combined chitosan with turmeric-extracted curcumin to create

the bioactive film (Kalaycioglu et al., 2017). From the previous studies, adding the curcumin can increase the properties barrier of coating, and by comparing to my study, it showed that the colour can be blended well with the curcumin and chitosan. So, it does show that curcumin is generally used a lot, basically in coating applications.

Table 8.9 Effects of heat exposure in 3 hours on colour degradation of chitosan-curcumin

Degradation percentage (%)				
		Curcumin concentration (g/L)		
CIE L*a*b*	°C	5	10	20
Colour saturation (s)	10	3.2	16.7	10.2
	20	29.6	18.9	17.4
	30	26.7	63.9	88.5
	40	35.1	50.4	76.1
	50	15.6	60.5	76.8
	60	30.1	88.7	63.0
	70	53.0	66.5	51.2
Yellow intensity (b*)	10	9.6	28.2	23.3
	20	37.4	13.4	26.6
	30	44.4	56.6	89.4
	40	51.3	18.0	77.9
	50	56.9	24.0	73.3
	60	63.8	88.1	67.6
	70	68.0	47.6	84.6
ΔE*	10	> 5.0		
	20			
	30			
	40			
	50			
	60			
	70			
ΔE* value	Indication			
< 1.0	No colour difference is visible			
< 2.0	colour difference is only slightly visible			
< 3.5	noticeable colour difference			
< 5.0	clear difference in colour is noticed			
> 5.0	indicate clearly two different colours			

Table 8.10 Effects of heat exposure in 3 hours on colour degradation of chitosan-curcumin

TEMPERATURE	0 HOUR OF EXPOSURE			3 HOURS OF EXPOSURE		
	5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
10 °C	 b* 71.88	 b* 46.56	 b* 48.86	 b* 65.00	 b* 52.23	 b* 37.49
20 °C	 b* 71.88	 b* 46.56	 b* 48.86	 b* 45.00	 b* 40.34	 b* 35.88
30 °C	 b* 71.88	 b* 46.56	 b* 48.86	 b* 40.00	 b* 20.21	 b* 5.17
40 °C	 b* 71.88	 b* 46.56	 b* 48.86	 b* 35.00	 b* 38.20	 b* 10.78
50 °C	 b* 71.88	 b* 46.56	 b* 48.86	 b* 31.00	 b* 35.3	 b* 13.03
60 °C	 b* 71.88	 b* 46.56	 b* 48.86	 b* 26.00	 b* 5.55	 b* 15.85
70 °C	 b* 71.88	 b* 46.56	 b* 48.86	 b* 23.00	 b* 24.39	 b* 7.51

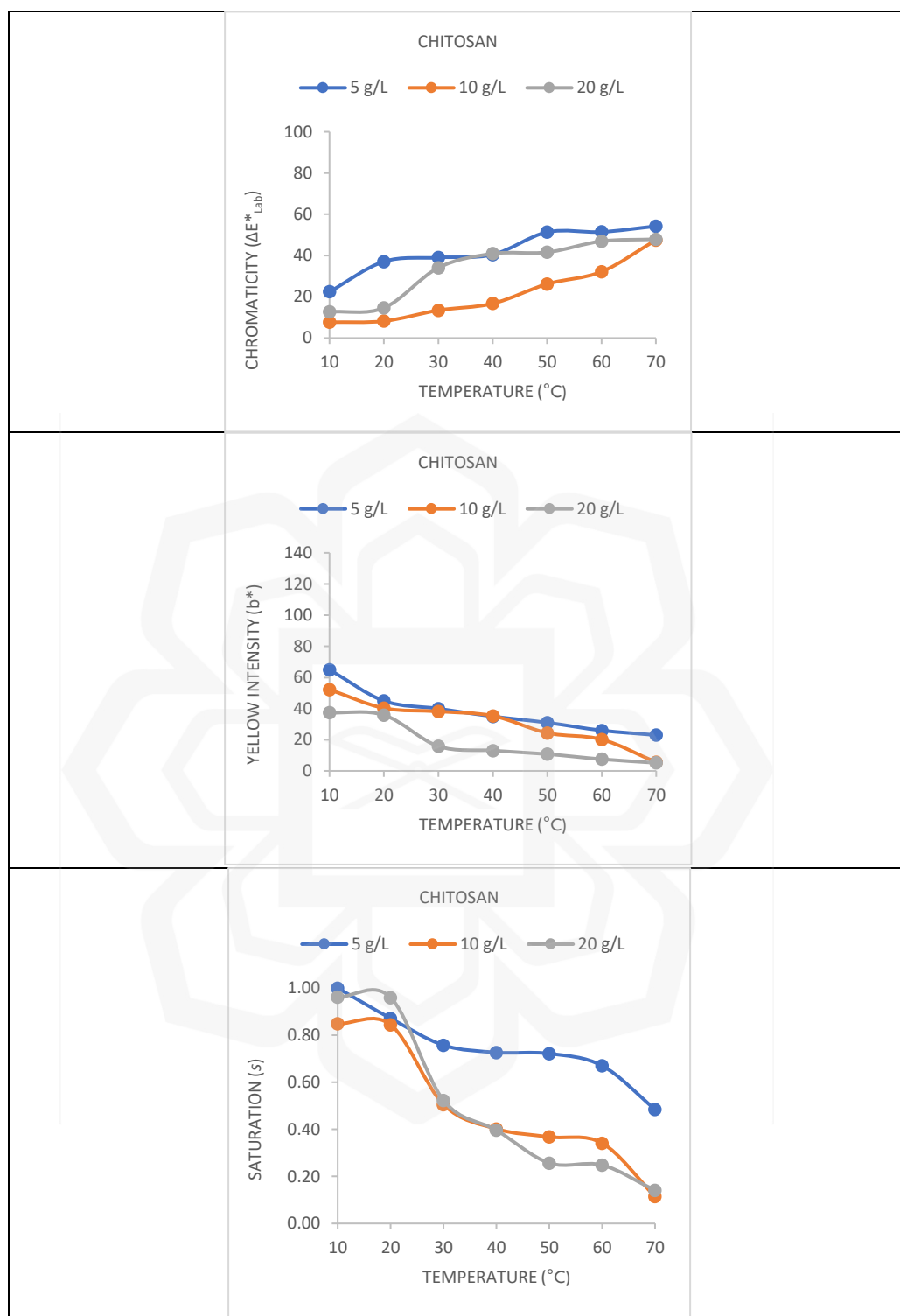


Figure 8.9 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of chitosan-curcumin when treated with different temperature for 3 hours

8.3 MULTIVARIATE DATA ANALYSIS

8.3.1 Sampling Adequacy for Multivariate Analysis

The results from 10 variables were analysed using multivariate data analysis to determine the significant variables contributing to chitosan-curcumin chromaticity stability. Data transformation is a component of the initial preparation of data prior to statistical analysis. The entire data set was normalized through the use of variable transformation. Figure 8.10 revealed the box and whisker plot to show the comparison in distributions of variables graphically to identify outliers. Outliers detected at L, a, b, C, H, ΔE , and pH in this boxplot signify unusual values representing the overall data distribution. In this plot, the outliers remained as the data is significant to show the highest values for each variable. The median score of H (hue) is the highest, and s (saturation) is the lowest.

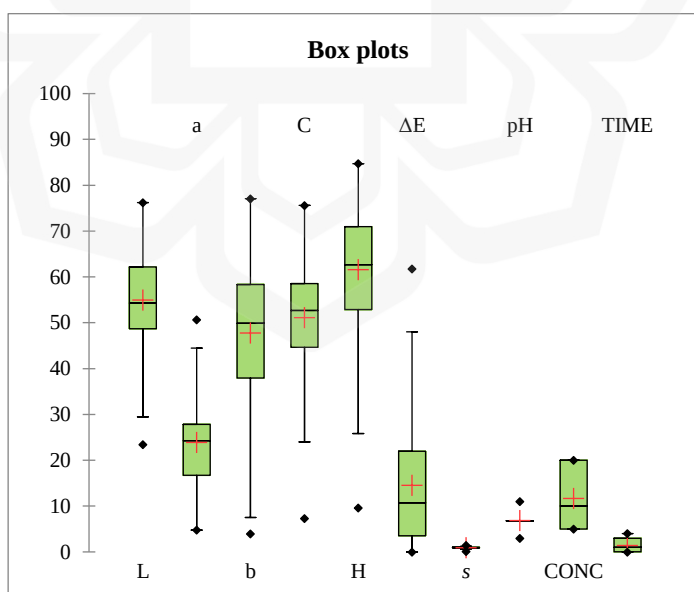


Figure 8.10 Box-and-whisker plots of chitosan-curcumin pigment chromaticity stability variables at different concentrations and time of exposure

Sampling adequacy for chitosan-curcumin pigment chromaticity stability data were further tested by using Kaiser-Meyer-Olkin (KMO) where the value must be greater than 0.5 followed by the Barlett's Test of Sphericity ($p < 0.05$) and Correlation Matrix Pearson where the value must be equal or greater than 0.01 (Atiqah et al., 2023). The KMO test was conducted to evaluate the dataset for adequacy at α of 0.05 to ensure the dataset is adequate for the multivariate data analysis. The KMO values for the 382 datasets produced a KMO score of 0.549 (Table 8.11) confirming the dataset is in mediocre category and adequate for PCA test. Barlett's Test of Sphericity is measured to ensure that the R-matrix does not equal the identity matrix. The p -value for the chitosan-curcumin pigment chromaticity stability data exhibited highly significant differences at 0.0001.

Table 8.11 Kaiser-Meyer-Olkin and Bartlett's test for the chitosan-curcumin pigment chromaticity stability

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.549
Bartlett's Test of Sphericity	Chi-square (Observed value)	3775.886
	DF	45
	p -value (Two-tailed)	< 0.0001

8.3.2 Correlation Coefficient Matrix (r^2)

Tables 8.12 and 8.13 revealed a positive and negative correlations strength between two variables among chitosan-curcumin pigment chromaticity stability test. A high positive correlation was identified among L versus H ($r^2 = 0.731$) b^* versus C, ($r^2 = 0.824$), b^* versus H ($r^2 = 0.802$) and C versus s ($r^2 = 0.741$). The tables also showed the relationship between 2 groups of L versus b^* ($r^2 = 0.624$), L versus C ($r^2 = 0.638$), b^* versus s ($r^2 = 0.506$), and C versus H ($r^2 = 0.692$) that produced moderate positive correlation. The significant negative correlation only at moderate strength shows among H versus a^* ($r^2 = -0.659$), ΔE

versus b^* and C^* ($r^2 = -0.626$ and -0.505 respectively), and concentration versus L^* and H ($r^2 = -0.515$ and -0.578 respectively). In general, the chitosan-curcumin pigment chromaticity stability variables can be grouped into three clusters of positive correlations. Whereas the chitosan-curcumin samples were scattered without any cluster as indicated in biplot (Figure 8.11).

Table 8.12 Correlation coefficient matrix for the chitosan-curcumin pigment chromaticity stability

Variables	L	a	b	C	H	ΔE	s	PH	CONC	TIME
L	1									
a	-0.445	1								
b	0.624	-0.179	1							
C	0.638	-0.187	0.824	1						
H	0.731	-0.659	0.802	0.692	1					
ΔE	-0.335	-0.064	-0.626	-0.505	-0.397	1				
s	-0.015	0.144	0.506	0.741	0.273	-0.349	1			
pH	-0.010	0.082	0.062	0.009	-0.017	0.002	0.012	1		
CONC	-0.515	0.462	-0.414	-0.429	-0.578	-0.131	-0.115	0.000	1	
TIME	-0.305	0.225	-0.171	-0.264	-0.232	0.282	-0.090	0.015	0.000	1

Values in bold are different from 0 with a significance level $\alpha=0.01$

Table 8.13 evaluated the correlation coefficient (r^2) or the strength of the correlation between two variables among chitosan-curcumin pigment chromaticity stability test. The correlation coefficient is measured on a scale from -1 to +1 as described by Guilford (1973).

Table 8.13 Correlation coefficient (r^2) strength between two variables among chitosan-curcumin pigment chromaticity stability test.

Variables	r^2	Size of Correlation	Interpretation
-	-	0.90 to 1.00	Very high positive correlation
b x C b x H C x s L x H	0.824 0.802 0.741 0.731	0.70 to 0.90	High positive correlation
C x H C x L b x L b x s	0.692 0.638 0.624 0.506	0.50 to 0.70	Moderate positive correlation
C x a	0.462	0.30 to 0.50	Low positive correlation
-	-	-0.70 to -0.90	High negative correlation
H x a ΔE x b ΔE x C Conc x L Conc x H	-0.659 -0.626 -0.505 -0.515 -0.578	-0.50 to -0.70	Moderate negative correlation
L x a Conc x C Conc x b ΔE x H ΔE x s ΔE x L t x L	-0.445 -0.429 -0.414 -0.397 -0.349 -0.335 -0.305	-0.30 to -0.50	Low negative correlation
t x ΔE s x H t x a s x a pH x a pH x b pH x t pH x s pH x C pH x ΔE pH x Conc. t x Conc. t x C t x H b x a t x b ΔE x C Conc x s t x s ΔE x a pH x H L x s L x pH	0.282 0.273 0.225 0.144 0.082 0.062 0.015 0.012 0.009 0.002 0.000 0.000 -0.264 -0.232 -0.179 -0.171 -0.131 -0.115 -0.090 -0.064 -0.017 -0.015 -0.010	0.00 to 0.30 (0.00 to -0.30)	Little if any correlation
Values in bold are different from 0 with a significance level $\alpha=0.01$			

8.3.3 Principal Component Analysis (PCA)

The PCA revealed two principal components (PCs) entailing 10 variables that represent cumulative variability of 60.73%. The eigenvalues (EV) were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 4.274, and for PC2 was 1.799 as showed in Table 8.14. Referring to the Table 8.14, L, b*, C, and H had strong FL (FL > 0.75) whereas for moderate Factor Loading FL ($0.500 < FL < 0.749$), it was proved from variables of a, s and concentration. The first principal PC1 identified for 42.73% of the total variance L, b*, C and H, whereas for PC2, it demonstrated moderate positive correlation, comprising 17.99% of the total variance for the data. The PCA analysis was conducted to identify the factors and proved in chitosan-curcumin pigment stability.

Table 8.14 The principal components factor loading and eigenvalues of the correlation matrix for chitosan-curcumin pigment chromaticity stability

Variables	PC1	PC2
L	0.791	-0.308
a	-0.465	0.715
b	0.896	0.239
C	0.897	0.294
H	0.913	-0.249
ΔE	-0.551	-0.549
s	0.482	0.644
pH	0.001	0.112
Concentration	-0.577	0.509
Time	-0.350	-0.020
Eigenvalue	4.274	1.799
Variability (%)	42.736	17.991
Cumulative (%)	42.736	60.727

The PCA analysis allows for identifying factors and provides insight in chitosan-curcumin pigment stability. Therefore, factor loadings can be utilised to determine the strength of the association between variables in chitosan-curcumin pigment chromaticity stability test. The degree of correspondence or their potential between the variables can be further explained in Figure 8.11. All variables are grouped into positive and negative correlated, that is depends on the close together of the variables. All variables were positively correlated as long as they were grouped close together.

Figure 8.11 showed the variables C and b* were grouped in group 1, whereas for group 2, the variables L and H was identified and a* and concentration were group together as group 3. Group 2 and group 3 were negatively correlated as there were at the opposite positioned. Whereas s variable shows negative correlation towards ΔE . The biplot in Figure 8.11 exhibited mixed and scattered samples with no clear cluster. It can be concluded that different chitosan-curcumin concentrations from 1 to 3 g/L did not necessary correlate with the curcumin chromaticity stability. In general, the variables for chitosan-curcumin pigment chromaticity stability behaviour blends under various conditions, particularly in terms of colour stability.

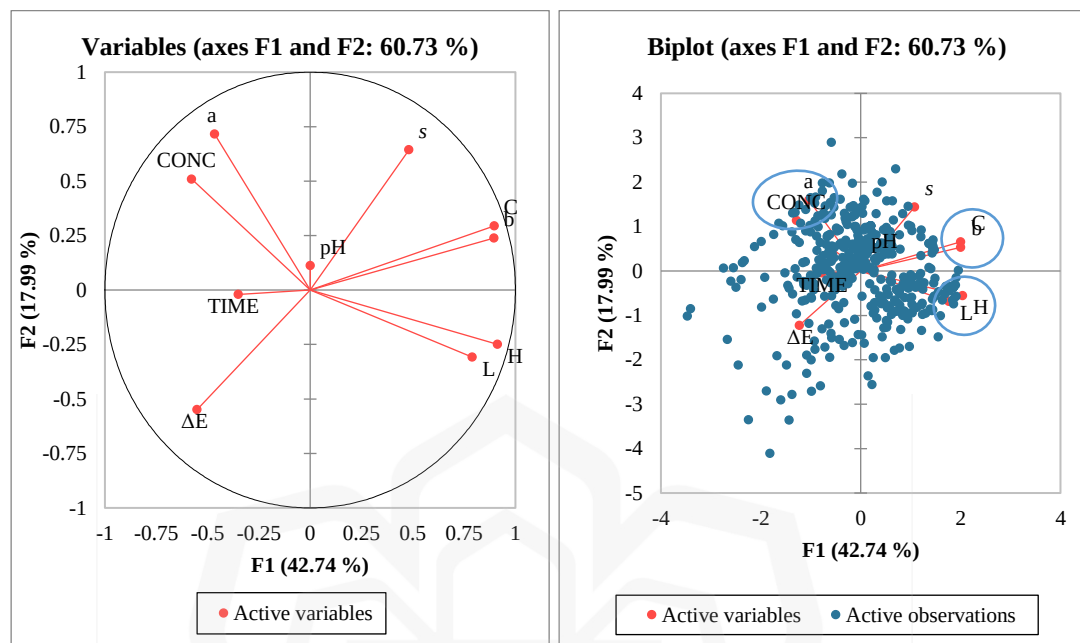
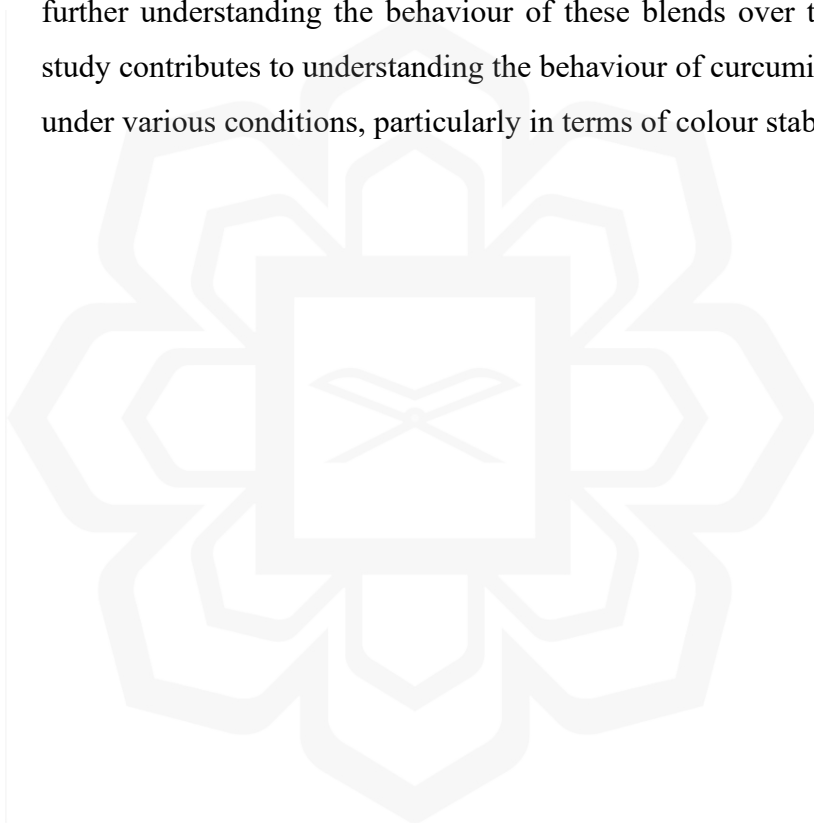


Figure 8.11 Variables plot and correlation biplot for chitosan-curcumin pigment stability test variables

8.4 SUMMARY OF THE RESULTS

1. The study demonstrates the highest degradation in 10 g/L of chitosan-curcumin exposed under light conditions, whereas in dark conditions, the lowest degradation is in chitosan-curcumin 5 g/L.
2. The highest degradation was in 5 g/L of NaCl in 5 g/L of chitosan-curcumin concentration when exposed under light conditions, whereas for dark conditions, the least degradation was in 20 g/L of NaCl in 20 g/L of chitosan-curcumin concentration.
3. pH 7 had the highest degradation in 10 g/L of chitosan-curcumin concentration in the light condition and showed the least degradation in the dark condition at pH 11 in 10 g/L of chitosan-curcumin concentration.

4. For UV-B irradiation, the highest degradation in 20 g/L of chitosan-curcumin for s values, and the highest degradation in 20 g/L of chitosan-curcumin at 30 °C of temperature for heat treatment.
5. Study highlights the influence of curcumin on the colour, water solubility, and mechanical properties of chitosan blends, making it a promising additive for film applications.
6. The statistical analysis supports the validity of the findings and provides insights into the relationships between various colour variables, aiding in further understanding the behaviour of these blends over time. Overall, the study contributes to understanding the behaviour of curcumin-chitosan blends under various conditions, particularly in terms of colour stability.



CHAPTER NINE

ASSESSMENT OF CURCUMIN CHROMATICITY STABILITY MODEL SYSTEM IN NATURAL SOAP AS AN ADDITIVE

9.1 INTRODUCTION

Current trends in consumer preference indicate an increased demand for natural ingredients in skin care and cosmetic products. This has led to an increase in the economy of small-scale homemade (artisanal) industries that offer a wide range of products with a variety of natural ingredients. One skincare product with natural ingredients that is now in vogue is natural bar soap. The global market for bar soap has been studied in key regions, including North and South America, Europe, China, Japan, India, the Middle East, and Africa. The market for bar soap in the Asia-Pacific Region is likely to show remarkable growth. Bar soap made from natural ingredients, such as vegetable oil, does not dry out the skin because this soap type contains glycerin. Natural herbal soap ingredients are generally vegetable or saponification oil (even animal fat), glycerin, natural fragrance, various organic additives, and sodium hydroxide. Natural herbal soaps usually contain a mixture of coconut oil, palm oil, olive oil, rice bran oil, and sunflower seed oil (Araseretnam and Venujah, 2019). The distribution of saturated and unsaturated fatty acids determines the hardness, aroma, cleansing, power, foaming, and moisturising ability of the soap. The properties of the resulting soap are also further influenced by the additives or fillers that are used. One common additive used in soap-making is a natural colorant (Moosavi, 2017). The production of solid bath soap using coconut oil and curcumin extracted from *C. xanthorrhiza* Roxb. as a natural colorant has not yet been reported. Therefore, the objective of the present research was to assess a soap formula using coconut oil and sodium hydroxide as the main raw materials and curcumin as an additive, resulting in a solid soap that contains glycerin and curcumin. The chromaticity stability properties of the resulting

solid bath soap, including the curcumin content, were analyzed using UV-Vis spectrophotometer and CIELAB colour parameters.

9.2 RESULTS AND DISCUSSION

9.2.1 Effect of light, pigment concentration and time of exposure on curcumin stability in natural soap

The chromaticity measurements of curcumin-soap pigment from 0.25 g/L to 2.5 g/L with the time of exposure from week 0 until week 4 in conditions of light and dark were presented in Figure 9.1. The results of chromaticity established that ΔE^* value > 5.0 for the concentrations from 0.5 g/L to 2.5 g/L exposed in response to light indicated clearly visible of two different colours, except for the 0.25 g/L of curcumin concentration, which estimated < 5.0 , that showed a clear difference in colour is noticed. Curcumin-soap pigment was observed for yellow intensity (b^*) and colour saturation (s) degradation percentage for all the concentrations and time of exposure from week 0 to week 4 developing under light and dark conditions (Table 9.1). The highest degradation of s (53.01%) and b^* (100%) was observed in 0.25 g/L in response to the light condition, whereas the curcumin pigment with the lowest degradation was found in the highest concentration of curcumin-soap (2.5 g/L) in response to the dark condition, with the percentage of degradation being s (16.26%) and b^* (0%). From the results in Table 9.2, it showed that the colour degradation of soap-curcumin was significantly fading due to exposure to light in which the colour change to the lighter tone after 4 weeks. If curcumin is exposed to sunshine, its breakdown can occur significantly more quickly (Wang et al., 2023a; Laneri et al., 2023b).

It is evident from the stains that curcumin causes, which are rapidly cleared when exposed to sunlight. The excited state of curcumin is what causes the degradation. It has

been claimed that curcumin's photobiological and photodynamic activities are what cause it to produce singlet oxygen and other reactive oxygen species upon photoexcitation (Praditya et al., 2019c). It is brought on by curcumin's degradation during photoexcitation, which is assisted by curcumin's triplet excited state (Kotha and Luthria, 2019b). According to reports from photophysical studies, curcumin's triplet excited state has a lifetime measured in microseconds, which aids in the rapid disintegration process and competes with the creation of singlet oxygen. The pace at which curcumin is biosynthesised during storage can have an impact on the content of curcumin. It is brought on by elements in the stored circumstances, such as air, humidity, sunlight, and room temperature for a certain amount of time. Curcumin's instability and susceptibility to light-induced degradation have also been demonstrated (Schieffer, 2002; Victor Thingujam and Saswat Nayak, 2019). It has been demonstrated that curcumin's photobiological and photodynamic activity is primarily caused by its photodegradation, which involves the oxygen creation of excited states and the generation from singlet oxygen (Priyadarsini, 2014b; Tonnesen et al., 1987).

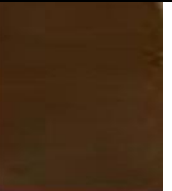
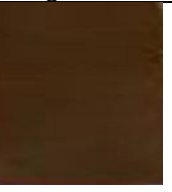
As a result, it was suggested that curcumin's degradation would occur after photoexcitation, which would be aided by curcumin's triplet excited state (Priyadarsini, 2014b). Curcumin, which has strong anti-inflammatory and antioxidant properties, has been utilised extensively in the cosmetics sector for several decades. It displays a broad range of beauty treatments, such as those for the face, lips, skin, hair, and nails, which have positive effects on ageing, inflammation, UV light damage, hair loss, and lip and nail care. Curcumin is used in cosmetics to shield the skin from the effects of moisture retention, anti-aging, sunscreen, and anti-wrinkles (Friedrich and Gokulakrishnan, 2015; Kaur and Saraf, 2011). When exposed to a variety of extreme environmental factors, such as UV and infrared radiation, physical strain, and chemical pollution, curcumin degrades. This process helps shield the skin from these damaging factors by preventing the production of oxygen free radicals and lipid peroxidation.

The skin's cellular absorption and penetration behaviour can be improved by the curcumin-loaded formulations by increasing their stability and bioavailability (Coradini et al., 2014; Friedrich and Gokulakrishnan, 2015). From the results, it showed that, in the presence of light, the degradation was much higher as compared to the absence of light. The chemical stability of curcumin under various processing and storage environments. Previous research has demonstrated that curcumin is susceptible to autooxidation, exposure to reactive oxygen species (Gordon and Fagundes-Klen, 2015; Schneider et al., 2015), and exposure to light (Priyadarsini, 2009b; Priyadarsini, 2014b). The physical state of curcumin and the circumstances of the light source are shown to have an impact on the composition and relative abundance of the resulting products (Tonnesen et al., 1986a; Huiying Amelia Zhang et al., 2023).

Table 9.1 Effects of exposure for 4 week in light and dark condition on colour degradation of soap-curcumin

Degradation percentage (%)							
CIE L*a*b*	Condition	Curcumin concentration (g/l)					
		0.25	0.5	1.0	1.5	2.0	2.5
Colour saturation (s)	Light	53.01	54.28	59.53	38.53	56.26	39.09
	Dark	28.08	27.70	29.31	21.54	21.58	16.26
Yellow intensity (b*)	Light	100	98.33	81.13	36.54	57.25	26.11
	Dark	31.20	27.99	21.21	0.32	9.56	0
Chromaticity (ΔE^*_{Lab})	Light	> 5.0					
	Dark	< 5.0	> 5.0	> 5.0	> 5.0	> 5.0	> 5.0
ΔE^*_{Lab} value	Indication						
< 1.0	No colour difference is visible						
< 2.0	colour difference is only slightly visible						
< 3.5	noticeable colour difference						
< 5.0	clear difference in colour is noticed						
> 5.0	indicate clearly two different colours						

Table 9.2 Effects of exposure for 4 week in light and dark condition on colour degradation of chitosan-curcumin

LIGHT / WEEK 0					
0.25 g/L	0.5 g/L	1.0 g/L	1.5 g/L	2.0 g/L	2.5 g/L
					
b* 8.28	b* 13.41	b* 19.24	b* 24.79	b* 28.89	b* 22.95
LIGHT / WEEK 4					
0.25 g/L	0.5 g/L	1.0 g/L	1.5 g/L	2.0 g/L	2.5 g/L
					
b* -2.05	b* 0.22	b* 3.63	b* 15.73	b* 12.35	b* 16.96
DARK / WEEK 0					
0.25 g/L	0.5 g/L	1.0 g/L	1.5 g/L	2.0 g/L	2.5 g/L
					
b* 12.04	b* 17.88	b* 25.75	b* 24.95	b* 31.25	b* 18.07
DARK / WEEK 4					
0.25 g/L	0.5 g/L	1.0 g/L	1.5 g/L	2.0 g/L	2.5 g/L
					
b* 8.28	b* 12.87	b* 20.29	b* 24.87	b* 28.26	b* 2.59

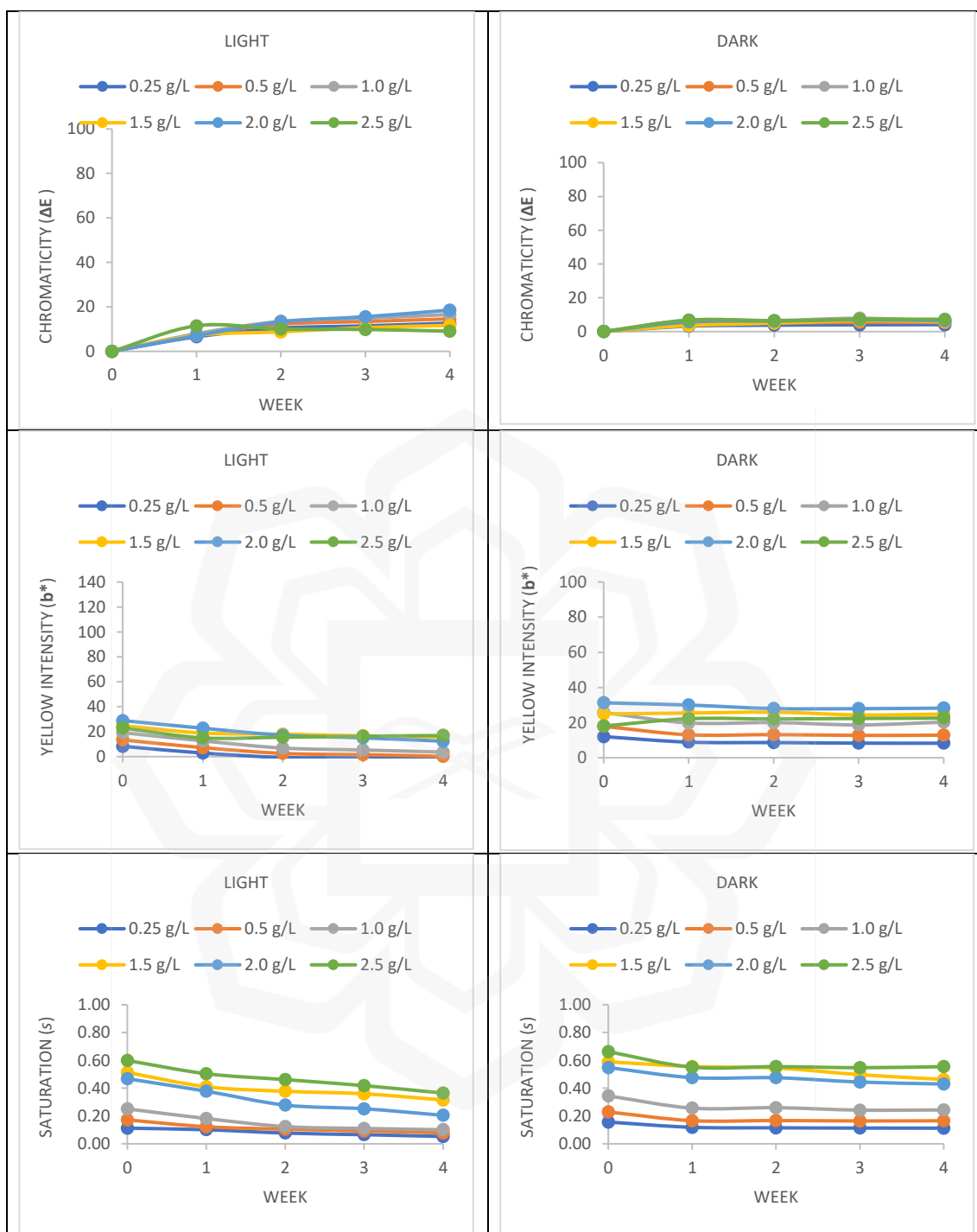


Figure 9.1 CIE L*a*b* colour difference or chromaticity (ΔE^*_{Lab}), yellow intensity (b^*), and colour saturation (s) values for different concentrations of curcumin in light and dark condition for different exposure of time in natural soap

9.3 MULTIVARIATE DATA ANALYSIS

9.3.1 Sampling Adequacy for Multivariate Analysis

The results from 9 variables were analysed using multivariate data analysis to determine the significant variables contributing to curcumin pigment chromaticity stability in solid bath soap. Data transformation is a component of the initial preparation of data prior to statistical analysis. The entire data set was normalised through the use of variable transformation. Figure 9.2 revealed the box and whisker plot to show the comparison in distributions of variables graphically to identify outliers. Outliers detected at H in this boxplot signify unusual values representing the overall data distribution. In this plot, the outliers remained as the data is significant to show the highest values for each variable. The median score of H (hue) is the highest, and s (saturation) is the lowest.

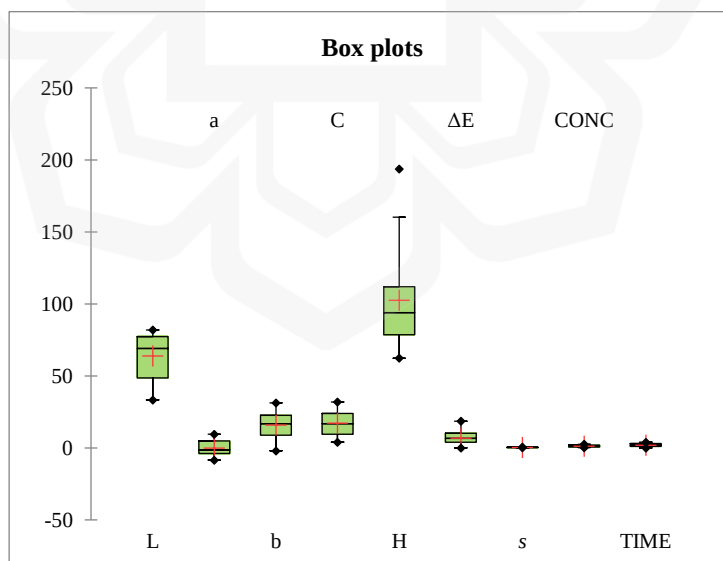


Figure 9.2 Box-and-whisker plots of curcumin pigment in solid bath soap chromaticity stability variables at different concentrations and time of exposure

Sampling adequacy for curcumin pigment in solid bath soap chromaticity stability data were further tested by using Kaiser-Meyer-Olkin (KMO) where the value must be greater than 0.5 followed by the Barlett's Test of Sphericity ($p < 0.05$) and Correlation Matrix Pearson where the value must be equal or greater than 0.01 (Atiqah et al., 2023). The KMO test was conducted to evaluate the dataset for adequacy at α of 0.05 to ensure the dataset is adequate for the multivariate data analysis. The average KMO value was ranked as detailed by Ismail et al. (2021a). The KMO values for the 378 datasets produced a KMO score of 0.604 (Table 9.3) confirming the dataset is in mediocre category and adequate for PCA test. Barlett's Test of Sphericity is measured to ensure that the R-matrix does not equal the identity matrix. The p-value for the curcumin pigment chromaticity stability data exhibited highly significant differences at 0.0001.

Table 9.3 Kaiser-Meyer-Olkin and Bartlett's test for the PLA-curcumin pigment chromaticity stability

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.604
Bartlett's Test of Sphericity	Chi-square (Observed value)	1062.777
	DF	36
	p-value (Two-tailed)	< 0.0001

9.3.2 Correlation Coefficient Matrix (r^2)

Table 9.4 and 9.5 revealed a positive and negative correlations strength between two variables among curcumin pigment in solid bath soap chromaticity stability test. A very strong positive correlation was observed between C^* with b^* , and a^* ($r^2 = 0.976$ and 0.842), s with a^* , C^* , and b^* ($r^2 = 0.974$, 0.888 , and 0.843), and concentration with s and a^* ($r^2 = 0.851$ and 0.809). Whereas other variables such as H with L ($r^2 = 0.659$), concentration

with C* and b* ($r^2 = 0.692$ and 0.671) as well as time with ΔE ($r^2 = 0.668$) have moderate positive correlations respectively. The tables also showed a high negative correlation between H with a*, b*, and C* ($r^2 = -0.876$, -0.873 , and -0.813), a* with L ($r^2 = -0.833$), s with L and H ($r^2 = -0.861$ and -0.834), and concentration with L and H ($r^2 = -0.873$ and -0.717). It can be concluded that the curcumin concentrations in solid bath soap from 0.25 to 2.5 g/L has correlation with the curcumin chromaticity stability. In general, the curcumin pigment in solid bath soap chromaticity stability variables can be grouped into two clusters with positive correlations and another three variables with negative correlations as indicated in Figure 9.3.

Table 9.4 Correlation coefficient matrix for the curcumin pigment in solid bath soap chromaticity stability

Variables	L	a	b	C	H	ΔE	s	CONC	TIME
L	1								
a	-0.833	1							
b	-0.535	0.818	1						
C	-0.564	0.842	0.976	1					
H	0.659	-0.876	-0.873	-0.813	1				
ΔE	-0.014	-0.426	-0.485	-0.475	0.471	1			
s	-0.861	0.974	0.843	0.888	-0.834	-0.340	1		
CONC	-0.873	0.809	0.671	0.692	-0.717	0.064	0.851	1	
TIME	0.018	-0.254	-0.270	-0.313	0.278	0.668	-0.241	0.000	1

Values in bold are different from 0 with a significance level $\alpha=0.01$

Table 9.5 evaluated the correlation coefficient (r^2) or the strength of the correlation between two variables among curcumin pigment chromaticity stability test. The correlation coefficient is measured on a scale from -1 to +1 as described by Guilford (1973).

Table 9.5 The correlation coefficient (r^2) strength between two variables among curcumin pigment in solid bath soap chromaticity stability test.

Variables	r^2	Size of Correlation	Interpretation
C x b s x a	0.976 0.974	0.90 to 1.00	Very high positive correlation
s x C Conc x s s x b C x a b x a Conc x a	0.888 0.851 0.843 0.842 0.818 0.809	0.70 to 0.90	High positive correlation
Conc x C Conc x b t x ΔE H x L	0.692 0.671 0.668 0.659	0.50 to 0.70	Moderate positive correlation
ΔE x H	0.471	0.30 to 0.50	Low positive correlation
H x b Conc x L s x L s x H a x L H x C Conc x H	-0.873 -0.873 -0.861 -0.834 -0.833 -0.813 -0.717	-0.70 to -0.90	High negative correlation
C x L b x L	-0.564 -0.535	-0.50 to -0.70	Moderate negative correlation
ΔE x b ΔE x C ΔE x a s x ΔE t x C	-0.485 -0.475 -0.426 -0.340 -0.313	-0.30 to -0.50	Low negative correlation
t x H ΔE x Conc. t x L t x Conc. ΔE x L t x s t x a t x b	0.278 0.064 0.018 0.000 -0.014 -0.241 -0.254 -0.270	0.00 to 0.30 (0.00 to -0.30)	Little if any correlation
Values in bold are different from 0 with a significance level $\alpha=0.01$			

9.3.3 Principal Component Analysis (PCA)

The next step involved analyzing the data set using PCA to identify variables with high factor loadings that explain the principal components significantly. A factor loading greater than 0.75 was considered strong, between 0.50 and 0.75 was moderate, between 0.30 and 0.49 was weak, and less than 0.30 was negligible (Rani et al., 2017). Eigenvalues were used to determine the principal components with more cumulative dataset variations. An eigenvalue of 1 or greater was considered significant for the PCA (Le et al., 2017; Zubir et al., 2016a; Yang et al., 2020a). PCA has been applied to the 10 variables taken from curcumin pigment chromaticity stability test to study which variables significantly contributed to curcumin pigment stability as detailed by Siudek and Frankowski (2018) and Vuong et al. (2020). The PCA exposed two principal components (PCs) entailing 9 variables that represent cumulative variability of 86.83%. The eigenvalues (EV) were used to determine the principal components (PCs) with more cumulative dataset variations. The EV obtained for PC1 was 6.034, and for PC2 was 1.78 as showed in Table 9.6. In principle, variables far away from the axes F1 and F2 had a strong factor loading (FL). Referring to the Table 9.6, all variables (L, a*, b*, C*, H, s, concentration, and ΔE) had strong FL (FL > 0.75).

Table 9.6 The principal components factor loading and eigenvalues of the correlation matrix for PLA-curcumin pigment chromaticity stability

Variables	PC1	PC2
L	-0.792	-0.458
a	0.970	0.051
b	0.913	-0.133
C	0.924	-0.128
H	-0.919	0.068
ΔE	-0.438	0.835
s	0.976	0.113
Concentration	0.832	0.467
Time	-0.314	0.775
Eigenvalue	6.034	1.780
Variability (%)	67.050	19.777
Cumulative (%)	67.050	86.827

The first principal PC1 accounted for 67.05% of the total variance, which comprised strong factor loadings of L, a, b, C, H, s, and concentration. Whereas for PC2, ΔE and time were the strong variables identified, comprising of 19.77 % of the total variance for the data. The PCA analysis allows for identifying factors and provides insight in curcumin pigment stability. Therefore, factor loadings can be utilised to determine the strength of the association between variables in soap-curcumin pigment chromaticity stability test. The degree of correspondence or their potential between the variables can be further explained in Figure 9.3. Figure 9.3 summarised that all variables were either positively or negatively correlated to each other can be grouped into three groups. Group 1 consist of b and C, group 2 is a and s, while group 3 is ΔE and time.

All variables were positively correlated as long as they were grouped close together. Whereas, group 1 variables were negatively correlate with H as there were at the opposite positioned. The others were no correlation. The samples cluster positioned at the right side of F1 axes and within group 1, 2, and 3 variables were all samples of soap-curcumin at 1.5 g/L to 2.5 g/L concentration which confirmed to be the most potential concentration for

curcumin pigment stability application for soap since their colour were maintained with minimum degradation after 4 weeks being exposed under light condition.

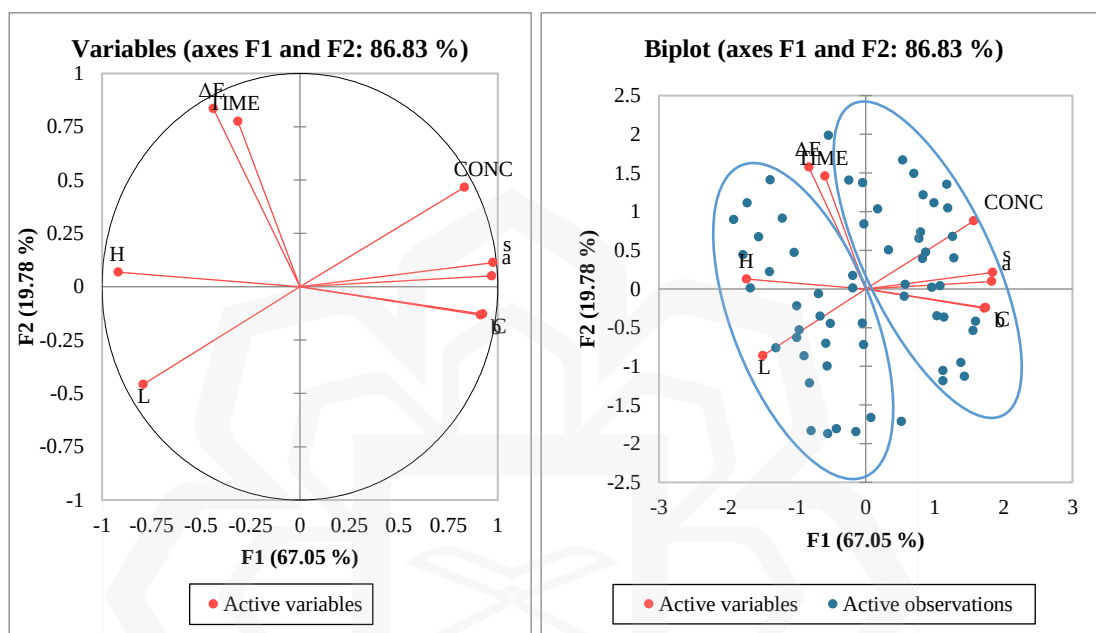


Figure 9.3 Variables plot and correlation biplot for PLA-curcumin pigment stability test variables

9.4 SUMMARY OF THE RESULTS

1. In 0.25 g/L of soap-curcumin, the highest degradation occurred under light conditions, whereas in dark conditions, the least degradation occurred in 2.5 g/L of soap-curcumin.
2. The chromaticity (ΔE^*) values > 5.0 , indicate clearly two different colours when exposed under light condition in all different soap-curcumin concentrations tested.

3. The degradation process seems to be accelerated by exposure to sunlight, leading to a rapid breakdown of curcumin molecules and subsequent loss of colour.
4. These findings are significant in various applications, particularly in cosmetics where curcumin is utilised for its anti-inflammatory and antioxidant properties. Understanding the degradation behaviour of curcumin under different environmental conditions, such as exposure to light, can help in formulating more stable cosmetic products that retain curcumin's beneficial effects over time.
5. The application of principal component analysis (PCA) to the dataset allows for a deeper understanding of the relationships between various colorimetric parameters (L^* , a^* , b^* , C^* , h^* , and s^*) and curcumin concentration in bar soap under light treatment. This statistical approach aids in identifying patterns and correlations within the data, providing insights into how different factors interact to influence colour stability.

CHAPTER TEN

GENERAL DISCUSSION AND CONCLUSION

10.1 RESEARCH AIM AND OBJECTIVES

The aim of this research is to explore curcumin pigment extracted from natural resources, specifically *Curcuma xanthorrhiza* Roxb. (temu lawak) rhizomes as potential application in halal food packaging materials and cosmetics. For the purpose of achieving the aims of the research, several objectives have been set. The first and second objectives have been investigated in Chapter 4. Through the extraction of an acetone and methanol mixture, NaOH solution and DMSO were used for solvent extraction of 12 ginger species with different rhizome flesh colours and antioxidant properties and antimicrobial activities for the selected ginger species. Then Chapter 5 has explored the topic further by studying the chromaticity stability properties of curcumin extracted from Temu Lawak (*Curcuma xanthorrhiza* Roxb.). Next, chapters 6, 7, and 8 have allowed the fourth objective to be achieved by investigating the effect of visual indicators on colour changes of curcumin-packaging material (PLA, PVA, and chitosan) in response to cumulative exposure to stress conditions. Finally, the effect of visual indicators on colour changes of curcumin-bar soap in response to cumulative exposure to stress conditions has been assessed.

10.1.1 Curcumin Properties, Stability and Potential Application

The stability of curcumin is significantly influenced by several environmental factors, including pH, temperature, light exposure, and concentration. It is particularly prone to degradation under alkaline conditions, where it rapidly breaks down into various

compounds, such as vanillin and ferulic acid. Research has demonstrated that curcumin maintain its structural integrity and therapeutic efficacy most effectively in acidic environments (pH 1-6). A study conducted by Ahmad et al. (2014a) revealed that curcumin's half-life diminishes as pH increases, with rapid degradation occurring in alkaline conditions (pH > 7). Furthermore, Priyadarsini et al. (2014d) noted that exposure to UV light compromises curcumin's stability, leading to photodegradation and a subsequent reduction in its antioxidant properties. Temperature is another critical factor, as significant deterioration occurs at elevated temperatures, particularly those exceeding 70 °C. Understanding these stability determinants is crucial for enhancing curcumin's effectiveness in various applications.

Curcumin, a polyphenolic compound predominantly found in the *Curcuma xanthorrhiza* Roxb. (temu lawak) rhizomes is widely recognised for its extensive medicinal properties, including potent antibacterial, anti-inflammatory, and antioxidant effects. According to Priyadarsini et al. (2014f), curcumin's antioxidant activity is attributed to its ability to scavenge free radicals, thereby reducing oxidative stress, which is linked to various chronic diseases. Furthermore, curcumin demonstrates significant anti-inflammatory effects by inhibiting pro-inflammatory cytokines and enzymes, such as lipoxygenase (LOX) and cyclooxygenase-2 (COX-2), which play critical roles in inflammatory pathways (Singh et al., 2016). Additionally, curcumin has exhibited remarkable antimicrobial properties against a diverse array of bacteria, fungi, and viruses, positioning it as a promising candidate for therapeutic applications in infectious diseases (Khan et al., 2017). Its ability to modulate multiple molecular targets further enhances its multifaceted biological actions, suggesting a significant potential for curcumin in various therapeutic contexts. Curcumin, the principal bioactive compound extracted from *Curcuma xanthorrhiza* Roxb. (temu lawak) rhizomes, exhibits several remarkable characteristics that enhance its therapeutic potential.

As a polyphenolic compound, curcumin is well-known for its distinctive vibrant yellow hue, rendering it a valuable natural dye in both the food and cosmetics industries. It demonstrates significant solubility in organic solvents but shows limited solubility in aqueous environments, especially at lower pH levels. Curcumin is distinguished by its potent antioxidant properties, which enable it to scavenge free radicals and mitigate oxidative stress associated with numerous chronic diseases. In addition, curcumin possesses notable anti-inflammatory effects, primarily through the inhibition of key pro-inflammatory enzymes and cytokines, thereby modulating various inflammatory pathways. Its broad-spectrum antimicrobial activity against a range of bacteria, fungi, and viruses further enhances its attractiveness as a therapeutic agent.

However, curcumin also exhibits a degree of chemical instability, particularly under alkaline conditions and upon exposure to light, which can result in degradation and a subsequent reduction in its biological efficacy. Overall, the diverse characteristics of curcumin highlight its promising applications across medicine, nutrition, and various other fields. It serves as the principal active ingredient responsible for turmeric's distinctive yellow colouration and has been extensively investigated for its numerous health benefits. Numerous studies have highlighted the potential applications of curcumin, a bioactive compound extracted from the rhizome of *Curcuma xanthorrhiza* Roxb. (temu lawak), across various sectors. The medicinal properties of curcumin have been extensively investigated, particularly concerning its role in treating chronic diseases such as cancer and cardiovascular disorders. For instance, a systematic review by Zhang et al. (2015) demonstrated curcumin's ability to inhibit tumour growth and induce apoptosis in various cancer cell lines. Additionally, Bhandari et al. (2020) reported that curcumin significantly reduces inflammatory markers and enhances cardiovascular health by modulating lipid profiles and preventing arterial plaque formation. Characteristic positions curcumin as a potential therapeutic agent for inflammatory conditions such as arthritis and inflammatory bowel disease. Moreover, curcumin exhibits broad-spectrum antimicrobial activity against various pathogens. Khan et al. (2017) found that curcumin effectively inhibits the growth

of bacteria, fungi, and viruses, suggesting its potential use as a natural food preservative and as a treatment for infections.

In the nutritional sector, curcumin is increasingly recognized as an ingredient in functional foods and dietary supplements. Research by Wang et al. (2018b) indicated that curcumin supplementation could enhance antioxidant capacity and improve overall metabolic health, making it an appealing option for promoting health and preventing chronic diseases. Furthermore, curcumin's antioxidant properties make it valuable in the cosmetics industry. A study by Mikaili et al. (2013) demonstrated curcumin's effectiveness in protecting skin cells from oxidative stress, which contributes to improved skin health and reduced signs of aging. Finally, the potential of curcumin in developing biodegradable packaging materials is being explored. Zhang et al. (2020a) showed that curcumin-loaded films exhibited antibacterial properties, which may help extend the shelf life of food products while minimising environmental impact. Collectively, these findings underscore curcumin's diverse applications and significant therapeutic potential across multiple domains.

10.2 QUANTIFICATION OF CURCUMIN CONTENT FROM SELECTED GINGER SPECIES, ANTIOXIDANT PROPERTIES AND ANTIMICROBIAL ACTIVITIES

Curcuma xanthorrhiza Roxb. was selected for extraction and quantification using various extraction methods, and HPLC analysis was conducted to determine the curcumin pigment from selected ginger species, specifically curcumin. The findings have concluded that kunyit (*C. longa*), a dark orange-fleshed rhizome, was found to have the highest curcumin content ($578.38 \pm 6.37 \mu\text{g/g DW}$) through the alkaline extraction method, substantially higher than all other ginger species and extraction methods tested. The amount of curcumin,

the method of extraction, and the intensity of the yellow colour in the rhizome flesh are all positively correlated. From the previous study, it showed that the use of ethanol as a solvent can have a higher yield of curcumin extraction. It showed the turmeric species extracted from the pigmented rhizomes that are having a higher yield and higher curcumin concentrations extracted from alkaline methods (Sidek et al., 2023).

The ethanol technique was preferred for generating higher curcumin content in *C. xanthorrhiza*, indicating how the choice of solvent influences the extraction yield (Halim et al., 2012). The previous study is in contravention with this study, in which the solvent used in this study is aqueous sodium hydroxide. The bioactivities like antioxidant, antibacterial, and antifungal have been tested on curcumin. The dark orange rhizome flesh of kunyit (*C. longa*) demonstrated substantially the highest DPPH scavenging activity of 98.43% inhibition at the concentration of 1 g/L. It can be suggested that the rhizome flesh colour especially most pigmented had a major effect on the percentage of DPPH scavenging activity. This is consistent with previous observations that more pigmented species had stronger antioxidant capabilities due to increased curcumin levels. Several research support the hypothesis that pigmentation intensity correlates with curcumin and phenolic content, resulting in increased free radical scavenging action. (Sidek et al., 2023). The highest antimicrobial inhibitory activity was obtained from the extract of temu emas (*C. heyneana*) which recorded the lowest MIC values ranged from 2 to 3 µg/µL against all five pathogenic microorganisms, which is in line with the previous study confirming *C. xanthorrhiza*'s effectiveness in antibacterial testing. The ethanol and hexane extracts of *C. xanthorrhiza* have shown strong suppression of bacterial growth, with low MIC values against many diseases. (Sidek et al., 2023). Furthermore, the type of solvent is the most important factor in determining extraction efficacy (Jayakumar et al., 2023b; Zannou et al., 2023). These findings emphasise the possibility of manipulating curcumin content produced in ginger rhizome for genetic manipulation objectives.

10.3 CHROMATICITY STABILITY PROPERTIES OF CURCUMIN EXTRACTED FROM TEMU LAWAK (*Curcuma xanthorrhiza* Roxb.)

Curcumin is well-known for its numerous therapeutic characteristics, including significant antioxidant, anti-inflammatory, and antibacterial activities. Despite its positive characteristics, curcumin's stability poses a significant difficulty in a variety of applications, particularly because it is susceptible to deterioration under certain conditions. Curcumin's stability in aqueous medium is pH-dependent, as demonstrated by its colour shift at different pH levels. Curcumin turns red in solution at low pH levels (<1) due to proton predomination. Curcumin turns yellow at pH levels ranging from 1 to 7, indicating that its molecules are neutral. Curcumin turns orange when the pH is higher than 7.5.

From this study, the results revealed that pH greater than 7 and curcumin concentration greater than 1 g/L can cause colour degradation and decrease the stability even in the dark condition. Curcumin, a bis- α,β -unsaturated β -diketone, exhibits keto-enol tautomerism and *cis-trans* isomerism based on the acidic state of the solution (Manolova et al., 2014). The keto-form is the prevalent form at acidic and neutral pH levels, as well as within cells. At such pH levels, curcumin may contribute an H-atom because its keto-form has a very active carbon atom in the heptadienone bond between the two methoxyphenol rings (Sharma et al., 2001). This carbon exhibits very weak C-H bonds because the unpaired electrons on the nearby oxygens are delocalised. At alkaline pH, however, the enol form predominates, where curcumin transfers electrons in a process that represents its antioxidant scavenging properties. Curcumin's enol structure is stabilised by resonance-assisted hydrogen bonding (Kolev et al., 2005). The stability of curcumin is strongly influenced by pH. It breaks down quickly in neutral or alkaline situations and is more stable in acidic environments.

This is confirmed from the previous study which showed that curcumin degrades quickly in alkaline situations (pH >7) but remains stable in acidic conditions (pH 1-6)

(Ahmad et al., 2014b). According to their findings, curcumin degrades into ferulic acid, vanillin, and other degradation products in a matter of minutes at an alkaline pH. These results were supported by the study from Bansal et al. (2017), which found that curcumin's half-life dramatically drops as pH rises. They recommended encasing curcumin in materials that react to pH in order to maintain its stability in alkaline and neutral environments. Curcumin contains multiple chemically reactive functional groups, allowing it to engage in a variety of metabolic pathways through interactions such as donating or accepting H-bonds, cation binding, and the Michael reaction.

Curcumin pigment was more stable in UV-B at higher concentration as compared to UV-A. Curcumin pigment degradation can be reduced with NaCl treatment at higher concentration of 3 g/L in dark condition. From this study, it showed, Curcumin concentration at 3 g/L showed better stability towards heating treatment. This has shown from the previous studied curcumin's thermal degradation and found that substantial deterioration occurs at temperatures higher than 70°C (Chen et al., 2016). Curcumin is extremely sensitive to temperature and light. It breaks down when heated over a specific point and is accelerated by UV light exposure. It is related from the previous study by, curcumin quickly photodegrades in the presence of UV light, producing oxidative byproducts (Priyadarsini et al., 2014d). According to the study, curcumin loses its antioxidant properties when exposed to heat for an extended period of time. On the other hand, keeping it stored in a dark place preserves its stability.

10.4 CHROMATICITY STABILITY OF PLA-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS

Polymer colour stability and degradation are influenced by various environmental factors such as light exposure, salinity, pH, and temperature. Curcumin-loaded poly (lactic acid)

(PLA) coatings, in particular, exhibit significant sensitivity to these conditions, with light exposure being a primary factor inducing photodegradation. Light presence is the main factor that considerably effects polymer colour fluctuations and stability, according to experiments on salinity, pH, and light exposure stability. The application of (PLA) for curcumin stabilisation in food packaging has garnered interest because of the antioxidant qualities of curcumin and its biodegradable nature. Food products are physically protected by PLA-based packaging films coated with curcumin, which also prolongs their shelf life by preventing oxidation, microbial development, and other degradation processes. When curcumin is added to PLA films, it keeps its antioxidant qualities, which helps keep food items from oxidising, especially when the food is lipid- or oxygen-rich. Curcumin's antibacterial qualities also aid in preventing the growth of microorganisms. Photodegradation of the coating was induced by the curcumin-PLA's low resistance to light exposure. By using PLA encapsulation, curcumin's inherent sensitivity to UV light can be reduced, improving the packaging's ability to preserve its contents when exposed to light.

According to Li et al. (2018), PLA films coated with curcumin remained stable in the presence of UV radiation and high temperatures. In 10 g/L, the highest degradation occurred under light conditions, whereas in dark conditions, the least degradation occurred in 5 g/L. From this study, it can be related from the previous study, when curcumin was added to PLA sheets, it significantly degraded when exposed to UV light (Li et al., 2018). As curcumin broke down into byproducts like ferulic acid and vanillin, the researchers noticed that the film's capacity to protect food decreased. Curcumin's yellow colour diminished after prolonged light exposure, according to research by Ravindran et al. (2017), suggesting a breakdown in the compound's molecular structure. The research revealed that curcumin's antioxidant potential also decreased when exposed to UV light, perhaps affecting its power to prevent food from oxidising. Although curcumin is sensitive to light, it can be protected to some extent by being physically shielded from direct UV light exposure by PLA films.

On the other hand, variables like the PLA film's thickness, curcumin content, and transparency affect how protected an area is. Although PLA offers some protection, curcumin still slowly deteriorates when exposed to light for prolonged periods of time (Jouki and Khazaei, 2019). To improve the stability of curcumin, it has been suggested to add more UV-blocking compounds to the PLA matrix. Curcumin's photodegradation was slowed down by thicker PLA films, which also offered superior light shielding (Songtipya et al., 2022). To further lessen UV penetration, the study also recommended including light-absorbing or light-blocking materials (like titanium dioxide) into PLA. Curcumin's ability to protect food against oxidation and microbiological growth—two processes that are essential for prolonging its shelf life—is diminished by photodegradation. Therefore, the protective effects of the packaging are likewise reduced when curcumin is broken down. It has examined the application of curcumin in PLA-based active packaging and discovered that the films' decreased antioxidant activity during light exposure accelerated the oxidation of foods high in fat (Cano et al., 2019). The study stressed that to sustain curcumin's efficacy over time, better light shielding is required.

Curcumin-loaded PLA films exhibited antibacterial properties; however, these effects were significantly reduced upon exposure to light (Espitia et al., 2016). This implies that curcumin's capacity to prevent microbiological spoiling is impacted by light degradation in addition to its antioxidant qualities. The highest degradation was at pH 9 in 10 and 20 g/L, whereas no degradation from colour saturation (s) was shown at pH 3 in 10 g/L. Curcumin in PLA sheets degrades significantly in very alkaline settings (pH > 9), turning brown and losing its antibacterial and antioxidant properties. This colour shift denotes the disintegration of curcumin's molecular structure in addition to a pH shift. Curcumin breaks down quickly at high pH levels, resulting in brownish-colored metabolites (Priyadarsini et al., 2014d). PLA-curcumin films exposed to these kinds of conditions show a similar brown discolouration, which is indicative of the bioactivity of curcumin degrading. PLA-curcumin films that change colour from yellow to brown at high pH also lose their capacity to stop microbial development, which reduces their usefulness as protective packaging materials (Santos et al., 2020).

The highest degradation at 30 g/L of NaCl in 5 g/L of PLA-curcumin concentration occurred under light conditions. In light conditions, it influences the degradation of the colour of curcumin, even it is tested in the salinity treatment. Elevated salinity has the ability to modify the mechanical characteristics of PLA films, resulting in alterations to their tensile strength, flexibility, or deterioration. The structure and durability of the polymer matrix can be affected by interactions between salts like sodium chloride (NaCl) and the matrix. High salt concentrations have been demonstrated by marginally decrease PLA film tensile strength and elongation at break (Reis et al., 2018). Salts have the potential to lessen the molecular connections inside the polymer matrix, which results in this impact. However, depending on the film composition, the effect of salt on PLA's mechanical properties may be less noticeable when mixed with curcumin. The effects of varying salt concentrations on PLA-based films and found that, in short-term usage situations, extended exposure to high salinity caused slight structural alterations but did not significantly deteriorate the film's integrity (Li et al., 2020). Although curcumin is known to break down in some settings, its behaviour in saline environments is more stable than that of light sensitivity and pH. Curcumin is relatively stable in saline circumstances when added to PLA, while its release and bioactivity may be affected by excessive salt concentrations. When curcumin was added to PLA films, found that the compound maintained its antioxidant qualities in saline settings. High salt (about 5–10% NaCl) did not dramatically break down curcumin, according to the researchers, indicating that the PLA matrix offers sufficient protection (Jouki and Khazaei, 2019).

In this study, it showed that, the PLA-curcumin showed the highest degradation in 5 g/L, whereas for the heat treatment, the least degradation occurred in 5 g/L at 10 °C. PLA-curcumin films may undergo colour changes as a result of curcumin's thermal degradation when they are heated. This can be seen clearly and could be a sign of decreased bioactivity. PLA-curcumin films that were subjected to heat treatments discoloured, which was associated with a drop-in antioxidant activity (Cano et al., 2019). Curcumin's vivid yellow hue dimmed, signifying deterioration. Researchers have looked into a number of formulations to improve the heat stability of PLA-curcumin films, such as adding coatings

or additives that are resistant to heat and can help shield curcumin from thermal degradation during heat processing or pasteurization. Through the addition of additional natural chemicals, PLA-curcumin films that were heat-stable while retaining the functionalities of curcumin (Zhang et al., 2020b). Heat exposure can have a substantial effect on PLA-curcumin films. It can cause thermal degradation of the curcumin and PLA, which can change the colour and cause it to lose its bioactivity as well as its mechanical qualities. The inclusion of additives or altered formulas are just two of the techniques that should be investigated in order to improve the thermal stability of PLA-curcumin films for effective applications in food packaging.

10.5 CHROMATICITY STABILITY OF PVA-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS

Polyvinyl alcohol (PVA) films that contain curcumin have shown great promise as food packaging materials because of their biodegradability, flexibility, and bioactive qualities. PVA-based packaging gains additional functional advantages from curcumin, a naturally occurring antioxidant and antibacterial compound. PVA is an eco-friendly option for packaging because it is a biodegradable polymer. By including bioactive ingredients that aid in food preservation, curcumin can improve the sustainability of packaging materials. PVA films are flexible and have strong tensile strength, which makes them appropriate for a range of packaging uses. Curcumin can affect the mechanical characteristics, which could make the films more resilient. Curcumin's potent antioxidant qualities are well-known. It can aid in preventing oxidative deterioration of food products when added to PVA films, increasing their shelf life and preserving their quality. It is crucial to take into account how UV-B radiation affects PVA films that have curcumin added, particularly when it comes to food packing and storage. The degradation of the bioactive ingredient and the polymer matrix can occur due to UV-B radiation, which can affect the functional and physical aspects of PVA-curcumin films. From this study, it can be concluded that the study

indicates that PVA-curcumin pigment was having the highest degradation at 10 g/L in UV-B as compared to the other concentrations. Curcumin is photosensitive, especially to UV-B rays. Curcumin can photodegrade when exposed to UV-B radiation, producing a variety of degradation products. This may cause it to lose its colour, antioxidant capacity, and antibacterial effectiveness. Curcumin rapidly photodegrades under UV light, producing oxidative byproducts that diminish its effectiveness.

According to one study, UV radiation significantly lowers curcumin's antioxidant activity (Priyadarsini et al., 2014d). PVA films exposed to UV light changed in colour and mechanical characteristics. The scientists pointed out that these modifications might make the film less useful for uses in food packaging (Baskaran et al., 2019). UV stabilisers or absorbers can be added to PVA-curcumin films to assist prevent UV-induced deterioration. These additions have the ability to absorb UV light, which lessens its effect on curcumin and the polymer. Curcumin's antioxidant qualities were preserved when UV stabilizers were added to PVA-curcumin films, greatly increasing their resistance to UV-B radiation (Zhao et al., 2021). The study highlights the colour stability of curcumin pigment under UVB exposure and its potential application in improving the barrier qualities of biodegradable films for food packaging.

The present study the degradation of PVA-curcumin was highest at pH 11 in 20 g/L in light condition, whereas in dark condition, the least degradation was at pH 7 in 5 g/L of curcumin concentration. In applications involving food packaging, the impact of light exposure on PVA films containing curcumin is crucial to take into account. It is well known that curcumin is light-sensitive, particularly to UV light. Curcumin can degrade in the presence of light, losing both its antioxidant qualities and vivid yellow colour. Different mechanisms, such as photolysis and oxidative reactions, may cause this deterioration. Curcumin breaks down quickly in the presence of UV light, producing photodegradation compounds that may have an adverse effect on the drug's bioactivity and functionality (Priyadarsini et al., 2014d). According to extended exposure to light reduced the

antioxidant activity of films containing curcumin, indicating curcumin's sensitivity to light (Sharma et al., 2020).

PVA films showed colour changes and decreased mechanical strength when exposed to light, which could potentially impair their usefulness in packaging applications (Baskaran et al., 2019). The application of protective coatings for PVA-curcumin films, they discovered that these coatings dramatically decreased the rate at which curcumin deteriorated when exposed to light (Singh et al., 2020). Curcumin is less prone to photodegrade in the absence of light. As a result, curcumin in PVA films may be more stable than it would be if it were exposed to light. The colour and bioactive qualities of curcumin are better preserved in dark environments. Research has indicated that curcumin exhibits enhanced antioxidant and antibacterial characteristics when exposed to low light levels. For example, curcumin's antioxidant activity persisted to be stronger while held in dark circumstances as opposed to being exposed to light (Sharma et al., 2020).

Curcumin's breakdown is slowed down in dark environments, prolonging the bioactive properties of the substance. PVA-curcumin films maintained in a dark environment had noticeably stronger antioxidant activity than those exposed to light, suggesting that the characteristics of curcumin were better preserved (Nawaz et al., 2020). Because PVA-curcumin films are less likely to undergo photodegradation, which can result in changes to the films' colour, flexibility, and tensile strength, dark environments can aid in maintaining the mechanical integrity of the films. PVA films held in the dark maintained better mechanical properties over time than those exposed to light, that are influencing the structural integrity of the film (Baskaran et al., 2019).

One of curcumin's distinguishing qualities in a variety of applications, such as food packaging, is its vivid yellow to orange colour, which also serves as a natural dye. Curcumin is susceptible to changes in temperature, light, and pH, which can cause colour

deterioration. Curcumin's colour is degraded by heat in particular, and it's necessary to understand this degradation for applications like food packaging where stability and appearance are important. The study demonstrates the highest degradation in 20 g/L at 70 °C, which showed the importance of environmental factors, such as temperature, in influencing the stability of PVA-curcumin. This is in line from a previous study, whereby curcumin rapidly loses colour at high temperatures, and this process speeds up as the temperature rises over 70 °C (Priyadarsini et al., 2014a). It is also supported by another study, which found that PVA-curcumin films significantly discoloured during heat treatment, suggesting that the packing material had deteriorated and could affect how consumers perceive the film and its functional qualities (Giménez et al., 2017). Research has indicated that curcumin has a rather stable behaviour at mild temperatures (below 50 °C, for example). However, when temperatures rise, especially beyond 70 °C, the rate of degradation and colour loss increases. The degradation accelerates and becomes more noticeable above 100 °C. This study also supported by the previous study that observed that curcumin films exhibited substantial colour fading when exposed to temperatures above 80 °C for extended periods (Zhang et al., 2016b). At temperatures above 100 °C, complete colour loss was seen, which is consistent with the chemical structure of curcumin breaking down.

The performance and stability of PVA-curcumin films are significantly influenced by temperature, particularly when it comes to food packaging applications. Heat sensitive, curcumin loses a great deal of its stability at high temperatures. Increased heat can cause curcumin to thermally degrade, losing its colour, antioxidant capacity, and antibacterial effectiveness. Degradation products including ferulic acid and vanillin can also be produced when curcumin breaks down at high temperatures. Curcumin rapidly degrades at high temperatures, especially over 70°C, where its antioxidant ability drastically decreases (Ahmad et al., 2014c). The mechanical characteristics of PVA films can be greatly impacted by temperature variations. PVA may soften and lose its tensile strength and flexibility at high temperatures. This may cause the packing material to distort or burst, impairing its ability to protect. PVA films lose mechanical stability and become more

malleable at higher temperatures, which may have an effect on their suitability as materials for food packaging when exposed to heat (Sundar et al., 2019). Because curcumin is sensitive to heat and the PVA matrix may distort at high temperatures, PVA-curcumin films are not as ideal for packing hot foods. The protective qualities of the packaging may be compromised by the antioxidant and antibacterial qualities of curcumin losing their potency at high temperatures.

Salinity's impact on PVA-curcumin films must be taken into account, particularly in applications where food preservation is involved or in settings where the packaging may come into contact with salt or other saline conditions. Salinity can affect the film's overall performance as well as the mechanical characteristics and curcumin stability. The study reveals the highest degradation at 5 g/L of NaCl concentration in 5 g/L of PVA-curcumin concentration in light conditions, whereas the least value is at 30 g/L of NaCl concentration in 10 and 20 g/L of PVA-Curcumin concentration. The presence of salt ions may have an impact on the solubility and stability of curcumin that caused the environments with high salinity may encourage curcumin aggregation, which over time may result in diminished activity and discolouration (Reddy et al., 2017). Curcumin deteriorated more quickly in conditions with higher ionic strength, particularly when oxidative stress was present, (Ahmad et al., 2014c). Conditions that are salinized could accelerate this deterioration process. Given their possible use in food packaging, PVA-curcumin films' resilience in the dark is a crucial factor to take into account.

Although curcumin is susceptible to temperature, light, and acidity, darkness can provide a more stable habitat for it. Due of curcumin's extreme sensitivity to light, especially UV light, photodegradation may result. Curcumin is shielded from light's damaging effects while it is dark, helping to maintain its chemical structure, colour, and bioactive qualities. PVA-curcumin films are therefore more stable and continue to work when there is no light for a longer period of time. From this study, it revealed that, the highest degradation occurred at 10 g/L in a light condition, whereas in a dark condition the

least degradation also occurred at 10 g/L in a dark condition. According to one previous report, curcumin is largely stable in the dark but breaks down quickly in the presence of light, especially UV radiation (Priyadarsini et al., 2014d). This suggests that curcumin's structural integrity and bioactive qualities can be preserved by storing PVA-curcumin films in dark conditions.

Curcumin's colour does not change in the dark because light does not create any oxidation or deterioration. This preserves the PVA-curcumin films' aesthetic appeal, which is beneficial in food packaging applications where aesthetics is crucial. Curcumin films stored in the dark held their colour much longer than those exposed to light, which started to fade after a few hours of exposure (Wang et al., 2018a). Curcumin cannot degrade in a dark environment, therefore food products are continuously protected against oxidation and microbiological contamination. The oxidation that results in colour deterioration can be avoided with dark storage. PVA-curcumin films kept in the dark effectively maintained the bioactive qualities of curcumin, allowing perishable foods like fruits and vegetables to last longer on the shelf (Kumar et al., 2021). PVA-curcumin films show noticeably greater stability in dark settings than in light-exposed ones. Curcumin retains its colour, mechanical integrity, and bioactivity (antioxidant and antibacterial qualities) when there is no light present because photodegradation is inhibited. PVA-curcumin films are therefore ideal for food packaging applications where the package will be kept in low-light or dark settings, including warehouses, refrigerators, or other storage facilities. These films provide food goods with extended protection, preserving their quality and prolonging their shelf life.

10.6 CHROMATICITY STABILITY OF CHITOSAN-CURCUMIN COATING USING CIELAB AND PRINCIPAL COMPONENT ANALYSIS

An inventive way to combine the advantages of natural antibacterial qualities with sustainability and longer shelf life for food goods is to blend chitosan with curcumin as a colourant in food packaging. Because chitosan and curcumin are both non-toxic and biodegradable, this combination is perfect for manufacturing environmentally friendly packaging materials. While curcumin is a naturally occurring pigment found in *Curcuma xanthorrhiza* Roxb. (temu lawak) rhizomes, chitosan is obtained from natural sources such as shrimp. As a result, packaging uses less artificial polymers and dyes.

Food packaging made of chitosan and curcumin has drawn a lot of interest from the food industry as a creative and ecological option. The natural qualities of chitosan and curcumin are combined in this mixture to meet the growing need for safe, effective, and biodegradable food packaging materials. A naturally occurring polysaccharide, chitosan is generated from chitin and is frequently present in the fungal cell walls and exoskeletons of crustaceans, including prawns and crabs. Being non-toxic, biodegradable, biocompatible, and having good film-forming properties, it is a potential material for environmentally friendly packaging. Additionally, chitosan is naturally antibacterial, which improves its usefulness as a food packaging material by shielding sealed goods from microbiological contamination and extending shelf life. Because of its antibacterial qualities, chitosan and curcumin both aid in preventing the growth of harmful bacteria and fungi in packaged goods. This is especially helpful for prolonging the shelf life of perishable goods including meat, fruits, and vegetables. Food is shielded from oxidative deterioration by the antioxidant action of curcumin, maintaining its nutritional content, flavour, and freshness. Because chitosan-curcumin packaging is completely biodegradable, it is a more environmentally friendly option than traditional petroleum-based plastics, which contaminate the environment. Since chitosan and curcumin are both accepted as safe for ingestion by humans, direct food contact with this packaging material is safe. The stability

and functionality of the curcumin component is greatly affected by light exposure, especially UV radiation, when chitosan and curcumin are combined to create food packaging films. The performance of the chitosan-curcumin blend may be impacted by curcumin's photosensitivity, which causes it to deteriorate when exposed to light. The following is a scientifically validated analysis of how light exposure affects chitosan-curcumin blends. UV and visible light sensitivity of curcumin is high. Curcumin rapidly breaks down in the presence of light, forming less effective metabolites like ferulic acid and vanillin that help preserve food.

The study demonstrates the highest degradation in 10 g/L exposed under light conditions, whereas in dark conditions, the lowest degradation is in 5 g/L. It showed in line from the previous study, curcumin breaks down in minutes when exposed to light, especially UV radiation (Priyadarsini et al., 2014d). Curcumin's ability to operate as an antioxidant and an antibacterial in active packaging is compromised by this photodegradation. Curcumin in a chitosan blend underwent a considerable degradation under UV radiation, which caused it to lose its bioactive qualities and change colour from yellow to brownish tones (Li et al., 2019). The colour fading of curcumin when exposed to light is one of the main effects of its photosensitivity. Curcumin's distinctive yellow-orange colour fades with degradation, which can have a negative effect on food packaging's aesthetic attractiveness. Chitosan-curcumin films could lose up to 50% of their original colour intensity after being exposed to UV light for 24 hours (Lu et al., 2020).

In practical applications, this can impact how consumers perceive the package because the colour shift might serve as a visual indicator of curcumin deterioration. When packaging salty foods or using it in high-salinity settings, the colour stability of chitosan combined with curcumin under salinity treatment is a crucial consideration. Through ionic bonding, salts can interact with both chitosan and curcumin. Curcumin's unique colour can be attributed to its light absorption characteristics, which may be affected by the presence of Na^+ and Cl^- ions in its molecular structure. Curcumin can degrade more quickly in

salinity, particularly when paired with additional elements like pH shifts or exposure to heat and light. Curcumin is more likely to break down in saltwater settings, which causes colour loss and fading over time. The way food packaging looks is important because it influences how consumers perceive it. The chitosan-curcumin packaging may become less appealing due to discolouration or fading brought on by saline. When promoting eco-friendly or bioactive packaging materials, for example, a loss of colour brightness may make the packaging appear less appealing or fresh.

Investigation into the stability of curcumin under varied circumstances, high ionic strength environments such as those with elevated salinity can cause curcumin to degrade and lose colour (Ghalandari et al., 2018). This implies that chitosan-curcumin packaging would probably lose part of its colour intensity in a salty environment. The natural colourants in biopolymer packaging films, such as curcumin, and found that salt concentrations can affect the color's overall stability, with higher salt concentrations causing discolouration to occur more quickly (Li et al., 2020). The highest degradation was in 5 g/L of NaCl in 5 g/L of chitosan-curcumin concentration when exposed under light conditions, whereas for dark conditions, the least degradation was in 20 g/L of NaCl in 20 g/L of chitosan-curcumin concentration. When added to chitosan films, salinity can dramatically hasten the breakdown of curcumin. Increased rates of photodegradation may result from the molecular interactions within the blend being influenced by the ionic environment formed by dissolved salts, such as sodium chloride (NaCl).

Research has demonstrated that when exposed to light, chitosan-curcumin films degrade more quickly at greater salinity levels. In one study, for instance, blends containing 5 mg/mL of NaCl showed the greatest degradation in response to light, resulting in a significant loss of colour intensity (Ghalandari et al., 2018). Na^+ and Cl^- ions can accelerate photodegradation by changing curcumin's properties related to light absorption. On the other hand, curcumin degrades much more slowly in gloomy environments. Studies revealed that when exposed to higher salt concentrations (20 g/L of NaCl, for example),

chitosan-curcumin films degraded the least, indicating that the process is slowed down by darkness (Li et al., 2020). For the package to continue to look good, curcumin's colour stability in chitosan films is essential. The effect of salinity on colour retention varies with exposure to light. Higher saline levels resulted in a noticeably more noticeable colour loss in light situations. In salty settings, chitosan-curcumin films may lose up to 50% of their initial colour intensity after 24 hours of light exposure (Lu et al., 2020). Curcumin's colour retention in chitosan films considerably improved in low light.

As an illustration of the protective impact of light exclusion, the curcumin colour on films exposed to saline environments maintained its vibrancy when preserved in the dark (Ghalandari et al., 2018). Understanding the material's stability in food packaging applications requires an understanding of the interactions between salt, light exposure, and the intrinsic properties of chitosan-curcumin blends. When curcumin is exposed to light, elevated salt levels can hasten its destruction, resulting in a notable reduction in both colour and bioactivity. Dark environments, on the other hand, have a protective effect that enhances colour retention and functionality. Subsequent investigations ought to concentrate on refining chitosan-curcumin mixtures and investigating efficient packaging approaches to augment the durability and functionality of these bioactive components under diverse environmental circumstances.

pH has a significant impact on chitosan-curcumin blends used in food packaging since both ingredients are sensitive to pH variations that may affect their functioning, stability, and colour. pH 7 had the highest degradation in 10 g/L of chitosan-curcumin concentration in the light condition and showed the least degradation in the dark condition at pH 11 in 10 g/L of chitosan-curcumin concentration. The surrounding pH has a significant impact on the stability of curcumin. It is crucial to comprehend how different pH levels can impact the integrity of curcumin and how it interacts with chitosan in the context of food packaging. When the pH is less than 7, curcumin is relatively stable. Because curcumin can withstand acidic surroundings and retain its colour and structural

integrity, it can be used to package acidic goods like fruits, juices, and pickles. Curcumin does not undergo significant degradation in solutions with a pH range of 1 to 6 (Ahmad et al., 2014b).

In alkaline settings, curcumin's colour might change from yellow-orange to a lighter tint or even brown. Curcumin's colour stability was enhanced by the addition of chitosan under mildly acidic conditions, while noticeable colour fading happened at higher pH values (Kumar et al., 2020). This colour shift could be seen by consumers as a decrease of curcumin's therapeutic qualities. Acidic environments improve the mechanical characteristics of chitosan films, leading to increased tensile strength and flexibility (Yadav et al., 2019). Under lower pH values, the addition of curcumin enhances these qualities even more. However, ionic interactions and curcumin degradation can disturb the molecular structure of chitosan in alkaline environments, perhaps leading to a deterioration of its mechanical capabilities. The stability, colour, mechanical characteristics, and antibacterial effectiveness of chitosan-curcumin blends used in food packaging are all strongly impacted by the pH of the surrounding environment. While curcumin remains stable in acidic environments, it breaks down quickly in alkaline ones, causing colour fading and loss of useful qualities. Comprehending these impacts is crucial for enhancing chitosan-curcumin combinations in food packing scenarios, especially for items with different pH values.

In this study, UV-B irradiation, the highest degradation is in 20 g/L curcumin concentration for s values which showed the degradation occurred when exposed under UV-B exposure. It was proven from the previous study regarding the food packaging's visual attractiveness may be impacted by the colour changes caused by the degradation of curcumin under UV-B radiation. Chitosan-curcumin films showed a decrease in colour intensity during UV-B irradiation, suggesting that extended exposure has a detrimental effect on the packaging's visual quality (Kumar et al., 2020). UV-B light has the ability to split the curcumin molecule, forming ferulic acid and vanillin among other breakdown

products. According to studies, curcumin quickly photodegrades in the presence of UV-B light, changing its colour dramatically. For example, Priyadarsini et al. (2014d) showed that when curcumin is exposed to UV-B light, it breaks down quickly and produces oxidative byproducts. The stability and performance of chitosan combined with curcumin for food packaging are strongly impacted by UV-B irradiation.

From this study, the highest degradation is in 20 g/L of curcumin concentration at 30 °C of temperature for heat treatment. This study is contrary to the previous study, which showed the colour of curcumin started to degrade at 60 °C of temperature, whereas in this study, the colour of curcumin started to degrade at 30 °C. Studies reveal that curcumin starts to break down at high temperatures. for example, showed that curcumin significantly degraded at temperatures higher than 60 °C (Bansal et al., 2017). Curcumin's brilliant colour and functional qualities are lost as a result of this deterioration, which is especially important for applications involving food packaging. Blends containing chitosan and curcumin may lose some of their mechanical qualities when heated. Extended exposure to high temperatures reduced the chitosan-curcumin films' tensile strength and flexibility, which may have an adverse effect on how useful they are as packaging materials (Khan et al., 2021). Curcumin has discernible colour changes as a result of heat-induced degradation, going from a brilliant yellow to softer hues of brown. Heating chitosan-curcumin films resulted in noticeable colour fading, which may have an impact on how consumers see food packaging and its aesthetic attractiveness (Zhang et al., 2019).

10.7 ASSESSMENT OF CURCUMIN CHROMATICITY STABILITY MODEL SYSTEM IN NATURAL SOAP AS AN ADDITIVE

Given curcumin's well-known antibacterial, anti-inflammatory, and antioxidant qualities, blending it into soap can give it a naturally vivid colour and offer further skin benefits. The

main ingredient in *Curcuma xanthorrhiza* Roxb., curcumin has long been utilised in traditional skincare products. Adding curcumin to soap enhances its medicinal and cosmetic properties. Natural polyphenolic substance curcumin, which is obtained from the rhizomes of *Curcuma xanthorrhiza* Roxb., has drawn a lot of interest due to its vivid yellow hue and a variety of bioactive characteristics, such as antibacterial, anti-inflammatory, and antioxidant capabilities. These qualities have led to the use of curcumin in a variety of industries, including food, cosmetics, and medicines. Due to customer desire for more environmentally friendly and non-toxic substitutes for synthetic dyes, its usage as a natural colourant and ingredient in soaps and other cosmetic goods has been investigated in the personal care industry.

However, curcumin's chromaticity stability presents a significant obstacle when using it in soap compositions. Due of curcumin's extreme sensitivity to light, temperature, and pH, it can eventually degrade and lose a large amount of its colour intensity. Curcumin's photodegradation, especially when exposed to light, causes colour fading and modifies the appearance of goods whose characteristic hue is essential to their design. The purpose of this research is to evaluate curcumin's chromaticity stability in natural soap formulations. We may more accurately assess curcumin's potential as a sustainable colourant in natural soap by comprehending how it behaves in a soap matrix under varied light levels and other environmental stressors. Due to consumer preferences shifting towards eco-friendly and chemical-free chemicals, its potential as a natural colourant has attracted increased interest in a number of industries, including cosmetics, medicines, and personal care goods. Among these uses, curcumin's use as a natural colourant in soap compositions provides a desirable substitute for artificial colours, in line with the expanding market for eco-friendly and skin-friendly goods.

Synthetic colourants, which have the potential to be hazardous to both human health and the environment, have historically been used by soap producers. Curcumin offers a more sustainable solution because it is a naturally occurring, biodegradable, and non-toxic

substance. Its vibrant colour can give soap an eye-catching yellow to orange tint, which may draw in customers searching for organic and natural personal care items. Curcumin's well-known antioxidant and antibacterial qualities also offer additional functional advantages, which may improve the soap's quality by guarding against oxidative stress and microbiological contamination. Curcumin is extremely light-sensitive, particularly to UV light. It may experience photodegradation in the presence of light, which results in colour fading or alterations. When exposed to oxygen and light, curcumin disintegrates into smaller molecules and loses its vivid yellow hue.

In this study, the results revealed that, in 0.25 g/L of soap-curcumin concentration, the highest degradation occurred under light conditions, whereas in dark conditions, the least degradation occurred in 2.5 g/L. The chromaticity (ΔE^*) values > 5.0 indicate clearly two different colours when exposed under light conditions in all different curcumin concentrations tested. The degradation process seems to be accelerated by exposure to sunlight, leading to a rapid breakdown of curcumin molecules and subsequent loss of colour. Curcumin stability in a variety of illumination scenarios, including fluorescent light was studied. The results of the study showed that although curcumin deteriorates more slowly in fluorescent light than it does in direct sunshine or ultraviolet light, the cumulative effect of light exposure causes noticeable colour fading over time (Jain et al., 2009). When kept in the dark, curcumin in soap can keep its vivid yellow-orange colour for long periods of time. The absence of light-induced energy in the dark inhibits the production of reactive oxygen species (ROS), which are principally in charge of curcumin's destruction in the presence of light. When ROS are not produced, curcumin molecules experience less oxidative stress, which keeps their colour and medicinal qualities intact in soap formulations. Curcumin gives soap its colour as well as antibacterial and antioxidant qualities. Not only does dark storage aid to prolong these functional benefits, it also helps to keep the colour. When photodegradation is prevented, curcumin's antimicrobial and antioxidative damage-causing properties in soap are preserved. Curcumin degraded very little and kept its bright colour for several weeks when kept in a dark environment (Jayaprakasha et al., 2002).

According to the study's findings, curcumin is mostly stable in the dark and breaks down when exposed to light. Curcumin becomes much more photostable when it is protected from light (Wang et al., 1997a). According to the study, items containing curcumin that were kept in the dark kept their yellow hue, but products that were exposed to light quickly lost it within a few days. It was shown in studies on curcumin-containing cosmetic formulations that goods kept their colour for significantly longer when kept in opaque or dark packaging. This emphasises how crucial it is to shield curcumin from light in order to maintain its bioactivity and chromaticity. These findings are significant in various applications, particularly in cosmetics where curcumin is utilised for its anti-inflammatory and antioxidant properties. Understanding the degradation behaviour of curcumin under different environmental conditions, such as exposure to light, can help in formulating more stable cosmetic products that retain curcumin's beneficial effects over time. When soap infused with curcumin is kept in a dark setting rather than one that is exposed to light, it shows noticeably greater colour stability. The soap retains its antioxidant and antibacterial qualities, along with its vivid yellow-orange colour, for a considerable amount of time because it does not photodegrade in low light. This means that keeping curcumin-blended soap dark in storage is crucial to maintaining its aesthetic and practical properties. Manufacturers should think about utilising opaque packaging for best results, and consumers should store curcumin-based soaps away from light to guarantee colour retention that lasts a long time.

10.8 CONCLUSION

Overall, the results emphasise how crucial extraction techniques and solvent selection are to maximising curcumin production and related bioactivities. Given the strong relationship between bioactive component levels and pigmentation, genetic modification of curcumin-rich ginger species may be a useful strategy for enhancing their health benefits. To create functional foods and natural medicinal agents, future study may concentrate on improving

extraction methods and investigating the genetic variables affecting curcumin production in *Curcuma xanthorrhiza* Roxb. rhizomes. The results of this investigation highlight the intricate relationship between curcumin's stability and the variables of pH, temperature, light exposure, and concentration. Curcumin's vulnerability to degradation, especially in alkaline and neutral environments where it quickly degrades into a variety of degradation products, including ferulic acid and vanillin, tempers its therapeutic benefits, which include its antioxidant, anti-inflammatory, and antibacterial properties.

For curcumin to be used as effectively as possible in a variety of applications—particularly in pharmaceuticals and functional foods—it is imperative to comprehend these stability aspects. Future studies should concentrate on creating efficient defenses against curcumin degradation, such as investigating cutting-edge encapsulating strategies and refining extraction processes. By resolving these issues, curcumin's full therapeutic potential can be achieved, improving patient outcomes and advancing the development of natural treatments. Important information about the durability and degradation of these materials under varied environmental circumstances, such as light exposure, salinity, pH, and temperature, is revealed by the research of curcumin-loaded (PLA) coatings. With its antibacterial and antioxidant qualities, curcumin improves the performance of PLA films used in food packaging, extending its shelf life and shielding food items from oxidation and microbiological development. Nonetheless, the results show that exposure to light continues to be the most important factor influencing curcumin's stability in PLA films, resulting in photodegradation and the loss of its bioactive qualities. The study emphasises how important it is to create compositions and approaches that improve PLA-curcumin films' thermal and photostability. Examining different coatings and additives could enhance their performance in food packaging applications while guaranteeing that curcumin's bioactive qualities are maintained even in harsh environments.

In the end, the results highlight the possibility of curcumin-loaded PLA as an effective material for active packaging, but they also highlight areas that require more

research to fully maximise its use in maintaining the safety and quality of food. The significance of pH, temperature, and salinity in influencing the stability of PVA-curcumin films is also emphasised in the study. High salinity, high temperatures, and extreme pH can hasten curcumin degradation and jeopardise the mechanical integrity of the films. On the other hand, curcumin is much more stable in dark settings, maintaining both its colour and bioactive qualities. For this reason, PVA-curcumin films are more suited for food packaging applications where light exposure is restricted. In summary, although chitosan-curcumin films show promise for use in food packaging, their effectiveness depends on the careful control of outside variables that impact their stability.

To meet consumer and environmental regulations and maximise their use in food packaging, more research and development aimed at improving these materials' resistance to light, temperature, salinity, and pH will be necessary. Chitosan-curcumin films, which provide both practical and visual advantages, may be a key component of the shift to environmentally friendly packaging options as the food sector places a greater emphasis on sustainability. This study concludes that, given curcumin's potential as a sustainable, biodegradable, and non-toxic colourant, it is imperative to comprehend its chromaticity stability in natural soap compositions. Although curcumin has several aesthetically pleasing and functional qualities (antibacterial, antioxidant, and brilliant colour), its sensitivity to light, especially UV radiation, makes it difficult to retain curcumin's integrity and performance in personal care products. The findings indicate that curcumin undergoes rapid degradation in the presence of light, resulting in significant colour fading and loss of bioactivity. On the other hand, curcumin keeps its therapeutic qualities and vivid yellow-orange colour for a longer amount of time when kept in dark storage conditions. These results highlight how manufacturers can improve the stability and efficacy of curcumin in soap formulations by implementing techniques that reduce exposure to light, like using opaque packaging.

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APPENDIX I

ANTIOXIDANT PROPERTIES

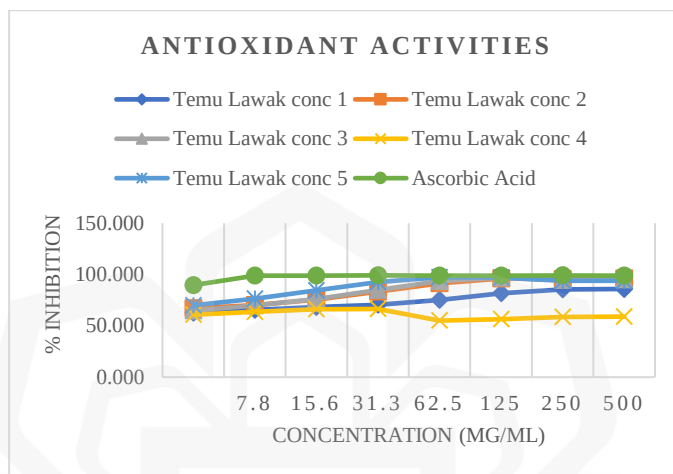


Figure 1. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *temulawak* rhizome and the ascorbic acid standard

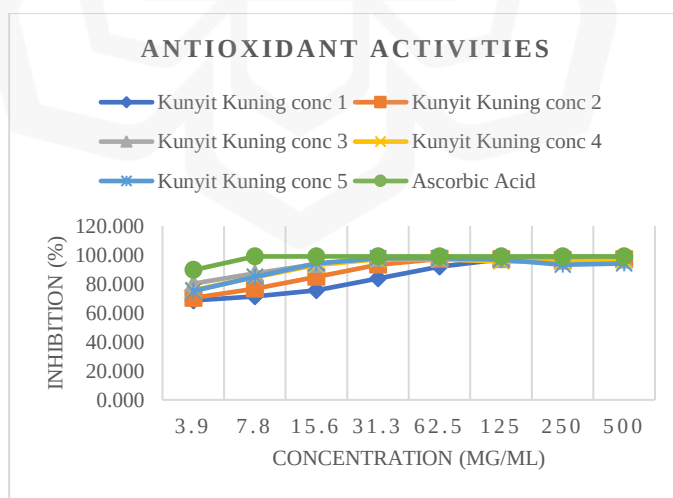


Figure 2. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *kunyit kuning* rhizome and the ascorbic acid standard

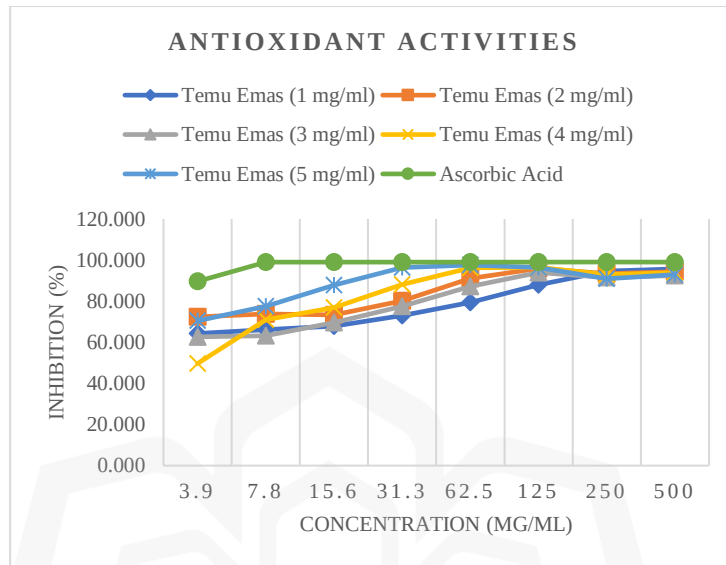


Figure 3. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *temu emas* rhizome and the ascorbic acid standard

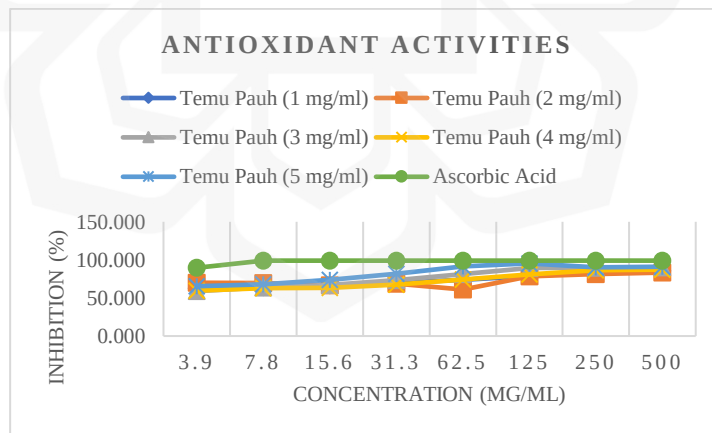


Figure 4. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *temu pauh* rhizome and the ascorbic acid standard

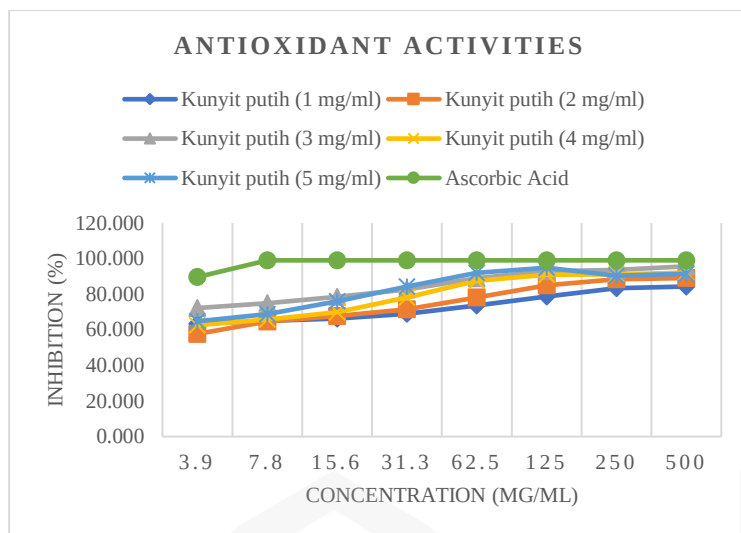


Figure 5. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *kunyit putih* rhizome and the ascorbic acid standard

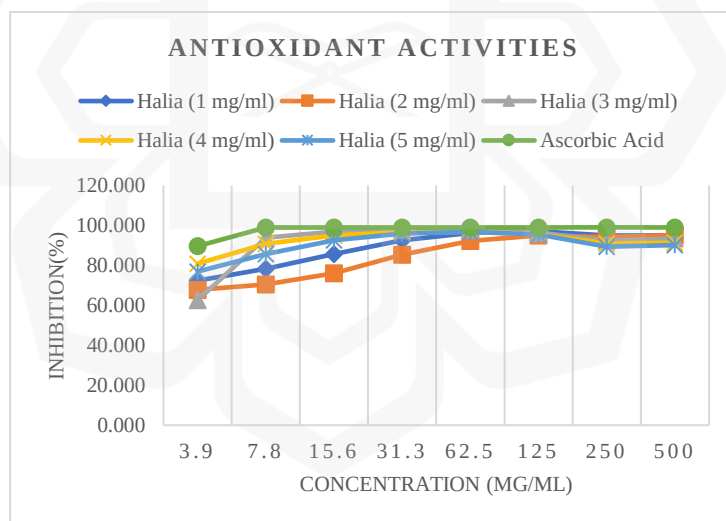


Figure 5. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *halia* rhizome and the ascorbic acid standard

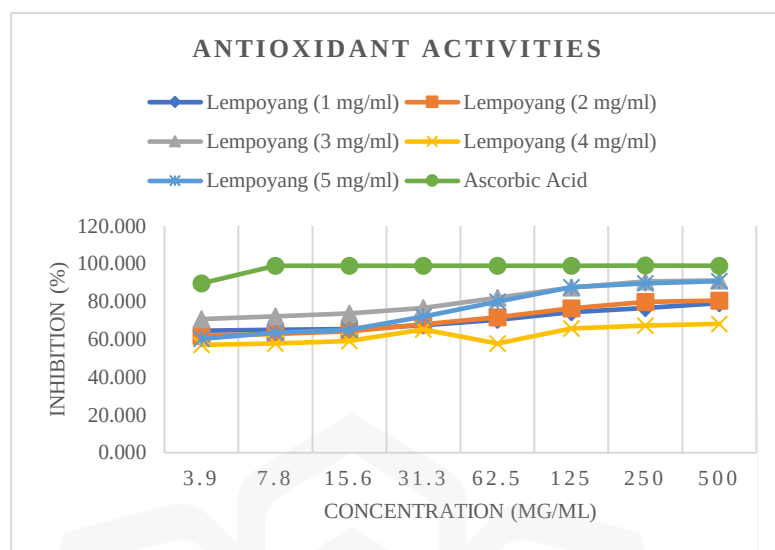


Figure 6. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *lempoyang* rhizome and the ascorbic acid standard

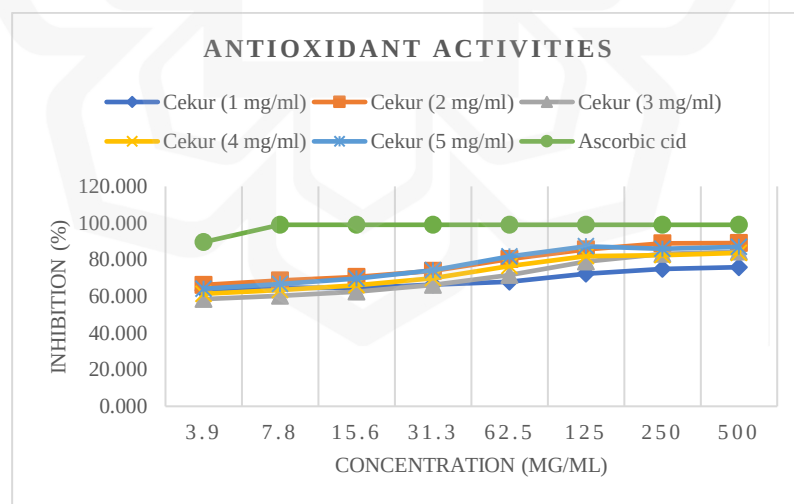


Figure 7. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *cekur* rhizome and the ascorbic acid standard

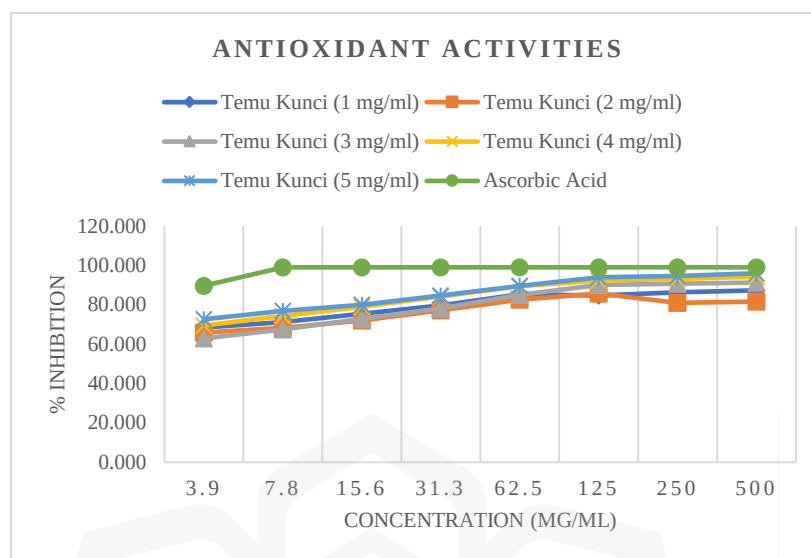


Figure 8. The antioxidant activity (inhibition percentage) of curcumin extracted from the flesh of the *temu kunci* rhizome and the ascorbic acid standard