INVESTIGATION OF COLOR CHANGE AND MOISTURE REGAIN

OF NATURALLY COLORED COTTON

by

SHO YEUNG KANG

(Under the Direction of Helen H. Epps)

ABSTRACT

Naturally colored cotton does not need to be dyed or bleached and does not require pesticide treatment, making the processing of colored cotton inexpensive and environmentally friendly. However, there are concerns about its unidentified pigment character and low moisture regain, which affect its commercialization. In this research, the characterization of pigments in three naturally colored (buffalo brown, coyote brown and green) cottons, and their moisture regain change during finishing and care processes were studied. An enzymatic scouring process using pectinase and cellulase did not significantly change color of naturally colored cottons. Two alkali scouring processes ($CaCO_3$ and NaOH) decreased lightness, and chroma (a* and b*) of naturally colored cottons. With color changes of cottons toward dark colors, scouring solutions after scouring were also strongly colored. Wax extraction, light microscopy analysis, scanning electron microscopy (SEM), X-ray surface analysis, and inductively coupled plasma (ICP) were used to determine mechanism of color changes. Results show that after alkali scouring, cross-sections of fiber became swollen for green cottons, pigment in the fibers moved to outer surface, and potassium content of fiber decreased significantly. Enzyme treatment as detergent and UV exposure were used to simulate possible color change after care. Color of scoured cotton was

stronger after enzyme treatment. Lightness of single enzyme treated cottons decreased after five hours UV exposure and then increased. Cottons treated with multiple enzymes showed continuous increase with UV exposure. The green cotton lost its green color and gained red color after UV exposure.

The scouring process and enzyme treatment increased the weight of naturally colored cottons. Statistical analysis on change in present moisture regain showed that the weight increase was due to higher moisture regain (%) after processes.

INDEX WORDS: Naturally colored cotton, Color, Pigment, Scouring, Enzyme treatment, Detergent, UV exposure, Moisture regain, SEM, LM

INVESTIGATION OF COLOR CHANGE AND MOISTURE REGAIN OF NATURALLY COLORED COTTON

by

SHO YEUNG KANG

B.S., Ewha Womans University, Seoul, Korea, 2000

M.A., Ewha Womans University, Seoul, Korea, 2002

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2007

© 2007

SHO YEUNG KANG

All Rights Reserved

INVESTIGATION OF COLOR CHANGE AND MOISTURE REGAIN OF NATURALLY COLORED COTTON

by

SHO YEUNG KANG

Major Professor: Helen H. Epps

Committee: J. Nolan Etters
Patricia Annis
John P. Shields
Lars G. Ljungdahl
Mary Meyer

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2007

DEDICATION

This dissertation is dedicated to my family for their loves and supports.

ACKNOWLEDGEMENTS

I would like to acknowledge many people for helping me during my doctoral work. I would especially like to thank my advisor, Dr. Helen H. Epps, for her generous time and commitment. Throughout my doctoral work she encouraged me to develop independent thinking and research skills.

I am also very grateful for having an exceptional doctoral committee and wish to thank Dr. J. Nolan Etters, Dr. Patricia Annis, and Dr. John P. Shields for their continual support and encouragement.

I am extremely grateful for the assistance, generosity, and advice I received from Dr. Mary Meyer and Dr. Lars G. Ljungdahl.

I extend many thanks to my colleagues, staffs and faculties in TMI department who supported me with their assistance and friendship.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES x

CHAPTER

1 INTRODUCTION 1

Significance of Study 3

Purpose of Study 4

2 Review of Literature 6

Cotton 6

Naturally Colored Cotton 9

Performance of Naturally Colored Cotton 17

Scouring 19

Laundering for Naturally Colored Cotton 20

Weathering effect on Naturally Colored Cotton 25

3 METHODOLOGY 29

Effect of Scouring 29

Effect of Enzymes as Detergent 34

Effect of UV Exposure 42

4 RESULTS AND DISCUSSION 45

Color Changes of Naturally Colored Cotton after Scouring 45

Mechanism of Color Change of Naturally Colored Cotton after Scouring 51

Color Change of Naturally Colored Cotton after Enzyme Treatment and UV Exposure 65

Changes of L*, a* and b* after Scouring, Enzyme Treatment and UV Exposure 92

Moisture Regain (%) Changes of Naturally Colored Cotton after Scouring and Enzyme Treatment 96

5 CONCLUSIONS AND FUTURE WORK 110

Conclusions 110

Future Work 112

REFERENCES 114

APPENDICES 124

A Statistical Analysis of Lightness Change of Naturally Colored Cotton after UV Exposure 124

B Change of a* and b* of Naturally Colored Cotton after UV Exposure 135

C Statistical Analysis of Moisture Regain (%) of Naturally Colored Cotton after Enzyme Treatment 169

LIST OF TABLES

Page

Table 2-1. Composition of typical mature cotton fiber....8

Table 2-2. Heavy metal analysis of cotton grown in India....9

Table 2-3. Heavy metal contents in cottons....10

Table 2-4. Percentage of wax in the cotton fiber samples....11

Table 2-5. Composition of green and white cottons (%)....13

Table 2-6. General properties of the cotton fiber sample....16

Table 2-7. Tensile Properties of the cotton fiber samples....17

Table 2-8. Components and functions of detergent....21

Table 2-9. Composition of an enzyme detergent....22

Table 3-1. Glucose standard solution....36

Table 3-2. Characteristics of enzymes used as detergent for naturally colored cottons....38

Table 3-3. Concentration and Types of Single Enzyme Treatment....38

Table 3-4. Concentration and Types of Mutiple Enzyme Treatment....39

Table 3-5. Weathering machine start-up condition by option E....42

Table 3-6. Six Levels of Exposure Duration....43

Table 4-1. Color changes of naturally buffalo brown cotton after scouring processes....45

Table 4-2. Color changes of naturally coyote brown cotton after scouring processes....47

Table 4-3. Color changes of naturally green cotton after scouring processes....49

Table 4-4. Color change of naturally colored cotton after wax extraction....51

Table 4-5. Potassium content on surfaces of naturally colored cottons (%).....62

Table 4-6. Weight percentage of heavy metal contents in three naturally colored cotton fibers (g) 64

Table 4-7. Statistical results of lightness of single enzyme treated samples after UV exposure...90

Table 4-8. Statistical results of lightness of multiple enzyme treated samples after UV exposure... 91

Table 4-9. Weight changes of buffalo brown colored cotton after enzyme treatment 97

Table 4-10. Weight changes of coyote brown colored cotton after enzyme treatment 97

Table 4-11. Weight changes of green colored cotton after enzyme treatment 97

Table 4-12. Duncan grouping of means of moisture regain (%) from single enzyme treatment on naturally colored cotton.....102

Table 4-13. Duncan grouping of means of moisture regain (%) from multiple enzyme treatment on naturally colored cotton.....106

LIST OF FIGURES

Page

Figure 2-1. Structure of cotton fiber 7

Figure 2-2. Caffeic acid 12

Figure 2-3. Cinnamic acid.....12

Figure 2-4. Electron micrograph of a transverse section through an ordinary white upland cotton fiber cell (never-dried).....14

Figure 2-5. Electron micrograph of a transverse section through a green colored section of upland cotton fiber.....14

Figure 3-1. CIELab color space 30

Figure 3-2. Plate for fiber cross sectioning.....32

Figure 3-3. Cross section of cotton fiber under SEM 33

Figure 3-4. Enzyme treatment process.....39

Figure 4-1. Buffalo brown cotton after scouring 46

Figure 4-2. Coyote brown cotton after scouring.....48

Figure 4-3. Green cotton after scouring.....50

Figure 4-4. Light microscopy image of raw buffalo brown cotton.....53

Figure 4-5. Light microscopy image of CaCO3 scoured buffalo brown cotton 53

Figure 4-6. Light microscopy image of raw coyote brown cotton 54

Figure 4-7. Light microscopy image of CaCO3 scoured coyote brown cotton 54

Figure 4-8. Light microscopy image of raw green cotton 55

Figure 4-9. Light microscopy image of CaCO3 scoured green cotton 55
Figure 4-10. SEM images of naturally buffalo brown cotton fibers.....58
Figure 4-11. SEM images of naturally coyote brown cotton fibers.....60
Figure 4-12. SEM images of naturally green cotton fibers.....62
Figure 4-13. Changes of L* of single enzyme treated naturally buffalo brown colored cottons after UV exposure 65
Figure 4-14. Changes of a* of single enzyme treated naturally buffalo brown colored cottons after UV exposure 66
Figure 4-15. Changes of b* of single enzyme treated naturally buffalo brown colored cottons after UV exposure 66
Figure 4-16. Changes of Δ E of single enzyme treated naturally buffalo brown colored cottons after UV exposure 67
Figure 4-17. Changes of L* of single enzyme treated naturally coyote brown colored cottons after UV exposure 68
Figure 4-18. Changes of a* of single enzyme treated naturally coyote brown colored cottons after UV exposure.....69
Figure 4-19. Changes of b* of single enzyme treated naturally coyote brown colored cottons after UV exposure 69
Figure 4-20. Changes of Δ E of single enzyme treated naturally coyote brown colored cottons after UV exposure 70
Figure 4-21. Changes of L* of single enzyme treated naturally green colored cottons after UV exposure.....71

Figure 4-22. Changes of a* of single enzyme treated naturally green colored cottons after UV exposure....72
Figure 4-23. Changes of b* of single enzyme treated naturally green colored cottons after UV exposure....72
Figure 4-24. Changes of Δ E of single enzyme treated naturally green colored cottons after UV exposure....73
Figure 4-25. Changes of L* of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure 74
Figure 4-26. Changes of a* of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure 75
Figure 4-27. Changes of b* of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure 75
Figure 4-28. Changes of Δ E of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure 76
Figure 4-29. Changes of L* of multiple enzyme treated naturally coyote brown colored cottons after UV exposure 77
Figure 4-30. Changes of a* of multiple enzyme treated naturally coyote brown colored cottons after UV exposure 78
Figure 4-31. Changes of b* of multiple enzyme treated naturally coyote brown colored cottons after UV exposure 78
Figure 4-32. Changes of Δ E of multiple enzyme treated naturally coyote brown colored cottons after UV exposure 79

Figure 4-33. Changes of L* of multiple enzyme treated naturally green colored cottons after UV exposure....80
Figure 4-34. Changes of a* of multiple enzyme treated naturally green colored cottons after UV exposure....81
Figure 4-35. Changes of b* of multiple enzyme treated naturally green colored cottons after UV exposure....81
Figure 4-36. Changes of Δ E of multiple enzyme treated naturally green colored cottons after UV exposure....82
Figure 4-37. Effect of single enzyme treatment concentration on color change (Δ E) of buffalo brown cotton....83
Figure 4-38. Effect of single enzyme treatment concentration on color change (Δ E) of buffalo brown cotton after 40 hours UV exposure....83
Figure 4-39. Effect of single enzyme treatment concentration on color change (Δ E) of coyote brown cotton....84
Figure 4-40. Effect of single enzyme treatment concentration on color change (Δ E) of coyote brown cotton after 40 hours UV exposure....84
Figure 4-41. Effect of single enzyme treatment concentration on color change (Δ E) of green cotton....85
Figure 4-42. Effect of single enzyme treatment concentration on color change (Δ E) of green cotton after 40 hours UV exposure....85
Figure 4-43. Effect of multiple enzyme treatment concentration on color change (Δ E) of buffalo brown cotton....87

Figure 4-44. Effect of multiple enzyme treatment concentration on color change (Δ E) of buffalo brown cotton after 40 hours UV exposure 87
Figure 4-45. Effect of multiple enzyme treatment concentration on color change (Δ E) of coyote brown cotton....88
Figure 4-46. Effect of multiple enzyme treatment concentration on color change (Δ E) of coyote brown cotton after 40 hours UV exposure 88
Figure 4-47. Effect of multiple enzyme treatment concentration on color change (Δ E) of green cotton....89
Figure 4-48. Effect of multiple enzyme treatment concentration on color change (Δ E) of green cotton after 40 hours UV exposure....89
Figure 4-49. Changes of L* of naturally colored cottons after scouring and enzyme treatment...92
Figure 4-50. Changes of a* and b* of naturally colored cottons after scouring and enzyme treatment....93
Figure 4-51. Changes of a* and b* of naturally buffalo brown colored cottons after scouring and enzyme treatment 93
Figure 4-52. Changes of a* and b* of naturally coyote brown colored cottons after scouring and enzyme treatment 94
Figure 4-53. Changes of a* and b* of naturally green colored cottons after scouring and enzyme treatment....94
Figure 4-54. Effect of single enzymes on moisture regain (%) changes of buffalo brown naturally colored cotton after scouring and enzyme treatment....98
Figure 4-55. Effect of single enzymes on moisture regain (%) changes of coyote brown naturally colored cotton after scouring and enzyme treatment....99

Figure 4-56. Effect of single enzymes on moisture regain (%) changes of green naturally colored cotton after scouring and enzyme treatment.....100
Figure 4-57. Effect of multiple enzymes on moisture regain (%) changes of buffalo brown naturally colored cotton after scouring and enzyme treatment 103
Figure 4-58. Effect of multiple enzymes on moisture regain (%) changes of coyote brown naturally colored cotton after scouring and enzyme treatment 104
Figure 4-59. Effect of multiple enzymes on moisture regain (%) changes of green naturally colored cotton after scouring and enzyme treatment.....105
Figure 4-60. Moisture Regain (%) Changes of Naturally Colored Cottons after Scouring and Enzyme treatment.....108

CHAPTER 1

INTRODUCTION

As demand for organic crop production has boomed, the US organic industry has shown annual increases in consumption of 20% per year in the past 5 years [1]. This is partially driven by consumers expressing concern over harmful chemical residues from food production such as pesticide processes [2]. The severe impact of non-organic, commercial crop production on the natural environment is seen as loss of natural resources, deforestation, soil erosion, salinization, fertilizer-pesticide contamination, and bio-magnification of pesticide residues to ground water depletion [3]. Fertilizers and pesticides can affect not only consumers but also farmers who come in contact with them. Although the concept of organic farming has become very popular, there has been economic concern due to low production. However, a premium of 12-60% is obtained from organic produce [4] and organic agriculture has become more attractive to farmers [5, 6].

White cotton is one of the most pesticide-dependent crops in the world. Almost one-third of a pound toxic chemical fertilizers and pesticides are applied for every pound of cotton harvested [7]. ‘Organic cotton’ is naturally grown cotton, based on the U.S. federal guidelines for organic crop production. According to these guidelines, soil fertility practices should meet organic certification standards including crop rotation, cover cropping, animal manure additions, and use of naturally occurring rock powders [8]. Six thousand tons of organic cotton is grown annually in the world but only accounts for 0.03% of global cotton production. Europe has the highest demand for organic cotton, 58% of total cotton production, and the United States utilizes 33% of total production of organic cotton [9]. Unfortunately, cotton processing such as bleaching,

scouring, and dyeing still leaves toxic chemicals in cotton after it is organically grown. ‘Textile dermatitis’, caused by contacting clothing, shows clinical symtoms usually on the neck, major skin folds, inner thighs or other friction and perspiration sites. There are many cases of textile dermatitis in textile industry workers, clothing store workers, and consumers who wear clothing dyed with disperse dyes [10, 11, 12, 13].

Naturally colored cotton is defined as cotton that produces lint in any color other than white. It does not need to be dyed or bleached, making the processing of colored cotton inexpensive and environmentally friendly. Also it is pest-resistant, so that it can be totally chemical free. Naturally colored cotton originated some 5,000 years ago in the Andes. Brown fibers have been unearthed at the most ancient levels of Huaca Prieta, a settlement on the northern Peruvian coast that was occupied between 3100 and 1300B.C. Because these fibers were shorter and weaker, naturally colored cotton never appeared commercially until after the industrial revolution which introduced spinning and weaving and development of chemical dyes. During World War II, when chemical dyes were in short supply, green and brown cottons were produced for a limited time. Naturally colored cotton suddenly reappeared as a fashion item in early 1990s. In 1981 the first serious consideration of naturally colored cotton as an alternative agriculture initiative on a legislative level was proposed by Senator Patrick Leahy and Representative James Weaver to study and compare organic and conventional farming techniques [14, 15, 16].

Currently, more than 27 countries conduct research on naturally colored cotton including the United States, China, Brazil, Egypt, India, and Peru. Various colors such as mocha, tan, gray, brown, black, mahogany, purple, orange, red, pink, blue, green, grey and cream are shades of naturally colored cotton commercially available after genetic development. There was only one

acre of naturally colored cotton grown in United States in 1988. By 2001, there were more than 11,000 acres of colored cotton, mainly in Texas, California, and Arizona. That number has dropped to under 5,000 acres, because competition from foreign growers such as China and some countries in South America has increased [14, 15]. After the first planting in the 1990s, China had more than 46,600 hectares of naturally colored cotton grown in 2005, which is more than 200% increase over the 20,000 hectares grown in 2004. Colored cotton output from China represents 30% of total cotton output of the world [17, 18, 19].

Significance of Study

Benefits of naturally colored cotton include flame resistance due to the presence of more heavy metals and a higher limited oxygen index value than conventional white cotton [20]. Also it has been shown that the pigments in naturally colored cotton provide excellent sun protection properties (high UPF values). The fiber's natural pigments provide protection from UV radiation for the embryonic cotton seed [21].

There are concerns regarding the commercialization of naturally colored cotton. Possibility of cross-pollination with white cotton is a major concern for farmers. The U.S. has developed rules for restrictions on delinting and ginning of naturally colored cotton, after several contamination cases. Colored cotton fields need to be located at some distance away from white cotton fields to prevent cross-pollination [22]. Quality characteristics of naturally colored cotton such as low strength, shorter length, and poor micronaire are also problematic. In the 1980s and 1990s, Sally Fox, who owned Foxfibre® for organic cotton and wool, started researching naturally colored cotton plants, breeding fibers that are stronger and longer than the original plants she worked with, allowing naturally colored cotton to be mechanically spun. Kimmel

(1996) in the Cotton Textile Engineering Research Unit at ARS' Southern Regional Research Center (SRRC) tried to develop improved colored cotton yarns and fabrics. Kimmel produced composite yarns using two ARS-patented methods, staple-core and filament-core spinning. Using these methods, yarns are constructed with naturally colored fiber at outer layer and stronger white cotton or synthetic fibers in cores. The fabrics produced from such yarns are made to look and feel like solid colored cotton with better strength than pure naturally colored cotton fabrics [23][24]. Color of naturally colored cotton is a critical concern as well as a great benefit. Color change of naturally colored cotton is very different from that of dyed cotton. When treated with chemicals, laundered or exposed to weather, the reaction of pigments in naturally colored cotton is not clearly defined yet. Naturally colored cotton has many benefits as an eco-friendly material that can be used as socks, T-shirts, underwear, towels or sheets that can touch human skin directly. But it has low moisture absorbency due to its different composition such as higher wax content than that of white cotton. Therefore, color change and moisture regain change in naturally colored cotton during production process and care process need to be investigated [25].

Purpose of Study

The purpose of this research was to characterize changes that occur in of pigment and moisture absorbency of naturally colored cotton, for processing the fiber during manufacturing and care of products made from the fiber.

Specifically, the objectives of this study were:

1. To determine if the scouring process affects color of naturally colored cotton.
2. To determine the mechanism of color change of naturally colored cotton after scouring process
3. To determine if laundering with enzymes affects color of naturally colored cotton.

4. To determine if UV exposure affects color of naturally colored cotton.

5. To determine if the combination of scouring and laundering with enzymes affects moisture absorbency of naturally colored cotton.

.

CHAPTER 2

REVIEW OF LITERATURE

It is necessary to introduce character of naturally colored cottons and the primary published research in this area, because work on character of naturally colored cotton is still ongoing. It is particularly important to compare the chemical composition and physical properties of naturally colored cotton to conventional white cotton for understanding the follwing work.

Cotton

Chemical composition and fiber structure of cotton

The cotton fiber is a single cell with several concentric layers. It is made up of cuticle layer, primary wall, secondary layer, and lumen. The cuticle layer is separable from the fiber and consists of wax and pectin materials. It covers the primary wall which is the outermost layer of raw cotton fiber and is composed of cellulosic crystalline fibrils [26]. The secondary wall represents the majority of cellulose within the fiber. It consists of three distinct layers that include closely packed parallel fibrils with spiral winding of 25-35°. The innermost part of cotton fiber is the lumen. Before cotton fiber is exposed to sun, the lumen is filled with cytoplasm of a living cell. The cytoplasm dies after boll opening. The twists and convolutions of the mature fiber are due to the removal of the liquid contained in the cytoplasm. The dried lumen is shown as a dark area under a microscope. The cross section of the raw fiber is typically bean-shaped as shown in figure 2-1. [27].

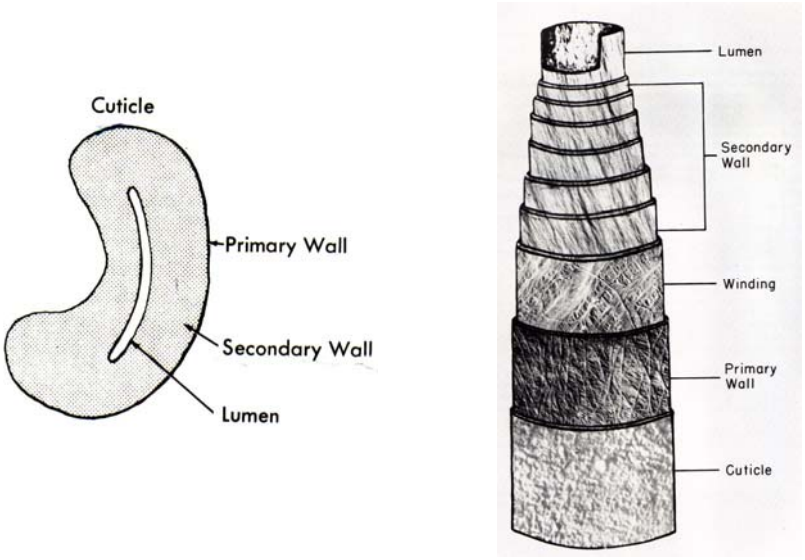


Figure 2-1. Structure of cotton fiber (left) [27], (right) [28]

Cellulose content in raw cotton fiber ranges from 88 to 96% of the dry weight. After it is scoured and bleached the percentage of cellulose becomes increased to 99%. Immature fibers contain low levels of cellulosic materials and thin walls. Table 2-1. shows typical composition of mature cotton fiber [29].

Possible diversity of cotton composition

Varieties of soil can affect the composition of cotton fiber and these effects can vary depending on the cotton plant tolerance [30]. Genetic and environmental conditions are important factors for yield and quality in Upland cotton [31, 32]. Nitrogen and potassium are the most important nutrition for cotton crop and the amount of these in soil can be different depending on locations of the farm. It was reported that potassium deficiency affects fiber quality, including lint yield, fiber elongation, and micronaire [33]. Acidic and naturally infertile soils that

Table 2-1. Composition of typical mature cotton fiber [29]

Constituent	Dry basis[a]		
	Typical, %	Low, %	High, %
Cellulose	94.0	88.0	96.0
Protein (N × 6.25)	1.3	1.1	1.9
Pectic substances	0.9	0.7	1.2
Ash	1.2	0.7	1.6
Wax	0.6	0.3	1.0
Malic, citric, and other organic acids	0.8	0.5	1.0
Total sugars	0.3	-	-
Other	0.9	-	-

[a] Moisture about 8% regain

are predominant in southeastern USA and other high rainfall areas around the world produce poor quality cotton fibers due to a deplaetion of potassium in soil [34, 35, 36, 37]. Qui *et al*. [38] reported that cotton fiber can have more lead and cadmium than rice under the same soil and aerosol condition. The environmental condition is a more important factor in heavy metal uptake by cotton plants than genetic deposition. Main factors for heavy metal uptake from soil can be the soil heavy metal content, sorptive capacity of soil, redox conditions, organic matter and pH [39]. Recent research shows how environmental conditions with heavy metals can change the heavy metal accumulation in cotton fiber. Heavy metal can be absorbed through cotton plant leaves. Lead remains as a surface precipitate, while zinc, copper and cadmium can penetrate into the leaf cells [40]. Angelova *et al.* [41] showed that cotton has heavy metal uptake mainly

through leaves from atmosphere and the heavy metal accumulation is concentrated on leaf and fiber parts rather than root system and stems.

Naturally Colored Cotton

Heavy metal content

Parmar *et al.* [20] reported that the total heavy metal content in naturally colored cotton fiber is higher than in white cotton fiber. Table 2-2 shows that the compositions of heavy metals in each of these three cotton fiber types are different. These cotton fiber samples were taken directly from a single cotton farm in India.

Table 2-2. Heavy metal analysis of cotton grown in India [20]

	White cotton		Brown cotton		Green cotton	
Heavy metals	Mg/Kg of fibers	No. of mole/Kg fiber × 10^{-5}	Mg/Kg of fibers	No. of mole/Kg fiber × 10^{-5}	Mg/Kg of fibers	No. of mole/Kg fiber × 10^{-5}
Cd	NT[a]	NT[a]	NT[a]	NT[a]	NT[a]	NT[a]
Cr	1.34	2.56	2.34	4.50	2.65	5.07
Co	3.95	6.70	2.70	4.58	4.68	7.92
Cu	4.19	6.59	4.25	6.68	3.05	4.80
Pb	10.10	4.86	7.90	3.80	7.44	3.60
Ni	10.67	18.17	12.07	20.55	11.18	18.17
Zn	3.78	5.78	7.83	11.96	4.42	6.76
Total		44.66		52.07		47.15

Other research shows heavy metal contents in cotton samples from a farm in China [41]. The results show very different heavy metal components and rate in three cottons from those grown in India (table 2-3).

Table 2-3. Heavy metal contents in cottons from China [42]

Chemical components	White cotton, ng/g	Brown cotton, ng/g	Green cotton, ng/g
Mn	1863.8	1364.3	1439.1
Cu	393.0	1180.1	1275.2
Zn	-	1052.5	399.1
Rb	2422.7	2015.5	1259.0
Sr	1623.6	2221.9	1239.8
Ba	2396.0	1864.5	1871.0
La	162.7	48.2	203.6
Pb	1202.1	596.2	101.3
Fe	203800	747800	249400
Ca	883400	1221500	860800
Mg	309500	567300	429700

Wax

Higher plants such as cotton have biopolymer suberin as a protective cell wall layer in various tissues and cells. The typically insoluble polymers in suberin that can be solubilized with organic solvents are together called waxes [43]. Early analysis of white cotton wax indicated that it consists of free fatty acids of about 25 percent, of a larger portion of esters, and of about 40 percent of higher alcohols (long-chain primary alcohol) [44]. According to the hypothetical model of Kolattukudy [45], the polymer includes aliphatic and aromatic domains. Other detailed study about cotton wax showed that the main compositions are 1-triacontanol, montanol, beta-sisterol, and some high molecular weight esters [46].

The wax of the fibers can affect processing in several ways. It is needed to provide lubrication for proper spinning of the fibers into yarn. If the cotton wax content is below 0.1

percent, the cotton is too crisp and harsh for being processed and used [47]. On the other hand, high content wax can reduce the absorbency and strength of the fibers.

Table 2-4 shows wax content of brown colored cotton and green colored cotton compared to that of white cotton. After 6 and 24 hours extraction with ethanol, the white and the brown colored cotton samples showed little difference. The percentage of wax is very high for green cotton, even after 24 hours of ethanol extraction.

Table 2-4. Percentage of wax in the cotton fiber samples [45]

Sample	Percentage of wax (by weight)	
	After 6 hours ethanol extraction	After 24 hours ethanol extraction
White cotton	0.76	1.24
Brown cotton	0.60	0.82
Green cotton	4.66	5.28

There are a few previous studies of the wax content of green colored cotton fiber. The main aliphatic monomers of green colored cotton suberin were found to be 70 percent of ω-hydroxydocosanoic and 30% of docosanedioic acids. The wax of green cotton is composed of long chain (>C16) 1-alkanols and alkanoic acids, and of a material with phenolic and polymeric characteristics [48, 49]. Caffeic acid [figure 2-2] and glycerol were also found to be constituents of the suberin layers in green cotton fibers [45]. Ryser (1994) isolated and characterized this caffeoyl-fatty acid-glycerol ester from wax associated with green cotton fiber suberin. All the constituents of the caffeoyl ester were found to be related to the antioxidant and radical-scavenging properties of caffeic acid [43]. It was noted that caffeic acid is a better antioxidant and has a higher radical-scavenging activity than cinamic, p-coumaric and ferulic acids that are

known to neutralize free radicals [50].

Pigment

The pigments in colored cotton have not yet been clearly defined. But there were previous studies on the composition and characteristics of the natural pigment in the colored cotton.

The brown color of colored cotton lints is obtained from tannin vacuoles in the lumen of the fiber cells [51]. The brown color does not appear until the seed pod opens when fibers are exposed to oxygen and sunlight [21]. It was revealed by thin layer chromatography analysis that the constituents of the pigment include the tannin precursor, catechin, and tannin derivatives [52]. Tannin is a large polyphenolic compound including sufficient hydroxyls and other possible groups that can form strong complexes with proteins and other macromolecules. The tea plant (Camellia sinensis) is an example of a plant with naturally high tannin content [53].

The color of green colored cotton is mainly from caffeic-acid [figure 2-2] that is a derivative of cinammic acid [figure 2-3] and found in the suberin layer. The caffeic acid is deposited in alternating layers with the suberin outside of the fiber [45].

Figure 2-2. Caffeic acid [54]

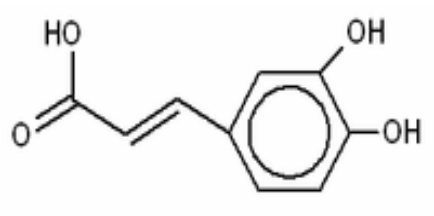
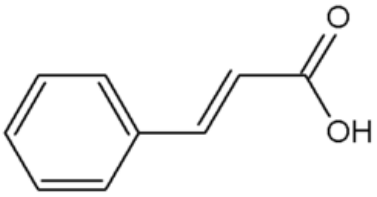


Figure 2-3. Cinnamic acid [55]



Caffeic acid is obtained naturally from oil of cinnamon, or from shea butter. It can be also obtained synthetically. Cinnamic acid is used in making synthetic flavours, synthetic indigo, and mainly certain pharmaceuticals such as the ethyl and methyl, and perfume such as benzyl esters

[56, 57]. It has also been shown that the isolated compound from green cotton is a fluorescent material [45] and it has been theorized that the purpose of fluorescence is to absorb UV radiation in order to protect the cotton seed [52].

The pigment amount is greater for brown cotton than for green cotton. Because these coloring materials are bonded to fiber molecules, they affect the formation of crystalline areas in the fiber, but the bonding effects are different for brown and green cotton [58].

Moisture absorbency

Compared with white cotton, brown colored cotton has a similar percentage of wax in its content but green cotton has much higher wax content. The higher wax content of green cotton with pectin and lignin can reduce the moisture absorbency of the fiber [17].

Table 2-5. Composition of green and white cottons (%) [17]

Substance	Green cotton	White cotton
Fat	4.34	0.6
Lignin	9.34	0.0
Pectin	0.51	1.2

Naturally colored cotton has the benefit of being identified as an environmentally friendly material. It is usually used for goods such as towels, T-shirts, socks and intimate apparel. Gu (2005) [17] treated green colored cotton with warm water and NaOH solution to reduce the hydrophobic content and increase moisture absorbency. After the caustic solution treatment, green colored cotton showed higher moisture absorbency and reduction of the crystalline region.

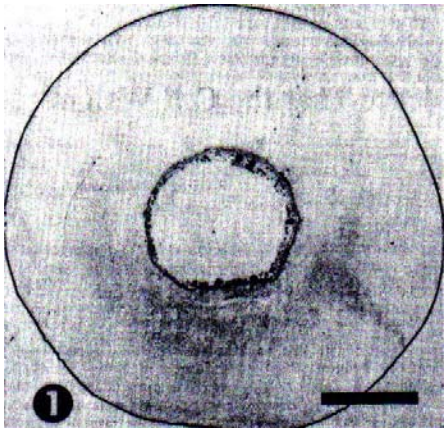
Fiber swelling

Richards *et al*.(1999) [59] noted that when a cotton fiber is swollen with a strong swelling

agent such as sodium hydroxide or cuprammonium hydroxide, the increased pressure within the secondary wall can result in the rupture of the primary wall. The white fiber swells into a characteristic spiral shape. But green fiber showed a different swelling with alternate colored concentric layers surrounding the lumen. There are uncolored layers between colored layers. The uncolored layers are assumed to be suberin layers.

Secondary wall

Yatsu *at al.*(1983) compared green colored cotton to white cotton using electron microscopy. The photomicrographs showed that white cotton has a very thin (0.02 μm) outer cuticle (Figures 2-4, 5). Green cotton, however, has at least two layered series of lines in its cross-section. One is a dark, continuous and heavy line. The other one is more wispy, discontinuous and lighter [45].



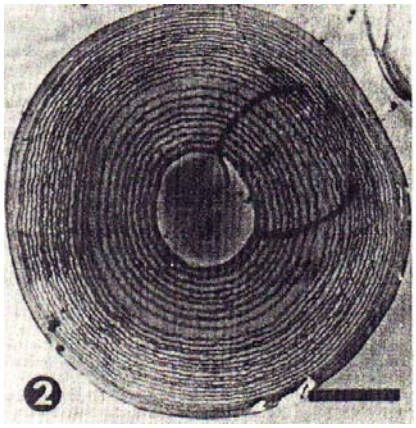


Figure 2-4. Electron micrograph of a transverse section through an ordinary white upland cotton fiber cell (never-dried). Bar=10μm [45]

Figure 2-5. Electron micrograph of a transverse section through a green colored section of upland cotton fiber. Bar=10μm [45]

Crystallinity

Previous research found that the degree of crystallinity of colored cotton is different from that of white cotton. Richard (1999) indicated that raw white cotton and raw colored cotton are consistent with the cellulose I structure normally found in cotton. But green colored cotton shows less crystallinity. When extracted with ethanol, the crystallinity of the green cotton became larger, with a greater volume similar to that of the brown cotton [59].

Chen and Yokochi (1999) reported that the crystallinity difference between white cotton and both brown and green colored cotton is too small to account for the special properties of naturally colored cotton such as strengthening of color after wet treatment or flame resistance [60]. However a recent study indicated that the crystallinity difference has a clear relationship with the pigment of naturally colored cotton. The cristallinity of white cotton was found to be 80.1% and this was a higher percentage than that of green colored cotton (71.5%) and brown cotton (67.3%). Also white cotton was found to have greater crystalline regularity than colored cotton. After supercritical CO_2 and alkali extraction, pigment materials were removed from naturally colored cotton fibers. After removal of pigment, the crystallinity of both colored cottons was higher [58].

Physical properties

In another study, fiber characteristics were evaluated by HVI (High Volume Instrument), AFIS (Advanced Fiber Information System), FMT (Shirley Fineness and Maturity Tester), and IA (Image Analyzer). Both HVI and AFIS measured the tensile, length, and fineness properties of the naturally colored cotton fibers. Fiber circularity and perimeter were measured using IA and fiber fineness and maturity ratio were measured using FMT. Results showed that white

cotton fiber is longer and stronger than colored cotton fibers. There were differences among colored fibers. The brown B14 fibers were much longer and stronger the brown B15 fibers. Compared to both brown fibers, the green fiber showed lower micronaire, lower surface friction, a less round cross section, and higher wax content [61]. Table 2-6 shows comparison of general properties between white cotton and colored cotton.

Green colored cotton showed lower micronaire than white cotton and brown colored cotton with lower fineness. Richards et al. (1999) evaluated general characteristics of colored cotton [60]. Tenacity and the specific work of rupture were low for both colored cotton but the values are still acceptable to be commercialized according to Meredith (1952) [62].

Table 2-6. General properties of the cotton fiber sample [59]

	Memphis white cotton	American brown cotton	American green cotton
Micronaire index[a]	3.7	3.8	2.6
Maturity ratio[b]	0.86	0.84	0.59
Percentage maturity[c]	76.8	74.4	50.8
Fineness (dtex)[d]	1.57	1.65	1.38

[a] fineness of a fiber derived from a function of the inverse of the specific surface area of the fiber, the area presented by a known mass of fibers

[b] Ratio of the actual degree of wall thickening to a standard degree of thickening equal to 0.577

[c] Percentage of matured fibers in the sample whose walls have developed to a greater than a specified degree

[d] Mass per unit length

Table 2-7. Tensile Properties of the cotton fiber samples [59]

	White cotton	Brown cotton	Green cotton
Tenecity (cN tex-1) CV (%)	14.33 (34.8)	9.78 (36.9)	9.12 (41.6)
Extension (%) CV (%)	5.78 (26.6)	4.96 (23.3)	6.18 (25.3)
Specific work of rupture (cN tex-1)	0.39	0.20	0.25

White cotton is superior to colored cotton in view of the typically measured fiber properties. Parmer et al. (2001) did an FTIR (Fourier Transform Infrared Spectroscopy) study on colored cotton fiber, comparing it to white cotton fiber. The study revealed that white cotton has more intermolecular hydrogen bonding than colored cotton. This helps to explain the higher mechanical strength of white cotton. However green colored cotton has higher intermolecular hydrogen bonding than brown colored cotton, and also lower mechanical strength, due to its higher wax content [20].

Performance of Naturally Colored Cotton

Abrasion resistance

Dickerson *et al.* (1996) evaluated the performance of green, brown, and red cotton knits against those made from white Pima and blends with colored cotton fibers. Abrasion resistance was evaluated according to ASTM D 3884. After 100 cycles with CS-10 wheels, the weight loss was measured. Overall abrasion resistance of blended knits was good with a maximum 0.5%

weight loss. The laundering process affected the weight loss but there was no significant difference between colored cotton knits and white and colored cotton blend knits [25].

Bursting strength

Bursting strength was evaluated using ASTM D 3787-89. The white pima cotton showed higher bursting strength than colored cotton both before and after laundering. Higher percentage of white cotton in blending showed higher bursting strength. But the effect of laundering on bursting strength was less important for the colored cotton. The decrease in bursting strength after laundering was less for colored cotton than for the white cotton [25].

Pilling resistance

Evaluation of pilling resistance was done according to ASTM D 3512-82. Laundered cotton knits were tested for 30 minutes in an Atlas Random Tumble Pilling Tester. The green cotton showed good resistance to pilling, and red cotton showed severe pilling before laundering. After 20 laundry periods, both the brown cotton and the white Pima showed the best pilling resistance and the red cotton showed the worst pilling resistance [25].

Dimensional stability

Dickerson *et al.* [25] also evaluated dimensional stability in their study of naturally colored cotton knitted fabrics. All fabrics had considerable shrinkage after laundering and the greatest dimensional change occurred after the first laundering. Shrinkage of the brown fabrics was similar to, and sometimes less than, that of the white Pima cotton. Both red and green colored fabrics shrank more than the white Pima and brown knits.

Scouring

Conventional scouring process using chemical agent

The cotton fiber has a multilayer structure. These layers are different physically and chemically, and contain approximately 10% of non-cellulosic substances by weight such as fat, waxes, pectic substances, organic acids, protein/nitrogeneous substances, non-cellulosic polysccharides, and other unidentified compounds [63, 64, 65, 66, 67]. These non-cellulosic materials become a physical hydrophobic barrier which protects the cotton fiber from the environment such as weather throughout growing. They provide lubrication during textile spinning, and also cause a decrease in moisture absorbency of the fiber [68, 69]. A scouring process is done for cellulosic fibers to remove these impurities. The usual process is done with 3% of NaOH on the weight of fibers in a boiling temperature with or without pressure. This alkaline process accelerates saponification of the esters present in cotton wax and neutralizes the free fatty acids. The alkaline reduces the interfacial tension of the remaining wax. Also electrolytic dissociation of the cellulose may reduce the adhesion of oil to the fiber surface [44].

Scouring assistants are required in addition to alkali. The chemicals usually used are an anionic surface active agent for wetting the substrate, a sequestering agent for chelating alkaline metals such as Mg or Ca, and reducing compound that helps protect the cotton from oxidative damage during the alkaline scouring [45].

Scouring process using enzymes

Alkaline scouring consumes a large quantity of alkali and needs an extensive rinsing process that produces waste matter with environmentally harmful chemicals [70]. Alkaline scouring may also cause fabric shrinkage and changes in physical properties of fabric such as

handle [71]. The enzymatic scouring of cotton known as bioscouring is a desirable alternative to conventional alkaline scouring [72]. Various individual enzymes and their mixtures for bioscouring were studied including pectinases, cellulases, proteases and lipases. Pectinases appear to be the most suitable enzymes for scouring. Pectinases are capable of depolymerising the pectin and breaking it into low molecular water-soluble oligomers [73, 74]. Pectinases can improve the absorbency of the textile material without causing cellulose destruction [66, 75].

Scouring process on naturally colored cotton

The color of naturally colored cotton was found to be more affected by alkaline solutions than acidic. It was reported that conventional caustic scouring caused the highest color loss compared to enzymatic scouring. Treatment with salts of iron, copper, zinc and other metal salts led to a washfastness shift in color of naturally colored cotton. Also, the quality of the water used for treatment of colored cotton affected the results, as salts present in the water were found to deepen the color of naturally colored cotton [76].

Laundering for Naturally Colored Cotton

Detergent

Synthetic detergents that are often called surfactants appeared in the 1930s. They are usually neutral (pH near 7) but sometimes provide a milder alkaline condition. Table 2-8. shows components and functions of general detergents [78].

Table 2-8. Components and functions of detergent [77]

Surfactant (LAS*, AS**, nonionic, or soap)	Loosen and disperse soil, provide or control suds; basic "cleaning" ingredient
Builder	Soften water, aid surfactant in dispersion of soil, buffer detergent solution in alkaline region
(1) Fluorescent whitening agent (2)Bluing agent	Overcome yellowness on fabric and provide whiteness of blue-white hue
Enzymes	Catalyze breakdown of protein or carbohydrate based stain to allow for removal by surfactant and builder
Antiredeposition agent	Prevent removed soil from going back on fabric
Sodium silicate, sodium sulfate, water	Carries free-flowing powder

Note: *Linear alkyl-benzene sulfonate; ** Alkyl-benzene sulfonate

Enzyme detergent

Though the addition of enzymes in detergent, energy can be saved by enabling a lower wash temperature. Enzymes that are biodegradable materials leaving no harmful residues can partly replace other less desirable chemicals in detergent. The idea of using enzyme in detergents was first described by Otto Röhm in a 1913 patent application [79]. After first appearing, the enzymatic detergent failed to be commercialized because a pancreatic extract enzyme from slaughtered animals was too sensitive to be applied in detergent. In the early 1960s, proteolytic enzymes from bacteria fermentation were produced. These enzymes have good resistance to alkaline environments and showed stability at temperatures up to 65°C for the time period required by normal laundering processes [80]. By 1969 almost 80% of marketed detergent in Europe contained enzymes but the use deceased to less than 50% after safety issues were noticed.

Table 2-9. Composition of an enzyme detergent [81]

Constituent	Composition (%)
Sodium tripolyphosphate (water softener, loosens dirt)	38.0
Sodium alkane sulphonate (surfactant)	25.0
Sodium perborate tetrahydrate (oxidising agent)	25.0
Soap (sodium alkane carboxylates)	3.0
Sodium sulphate (filler, water softener)	2.5
Sodium carboxymethyl cellulose (dirt-suspending agent)	1.6
Sodium metasilicate (binder, loosens dirt)	1.0
Bacillus protease (3% active)	0.8
Fluorescent brighteners	0.3
Foam-controlling agents	Trace
Perfume	Trace
Water	To 100%

Now the enzymes are prepared in safer ways such as granulates, or pellets [79]. More than half of all detergents presently available contain enzymes. Enzymes have the biggest share as a single item in the detergent industry with 25 - 30% of total sales. The amount of enzymes used in detergent preparations is only 0.4 - 0.8% by weight and about 1% by cost. Their ability to withstand breakdown in the alkali solution with other detergent component is an important criterion. Once released to the laundering solution from its granulated or pellets form, the enzyme must endure anionic and non-ionic surfactant, builders, various other agents, and sodium silicate. The pH values can be between 8.0 and 10.5. Enzymes are required to be retained up to 60°C [81].

Amyalse

Amylases are used as desizing agents to remove starch from fabrics after weaving. Amylase is also added to detergent to remove starch soils such as chocolate, gravy, rice, pasta, baby food, sauces and potato that act as a glue and bind particulate soil to the surface [81]. The use of amylases in detergent has caused concerns because the enzymes need stability. Also an optimal level of activity of protease which can hydrolyse other enzymes as well as dirt during laundering can be a problem. Mitidieri et al.(2005) tested various microorganisms and culture media for amylase production for detergent. The study indicated that *Aspergillus niger* L119 is the best producer of amylase. The amylase from *Aspergillus niger* L119 has good activity at 50-60°C and pH 4.0-7.0 [82].

Cellulase

Cellulases are widely used in textile processing mainly for depilling and stone washing effects. Cellulases produced in an alkaline-stable fungal cellulase preparation were introduced for use in cellulosic fabrics [81]. During use for cotton fabric, small fibers are raised from the surface of cotton thread, resulting in a change of hand feel, and improving the brightness of colours. Treatment with cellulase removes the small fibers without apparently damaging the major fibers in the fabrics and restores the fabric to its new appearance. The cellulase also aids the removal of soil particles from the wash by hydrolyzing associated cellulose fibers.

Lipase

Esterases are a useful additive for detergent formulations because they help in removing

lipid-based stains from fabrics. Some esterases such as carboxylesterases or arylesterases hydrolyse carboylesters or phenylacetate. Triacylglycerolesterases hydrolyse triacylglycerol, giving a diacyl glycerol and fatty acid anion, which is known as lipase [81]. Lipase activity is excellent at temperature of 30°C and is inhibited by anioninc surfactants in the detergent strongly. The ability of stain removal of lipase depends on the amount of soil, the concentration of lipase and other washing condition such as hardness of water, temperature and time [83].

Protease

Proteases are useful in processing of silk and wool. Proteases are also important components of detergent formulations for removing protein stains such as grass, blood, egg and perspiration. The most common proteases in the detergent industry are subtilisins from *Bacillus* species because of their high stability in various environments and relatively low substrate specificity [84]. There are many commercial protease enzymes. Alkaline protease 201 by Wallerstein Company is one of the proteases, which has been characterized. It has a broad pH range for activation from 5 to 11, but shows an excellent pH optimum of 10 to 11 on casein substrate at 37°C. It has a high optimum temperature of 60°C, and the optimum temperature can be changed by pH. Also it shows very good compatibility with other components of detergent such as sodium tripolyphosphate [85]. Other research (Joo & Chang, 2005) also showed good stability and compatibility with other detergent components of the alkaline protease [86].

Effect of chemical laundry aids on naturally colored cotton

Dikerson *et al.*(1999) evaluated the effects of various laundry aids-detergents with optical brighteners, phosphates, and chlorine and non-chlorine bleaches-on color retention of naturally

colored cotton. The study indicated that the most color change occurred within five launderings. Presence of bleach was found to affect the color change of naturally colored cotton up to five launderings. Naturally colored cotton showed an increase in lightness (L*). After laundering, presence of optical brighteners in laundry detergents did not show any significant effect on the color of naturally colored cotton. The effect of phosphates in detergents was even less than that of optical brighteners. These results indicate that optical brighteners and phosphates present in laundry detergents may not be instrumental in shade change of naturally colored cotton [87].

Williams and Horridge (1996) also did laundry tests to compare the colorfastness of coyote brown fiber, green fiber and white cotton fiber. The researchers noted that ammonia instantly darkened and blued green colored fiber and darkened coyote brown fiber. Chlorine bleach reduced the hue of the colored cotton [88].

Effect of drycleaning on naturally colored cotton

Commercial drycleaning using Stoddard (petroleum distillate) and perchloroethylene solvent was performed on naturally colored cotton fabric. The original color of the cotton fabrics was maintained after drycleaning. The hand was less affected and formation of pilling was less by drycleaning than by laundering in water. Drycleaning colored cotton may be preferable to laundering to retain the original character of the fiber. The rich and deep color that is possible in colored cotton after wet treatment did not occur after drycleaning [89].

Weathering Effect on Naturally Colored Cotton

Weathering

In 1729 Dufray in France developed the first system of controlled lightfastness evaluation.

During 1892-1899, Hummel evaluated the fastness of almost all the dyes available at that time [90]. Now each automobile manufacturer has developed and specified unique methods of tests for their automobile fabrics these methods can be greatly different in equipment, procedure and result. Xenon arc equipment provides flexibility in measuring the results and automatically controlling a wide rage of temperature and humidity conditions in addition to monitoring and controlling the irradiation level of the light source [90].

Lightfastness of dyed fiber

Senebier (1782) and Bancroft (1813) first discussed the chemical mechanism of fading resulting from oxidation and reduction. Chevreul (1837) found that dyes fade readily only in presence of oxygen and moisture [89]. Now more research on fading has shown that there are many factors which can affect the fading even though some interrelationships are not predictable. Lightfastness is determined by both internal and external factors. Internal factors include the chemical and the physical state of dye, the dye concentration, the nature of the fibers. External factors include the source and the intensity of illumination, the temperature and the humidity and atmospheric pollution [91]. Almost 50% of natural dyes contain flavonoid compound which is also found in naturally colored cotton. Although flavonoid compounds are not very lightfast, anthraquinones and indigoids that are other common components in natural dye are known as having excellent lightfastness. Chemical structure of the color factor in fibers can affect fading. Symmetrical dye molecules usually have greater lightfastness than non-symmetrical dye molecules [91]. Colorfastness increases with the increase of dye concentration [89].

Dyes with low lightfastness are sensitive to ultraviolet and visible light, whereas dyes with higher lightfastness are sensitive mainly to ultraviolet light [92]. Humidity and temperature

are very important external factors that affect colorfastness to light. Hydrophilic fibers under higher humidity showed higher rates of color loss than hydrophobic fibers [89]. The oxidation index and moisture regain characteristic of a fiber are important factors in this research. It is because the swelling effect of the absorbed moisture in the fiber, which facilitates oxygen transport to the excited dye molecules, involves the breakdown of the dyes [93].

Lightfastness of naturally colored cotton

A study examined the lightfastness and fading of 11 colored cottons. Lightness remained constant or decreased for nine brown cotton varieties exposed to light from a 400 watt mercury vapor filament phosphor coated fluorescent lamps at a distance of 20 centimeters for up to 300 hours. Lightness increased after 300 hours of exposure and reached saturation at approximately 500 hours. Redness (a*) values of the brown cottons increased up to 300 hours of exposure and decreased at 300-500 hours. For green samples, lightness and greenness increased after 50 hours of exposure [94].

Naturally colored cottons of camel brown and olive green color have been blended with conventional white cotton and then treated with various chemicals to develop various shades and colors. Ferrous sulphate, potassium aluminum sulphate, copper sulphate, and tannic acid were used separately and together. The chemically treated colored cotton showed very good colorfastness properties to light and washing compared to 100% white cotton samples [95].

Hustvedt and Crews (2005) evaluated lightfastness of wet treated naturally colored cotton. The treatment factor was type of laundering, which had three levels: none, laundered with detergent alone, and laundered with detergent plus non-chlorine bleach. After the wet treatment, samples were exposed to light of 40 and 80 AFUs according to American Association of Textile

Chemists and Colourists (AATCC) test Method 16: Color Fastness to Light, Option E using an Atlas Ci65A Xenon Weather-Ometer. The results show that the majority of the color loss in the naturally colored cotton occurred during the first 40 AFUs of light exposure. The green cotton samples show ΔE 9.7. This was the largest amount of color change of all of the samples. The color loss was visually perceptible and equivalent to a step 2 on the AATCC Gray Scale for Color Change (AATCC, 2002). The brown cotton samples also showed significant change in color (ΔE) and visually perceptible color change with a step 3.0 on the AATCC Gray Scale for Color Change (AATCC, 2002). Tan cotton samples showed significant changes in color (ΔE) but they were not visually perceptible with a step 4.5 on the AATCC Gray Scale for Color Change (AATCC, 2002). The color loss indicated that some damage occurred to the chromophores, such as the tannins and catechin, a tannin precursor [21].

Summary

Naturally colored cottons began to be considered as commercial product as interest in eco-friendly material increased. Previous research reported its special advantages such as good UV protection factor and flame resistance. Research on pigment in naturally colored cotton revealed part of its character. Wet laundering process changed color of naturally colored cotton to stronger color. Dry cleaning process showed less affect on color of naturally colored cotton. Generally, cotton processing includes scouring. Bio-scouring processes using enzymes are popular currently. Also enzymatic detergent is widely used. This research studied the character of pigment in colored cotton through scouring, enzymatic laundry, and UV exposure process. Various methods such as light microscopy, SEM, X-rays, ICP and wax extraction were used.

CHAPTER 3

METHODOLOGY

Effect of Scouring

Scouring process

Buffalo brown, Coyote brown and green fiber samples (FoxFiber®) were scoured three ways:

A. 0.5g/L sodium carbonate and 1g/L Tween 80: 40:1 liquor ratio at 95°C for 30 minutes

B. 0.5g/L sodium hydroxide and 1g/L Tween 80: 40:1 liquor ratio at 95°C for 30 minutes

C. 100g/L pectinase and 50g/L cellulase: 0.05M acetate buffer solution of pH 5.0 at 50°C for 3 hours.

After each scouring, the fiber samples were washed thoroughly in distilled water to remove any alkali present and air-dried. The fiber samples were then conditioned at 21±1°C and 65±2°RH for a period of 8 hours.

Analysis methods

Color measurement

The CIELAB color space can be visualized as a three dimensional space, where every color can be uniquely located. The location of any color in the space is determined by its color coordinates; L*, a*, and b*. L* indicates lightness (where L*=100) or darkness (where L*=0). The term of a* indicates the greeness (-a*) to redness (+a*). The term of b* indicates the

blueness (-b*) to yellowness (+b*).

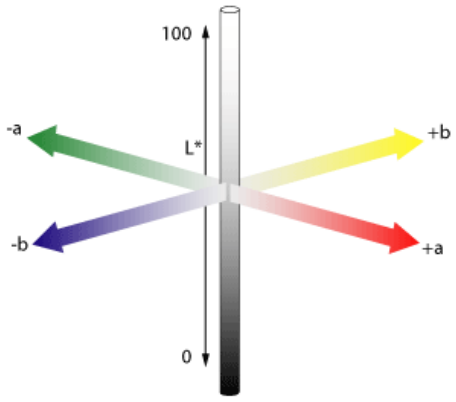


Figure 3-1. CIELab color space [96]

CIELAB color difference, between any two colors in CIE space, is the distance between the color locations. This distance can be expressed as ⊿E CIE L*a*b*, where:

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

Scoured and unscoured fiber samples were put into the glass sample compression cell with air pressure (30psi) and the CIEL*a*b* color coordinates were measured with Lab Scan XE (Hunter Lab) using D65 standard daylight as the illuminant and 10 degree observer. Every sample was conditioned before color measurement.

Moisture regain (%)

Scoured and unscoured fiber samples were conditioned at 21±1°C and 65±2°RH for a

period of at least eight hours at least before the weight measurement for moisture regain test. The measured samples were then oven-dried at 105±2°C for a period of 12 hours according to American Society for Testing and Materials (ASTM) D 2495-01: Standard Test Method for Moisture in Cotton by Oven-Drying. The samples were reweighed after oven-dried. The drying, cooling, and weighing processes were repeated until the change in mass between two successive weights was less than 0.1% of the specimen mass. The difference between the original mass and the oven-dry mass was calculated in percent, as moisture content and moisture regain, according to these equations:

$$M = G - C$$

Where:

M = mass of specimen as received, g

G = gross mass, specimen and container, g, and

C = mass of empty container, g.

$$D = B - T$$

Where:

D = oven-dry mass of specimen, g

B = mass of specimen and basket or weighting bottle, g, and

T = mass of empty weighting container, g.

$$\text{Moisture content, \%} = [(M-D)/M] \times 100$$

$$\text{Moisture regain, \%} = [(M-D)/D] \times 100$$

Wax extraction

Raw naturally colored cottons have higher wax content than conventional cotton. Wax was removed from naturally colored cotton fiber to determine the effect of the wax on color change. This process was to explain the color change of naturally colored cotton after the alkali scouring process. Wax extraction was processed according to Conrad's method [97] using Soxhlet extractor described in 1944.

Five grams of naturally colored cotton fiber was placed in a coarse thimble (Whatman, Maidstone England) in a Soxhlet (60×180 mm) (Kimax, USA) extractor. Two hundred fifty ml (250 ml) of hexane was added to the extraction flask and the heat was adjusted until the liquid siphoned over at 3 to 4 minute intervals. The extraction process then was continued for 6 hours. The fiber was taken out of the thimble and rinsed with distilled water thoroughly to remove excess wax on the surface. Fiber was dried and conditioned at 21±1°C and 65±2°RH for a period of 8 hours at least before color measurement according to ASTM method.

Light Microscopy

Figure 3-2. Plate for fiber cross sectioning

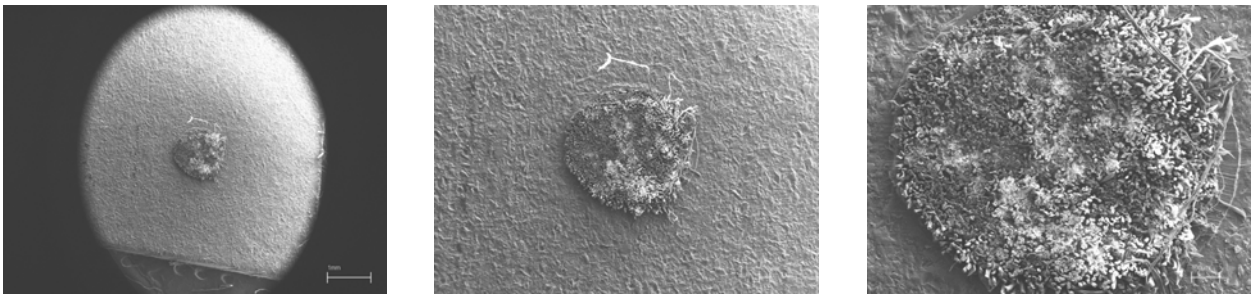
Raw and alkali (Na_2Co_3) scoured fiber was observed through light microscopy. An Olympus BX50 (Olympus Optical CO. LTD., Japan) was used for observation. For longitudinal section, one or two fibers were picked from the sliver and placed onto the glass slides. The fiber

cross-section was prepared in a plate. Fibers were fixed into cross-sectioning plate (figure 3-2) and the excess fiber bundles were trimmed. Light microscopy images were captured by camera.

Scanning electron microscopy (SEM)

There can be two possible reasons that can be explained by SEM to investigate the reason for changes in colors of three naturally colored cotton fibers. First, the surface of the fibers could be changed after scouring, which might affect the outer part of the fibers. Second, the location of pigment in the fibers might be changed affecting interior components of the fibers.

Raw and scoured fibers were prepared for comparisons with SEM. Samples were cut into small pieces and mounted onto the stub fixed on carbon adhesive for longitudinal side observation. For cross-section observation, the fibers were fixed into cross-sectioning plate and the excess fiber bundles were trimmed. Then the samples were coated with 30nm of gold in a sputter-coater. Both longitudinal and cross-sectional sides were observed. Figure 3-3 shows how the cross-sectioning plate was seen under SEM.



(a) (b) (c)

Figure 3-3. Cross section of cotton fiber under SEM

ICP (Inductively Coupled Plasma) analysis

Water, soil extraction, plant or animal tissue, food and chemical solutions can be analyzed by ICP when they can be brought into aqueous solution [98]. Samples for ICP analysis must be fully prepared by extraction, dissolution or acidification. Naturally colored cotton fibers were dissolved for preparation. Amount of 0.1g fiber was picked from various parts of the sample and placed in a beaker filled with 2ml of 98% H_2SO_4 and 10ml of H_2O_2. This mixture was heated to 380°C on a heating plate until all fiber was dissolved. When the solution became into transparent liquid and dense fume coming out of the solution was no longer visible, the heating was stopped. The liquid was moved into a 50ml flask and distilled water was added to make 50ml of solution. The samples were then ICP analyzed and amount of 23 heavy metals in the fiber solution were obtained.

X-ray surface analysis

When the primary x-ray beam hits atoms, electrons at the inner shell of an atom can be displaced and electrons from the outer shell can fall into the inner shell. Source of excess energy generated is in the form of X-rays, which can be detected and provide elemental information [99]. An X-ray detector (INCA X-sight, Oxford) detects the position and energy of X-ray. For this type of analysis, the fiber samples are prepared the same way as for SEM image analysis.

Effect of Enzymes as Detergent

Enzyme assay

Cellulase

Activity of cellulose was estimated by determining the amount of reducing sugars present

in supernatant by the dinitrosalicylic (DNS) acid method [100]. The DNS method is used to test the reducing sugars by the action of cellulolytic enzymes. A free carbonyl group present in glucose is oxidized to a carboxyl group and simultaneously 3,5-dintrosalicylic acid (DNS) is reduced to 3-amino, 5-nitrosalicyclic acid under alkaline conditions. The color that appeared by 3-amino, 5-nitrosalicylic acid was read at 540 nm. DNS solution was prepared as follows:

Sixteen grams of sodium hydroxide pellets were dissolved in water and ten g of dinitrosalicylic acid was quickly added. Three hundred grams of potassium was added after the other two components were dissolved. Deionized water was added to make the volume of 1 liter. DNS solution was stored in a brown glass bottle until the assay was performed.

The acid cellulose assay was processed as follows:

Carboximethylcellulose solution at 1% (CMC, low viscosity, Sigma Chemical) was prepared. Fifty μL of diluted enzyme was added to 200μL of 1% CMC. This mixture in a test tube was incubated for 15 minutes at 50°C. The reaction was stopped by adding 1.0mL of DNS reagent and boiled for five minutes. The reaction result was measured by reading ABS_{540}.

Standard solution was prepared as follows:

Ten g anhydrous D-glucose was dissolved into 1.0L 50mM SAB (Sodium Acetate Buffer), pH 4.8 and standard solutions were made according to table 3-1.

A standard plot of A_{540} standard vs μmoles of glucose was obtained from standard. The sample was determined by A_{540} test minus ABS_{540} sample blank. CMCase activity was obtained as follows:

Cellulase activity (Units/g) = (amount of glucose equivalent produced/180/15/0.05) × dilution factor

The dilution factor was 10 mL in this example.

Table 3-1. Glucose standard solution

	Standard solution 1	Standard solution 2	Standard solution 3	Standard solution 4	Standard solution 5	Standard solution 6
1.0% CMC	200	200	200	200	200	200
10 mg/mL glucose solution (μL)	0	2	3	5	10	15
50 mM SAB, pH 4.8 (μL)	50	48	47	45	40	35
Total volume (μL)	250	250	250	250	250	250
Amount of glucose (μg)	0	20	30	50	100	150

Amylase

Activity of amylase was obtained with the same method for cellulase. The substrate was 1% starch and this was applied on the stirring plate always because starch is not water soluble.

Lipase

Eleven percentage olive oil emulsion (olive oil into 5.0% Titron X-100 solution and to make emulsion, 4.0% bovine serum Albumin and potassium phosphate buffer, pH 7.0, are added.) was used as substrate. The reaction was started by adding of lipase solution in 20 mM potassium phosphate buffer, pH 7.0. Incubation was processed at 37°C for 15 min. Two hundred mM trichloroacetic acid solution (TCA) was added to the test solution and filtered through Whatman No. 42 filter paper. Color reagent (prepared in 50 mM MES NaOH buffer by adding Titron X-100 solution, 4-aminoantipyrene, adenosine 5'-triphosphate, magnesium chloride, glycerokinase, peroxinase and glycerol-3-phosphate oxidase) was added and incubated at 37°C for 15 min. The reaction result was measured by reading A_{545}. Lipase activity was obtained with

results from both test and blank tubes using a proper equation.

Protease

A 0.65% Casein solution was used as the substrate. The casein solution was made with Casein (Sigma) dissolved into 50 mM potassium phosphate buffer (pH 7.5) at 85 °C for 10 minutes. Tris-HCl was used as the incubation buffer, made by a mixture of tris (hydroxymethyl) aminomethane hydrochloride and calcium chloride dehydrate. The pH was controlled to 7.8 by adding sodium hydroxide solution (2 mol/l). Stop reagent was 110 mM Trichloroacetic Acid (TCA). Trypsin was dissolved into distilled water to be used as standard solution. Incubation was processed at 37°C for 2 hours and stopped by the stop reagent. The reaction result was measured by reading ABS_{574}. Protease activity (units/mg) was obtained according to standard plot.

Enzyme treatment process

Naturally colored cotton fibers scoured were treated with four kinds of enzymes separately. Amylase (from *Bacillus lichniformis*), cellulase (from *Aspergillus species*), lipase (from *Thermomyces lanuginosus*) and protease (from *Bacillus sp*) were selected for this research (Table 3-2). They were used in 0.05M borate buffer solution of pH 8.0 (Table 3-3).

Table 3-2. Characteristics of enzymes used as detergent for naturally colored cottons

Enzyme	Origin	Activity[a] ($U/g \times 10^{-3}$)	Supplier
Amylase	Bacillus lichniformis	75	Sigma-Aldrich
Cellulase	Aspergillus species	1.0	Sigma-Aldrich
Lipase	Thermomyces lanuginosus	0.02	Sigma-Aldrich
Protease	Bacillus species	1.0	Sigma-Aldrich

[a] Activities were determined by the methods that were mentioned above.

Table 3-3. Concentration and Types of Single Enzyme Treatment

Cotton	Enzyme	Concentration
Buffalo brown	Amylase Cellulase Lipase Protease	2, 5, 10, 15 U/g
Coyote brown	Amylase Cellulase Lipase Protease	2, 5, 10, 15 U/g
Green	Amylase Cellulase Lipase Protease	2, 5, 10, 15 U/g

There were three steps for the enzyme treatment processing. The treatment was started at 50°C for 1 hour and then the temperature of treatment was increased to 80°C for 10 min. to deactivate the enzyme. Then the temperature was decreased to 30°C before finishing treatment

(Figure 3-4). The time and temperature were controlled automatically by Ryrotec laboratory dyeing machine (Roaches). After the enzyme treatment each sample was thoroughly rinsed and air-dried. The samples were then conditioned at 21±1°C and 65±2°RH for a period of 8 hours.

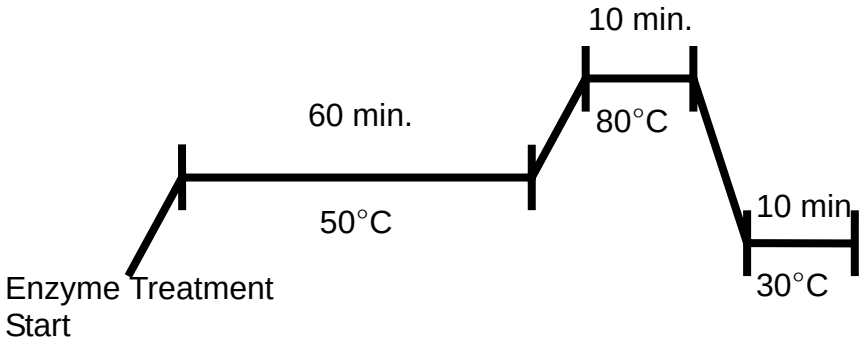


Figure 3-4. Enzyme treatment process

In addition to treatment with separate enzymes, the colored cotton fibers were also treated with multiple enzymes, as shown in table 3-4.

Table 3-4. Concentration and Types of Mutiple Enzyme Treatment

Cottons	Enzymes	Concentration
Buffalo Brown Cotton Coyote Brown Cotton Green Cotton	Amylase + Cellulase	6 & 12 U/g
	Amylase + Lipase	
	Amylase + Protease	
	Cellulase + Lipase	
	Cellulase + Protease	
	Lipase + Protease	
	Amylase + Cellulase + Lipase + Protease	6 & 12 U/g

Analysis methods

Color measurement

Cotton fiber samples were put into a glass sample compression cell with air pressure (30psi) and the CIEL*a*b* color coordinates were measured with Lab Scan XE (Hunter Lab) using D65 standard day light as the illuminant and 10 degree observer. Every sample was conditioned after wet treatment before color measurement.

Moisture regain (%)

Cotton fiber samples were conditioned at 21±1°C and 65±2°RH for a period of 8 hours before the weight measurement for moisture regain test. The measured samples were then oven-dried at 105±2°C for a period of 12 hours according to American Society for Testing and Materials (ASTM) D 2495-01: Standard Test Method for Moisture in Cotton by Oven-Drying. The samples after oven-dried were reweighed. The drying, cooling, and weighing processes were repeated until the change in mass between two successive weights was less than 0.1% of the specimen mass. The difference between the original mass and the oven-dry mass were calculated in percent, as moisture content and moisture regain according to these equations:

$$M = G - C$$

Where:

M = mass of specimen as received, g

G = gross mass, specimen and container, g, and

C = mass of empty container, g.

$$D = B - T$$

Where:

D = oven-dry mass of specimen, g

B = mass of specimen and basket or weighting bottle, g, and

T = mass of empty weighting container, g.

$$\text{Moisture content, \%} = [(M-D)/M] \times 100$$

$$\text{Moisture regain, \%} = [(M-D)/D] \times 100$$

Statistical analysis

To study determine significance of data sets of fiber types and enzyme treatments on increases of moisture regain, Analysis of Variance (ANOVA), Tukey, and Duncan groupings were tested. Predictor variables were fiber types, enzymes and interaction of fiber types and enzymes. Response variables were moisture regain (%). The hypothesis was,

H_1: Enzymes, fiber types and interactions between enzymes and fiber types are not significant effects on moisture regain (%).

Effect of UV Exposure

UV exposure process

An Atlas Weather-Ometer®, model C135A was used as the weathering chamber. The apparatus used a 2500 watt water-cooled xenon lamp with borosilicate inner filter and soda lime outer filter. Samples were mounted consistently in weather-Ometer® frames with a white cardboard backing and placed in the chamber in random positions. The standard procedure for weathering textiles in this instrument was based on AATCC Test Method 16-2002 Colorfastness to Light. Option E was chosen with 30±5% of relative humidity (Table 3-5).

Table 3-5. Weathering machine start-up condition by option E

Component	Option E
Light source	Xenon
Black panel temperature, light cycle	63±1°C
Dry bulb temperature, light cycle	43±1°C
Relative humidity, %	30±5%
Light cycle, hours	Continuous
Filter type: outer inner	Soda lime Borosilicate

Preliminary tests were conducted on each naturally colored sample to determine the most appropriate exposure duration to use in the study. The preliminary tests showed a visible color change after only five hours for green cotton. Consequentlt, five hours exposure was chosen as the first level of 6 levels exposure duration, as shown in table 3-6.

Table 3-6. Six Levels of Exposure Duration

Level	Duration	Light Cycle
1	0 hours	none
2	five hours	continuous
3	10 hours	continuous
4	20 hours	continuous
5	30 hours	continuous
6	40 hours	continuous

Analysis methods

Color measurement

Cotton fiber samples exposed to light were conditioned at 21±1°C and 65±2°RH for a period of 8 hours. Conditioned samples were put into the sample compression cell with pressure (30psi) and the CIEL*a*b* color coordinates were measured with Lab Scan XE (Hunter Lab) using D65 standard day light as illuminant and 10 degree observer.

Statistical analysis

Relationship between lightness and enzyme treatment condition were analyzed using Statistical Analysis System (SAS) program. This analysis was processed for each fiber type separately due to their different characters of lightfastness. Predictor variables were UV exposure hours, enzymes and enzyme concentrations. Response variables were values of lightness. When repeated measurements are taken on the same experimental unit, and these repeated measurements are correlated or exhibit variability that changes, the SAS procedure “Proc Mixed” can be used. Here, ‘Mixed procedure’ was used because values of lightness were

measured repeatedly for a same sample with increase of UV exposure hours. The hypothesis was,

H_1: UV exposure hours, enzymes and enzyme concentrations are not significant effects on moisture regain (%).

CHAPTER 4

RESULTS AND DISCUSSION

Color Changes of Naturally Colored Cotton after Scouring

Table 4-1 shows color change of buffalo brown cotton after different scouring methods. After each scouring treatment, lightness decreased. Enzymatic scouring process resulted in less change in color than conventional scouring. The amount of color change after enzyme treatment was similar to the change after only water (H_2O) boiling. However, alkali treatment showed large changes in color. Both $CaCO_3$ and NaOH treatment decreased lightness significantly. The b* values decreased (decreased yellowness) after both alkali treatments. This corresponded with change toward a deep brown color. Figure 4-1 shows how raw buffalo cottons and scouring solutions are changed after each scouring process. Decreases in lightness are clearly observed after alkali scouring processes. Scouring solutions after scouring process are shown in the beakers at the center of each figure. After alkali scouring processes the scouring solutions were

Table 4-1. Color changes of naturally buffalo brown cotton after scouring processes

	L*	a*	b*	ΔL	Δa	Δb	ΔE
Raw	51.17	12.33	24.04	-	-	-	-
Water only	48.81	13.01	25	-2.36	0.68	0.96	2.636968
$CaCO_3$	41.08	11.42	22.06	-10.09	-0.91	-1.98	10.32263
NaOH	42.7	11.73	20.72	-8.47	-0.6	-3.32	9.117198
Pectinase + Cellulase	47.82	13.02	24.07	-3.35	0.69	0.03	3.420453

(a)

(b)

(c)

Figure 4-1. Buffalo brown cotton after scouring; (a) $CaCO_3$, (b) NaOH, and (c) Pectinase and Cellulase

deeply brown colored with the result of strongly brown colored fiber. It is assumed that the colored solution is due to the released pigment from naturally colored cotton.

Table 4-2 shows color change of coyote brown cotton after different scouring methods. Similar to the result for the buffalo brown cotton, lightness of coyote brown cotton also decreased after each wet treatment, the enzymatic scouring process resulted in less change in color, which was similar to the change after water (H_2O) boiling. Both $CaCO_3$ and NaOH treatments showed large changes in color. Lightness decreased significantly after alkali treatments and the change was largest after $CaCO_3$ treatment (ΔL:-12.89). After $CaCO_3$ treatment, a* and b* also decreased significantly, which means the color become less red and less yellow.

Figure 4-2 shows how raw coyote brown cottons and scouring solutions are changed after each scouring process. Decreases in lightness are clearly observed after alkali scouring processes. The scouring solutions are deeply colored with dark brown even though the color of fiber also became darker. This change was less after enzymatic scouring process.

Table 4-2. Color changes of naturally coyote brown cotton after scouring processes

	L*	a*	b*	ΔL	Δa	Δb	ΔE
Raw	53.37	13.08	26.18	-	-	-	-
Water boiling	51.57	13.54	26.86	-1.8	0.46	0.68	1.978
$CaCO_3$	40.48	10.01	19.4	-12.89	-3.07	-6.78	14.884
NaOH	46.69	12.3	22	-6.68	-0.78	-4.18	7.919
Pectinase + Cellulase	51.18	13.66	26.65	-2.19	0.58	0.47	2.314

(a)

(b)

(c)

Figure 4-2. Coyote brown cotton after scouring; (a) $CaCO_3$, (b) NaOH, and (c) Pectinase and Cellulase

Table 4-3 shows color change of green cotton after different scouring methods. Green cotton also showed less color change after enzymatic scouring and the amount of color change with enzyme scouring was similar to that of water boiled green cotton. After both alkali treatments, the overall color changes of green cotton were larger than those of both brown colored cottons. The major reason of the high ΔE with the alkali scouring is decreased lightness. After $CaCO_3$ treatment, the lightness decreased -17.79. The a* and b* values also showed decreased, indicating a hue shift toward green and blue. After NaOH treatment, the a* value was changed to a negative value from a positive value, which indicates that the color lost its red and turned to green. Figure 4-3 shows that the scouring solutions are also colored with yellowish green after scouring processes. But color changes of the scouring solutions were not noticeably different from the color change of scouring solutions from both brown cottons.

Table 4-3. Color changes of naturally green cotton after scouring processes

	L*	a*	b*	ΔL	Δa	Δb	ΔE
Raw	68.62	2.36	22.16	-	-	-	-
Water boiling	66.77	2.88	23.57	-1.85	0.52	1.41	2.383
$CaCO_3$	50.83	0.36	16.74	-17.79	-2	-5.42	18.705
NaOH	55.29	-0.78	17.47	-13.33	-3.14	-4.69	14.476
Pectinase + Cellulase	66.95	2.5	22.42	-1.67	0.14	0.26	1.696

Results show that alkali scouring process makes color of naturally colored cotton stronger. Wet treatment makes the color of naturally colored cotton stronger, but decrease in lightness after alkali scouring process also occurs. Also, the very strong color of the scouring solution after the scouring process is an unexpected result. When the color of fiber was darkened, the scouring solution was also more colored. To find cause of this colored solution would be helpful to understand the character of naturally colored cotton.

(a)

(b)

(c)

Figure 4-3. Green cotton after scouring; (a) $CaCO_3$, (b) NaOH, and (c) Pectinase and Cellulase

Mechanism of Color Change of Naturally Colored Cotton after Scouring

Wax Extraction

Table 4-4. Color change of naturally colored cotton after wax extraction

	Buffalo brown			Coyote brown			Green		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Raw	51.17	12.33	24.04	53.37	13.08	26.18	68.62	2.36	22.16
After extraction	50.46	12.49	23.74	53.4	13.18	26.09	67.97	1.71	21.28

Naturally colored cottons have higher wax content than conventional white cotton. Wax extraction was done to study the effect of wax on color. Two possible explanations are provided. First, wax that is located on the surface of the fiber can cover the coloring component. In this explanation scouring process which removes wax with other impurities can expose more of the color of the fiber surface. The second possibility is that part of the wax layer contains the pigment. Scouring process can remove or pare layers and expose more colored wax layer.

Figure 4-4 shows the color changes of fiber after wax extraction of each of the three naturally colored cottons. After wax extraction, fibers did not show any significant change in color. Lightness was the same as before wax extraction. The extraction solutions from two brown cottons did not show any change in color. Only the extraction solution from the green cotton showed a slight increase in green color corresponding with a small decrease in a* value. The result shows that the brown colors of buffalo brown and coyote brown cotton are not related to their wax content. Wax content appears to be related to fiber color only in the case of the green cotton.

Light Microscopy

Color light microscopic images of colored cotton fibers are shown from figure 4-4 to figure 4-9. Figure 4-4 shows a flat and ribbon-like longitudinal image of raw buffalo brown cotton. Coloring material is scattered and appears faintly. After the alkali scouring process (figure 4-5), fiber swelling was noticed. The dark brown color of buffalo brown is noticed clearly and appears to be mostly on the surface part of the fiber. Raw coyote brown cotton also appeared very flat and had weak color (figure 4-6). The fiber became swollen after the scouring process (figure 4-7). The dark brown color was concentrated in the outer surface of the fiber and a faint green color was noticed at the center of the fiber. Raw green cotton had a greenish dark color at the center of the fiber (figure 4-8). The green color was clearly visible before the scouring process, which was different from raw brown cottons that were faint in color before scouring. After the scouring process, the coloring materials moved toward the edge of the fiber, so that green color was noticed only in the outer part of the fiber (figure 4-9).

These results suggest the movement of fiber pigment during the alkali scouring process. Movement occurred toward outer side of a fiber from center of a fiber. The color in the scouring solution can be explained by released pigment from fiber during this movement.

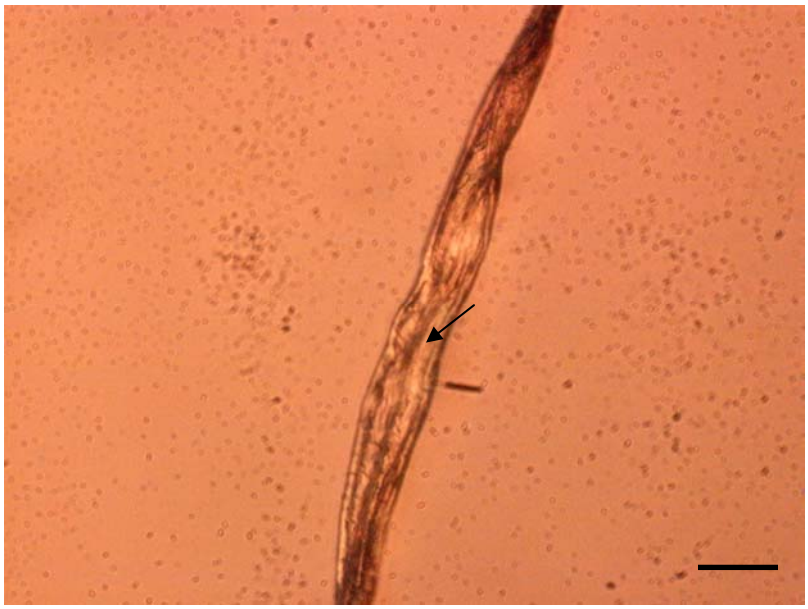


Figure 4-4. Light microscopy image of raw buffalo brown cotton, Bar: 20µm

Figure 4-5. Light microscopy image of CaCO3 scoured buffalo brown cotton, Bar: 20µm

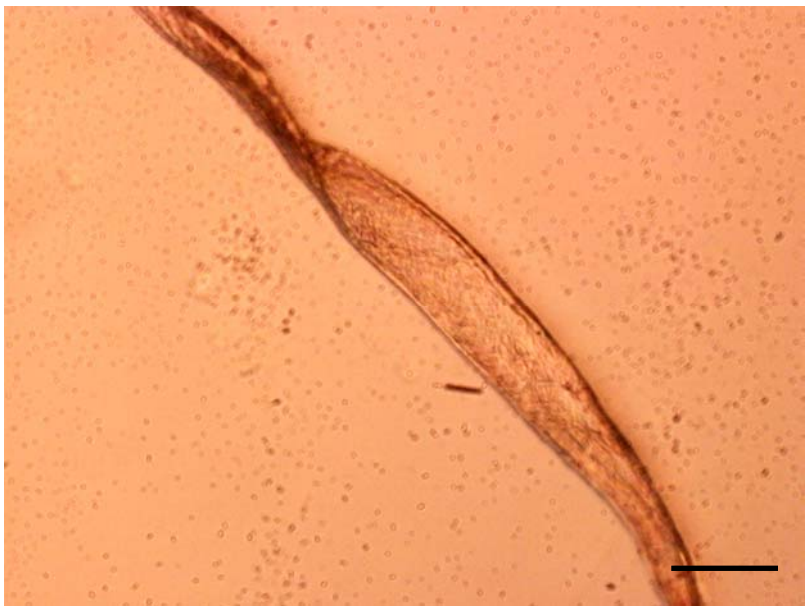


Figure 4-6. Light microscopy image of raw coyote brown cotton, Bar: 20µm

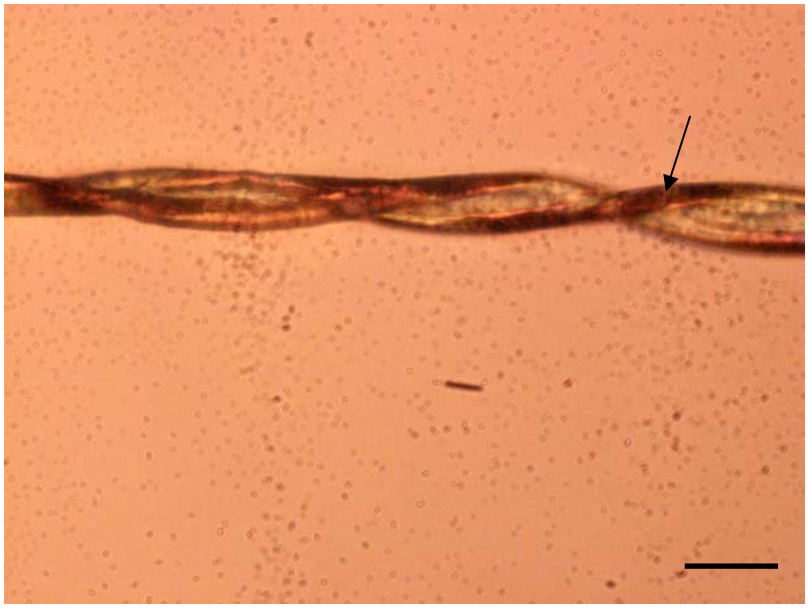


Figure 4-7. Light microscopy image of CaCO3 scoured coyote brown cotton, Bar: 20µm

Figure 4-8. Light microscopy image of raw green cotton, Bar: 20µm

Figure 4-9. Light microscopy image of CaCO3 scoured green cotton, Bar: 20µm

Scanning Electron Microscopy

Figure 4-10 (a)-(f) show SEM pictures of buffalo brown cotton. Figure (a) shows the cross-sections of raw buffalo brown cotton fibers. Because they are air-dried, the cross-section have a bean shape. The lumen area does not have its round shape. Figure (b) shows swollen cross-sections after alkali scouring. The surface area became uniform after the scouring process compared to that of raw buffalo brown fibers. Figure (c) and (d) are magnified pictures of buffalo brown cotton fibers. There is a slight increase in cross-sectional surface area after the scouring process. Figure (e) and (f) show longitudinal changes after scouring process. Because fibers are slightly swollen, the fiber has less twist and is less flat after scouring.

Figure 4-11 (a)-(f) show SEM images of coyote brown cotton fibers. After alkali scouring cross section became round and more regular. However, the difference was less than the changes of buffalo brown cotton fibers.

Figure 4-12 (a)-(f) show SEM pictures of green cotton fibers. Figure (a) shows the cross-sections of raw green cotton fibers with bean shape and lumen areas are compressed with drying of liquid inside after seed pod is opened. Figure (b) shows swollen cross-sections after alkali scouring. The surface area became uniform after scouring compared to that of green fibers. Figure (c) and (d) are magnified pictures of green fibers. The large lumen area of green cotton fiber is observed in figure d even though it is compressed. Clear differences between two pictures were notified. There was an increase in cross-sectional surface area after scouring. Figure (e) and (f) illustrate the longitudinal change after scouring. Figure e showed a very flat, ribbon-like surface of raw green fiber due to compressed large lumen area. Because fibers were swollen, the fiber was less convoluted and rounder after scouring.

The fiber surface is one of several important factors to give a certain color to the fiber. A smooth surface can result in a more brilliant color to fibers. For example, the mercerization process deepens the color of the yarn, because the surface becomes more round after treatment. A swollen surface can explain the stronger color after scouring. However, surface change in fibers does not explain the result of the scouring treatment of buffalo brown cotton fibers.

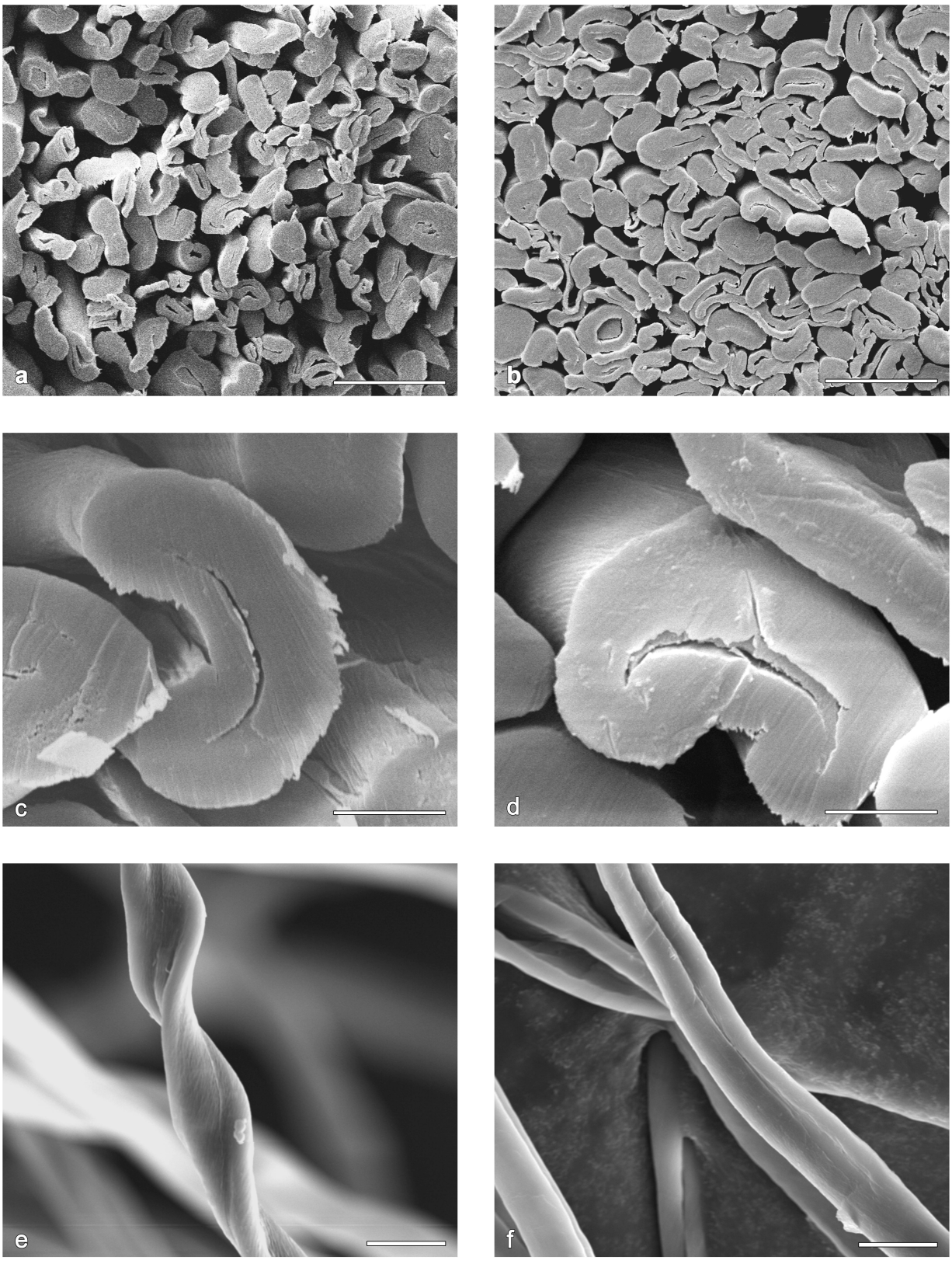

a
b
c
d
e
f

Figure 4-10. SEM images of naturally buffalo brown cotton fibers

(a) Cross-sections of raw buffalo brown cotton fibers. Magnification: X648; Bar=200μm

(b) Cross-sections of scoured buffalo brown cotton fibers with Na_2CO_3. Cross–sections show slightly swollen and more uniformed shape after scouring process. Magnification: X648; Bar=200μm.

(c) Cross-sections of raw buffalo brown cotton fibers. Magnification: X4000; Bar=30μm

(d) Cross-sections of scoured buffalo brown cotton fibers with Na_2CO_3. Cross–sections show slightly swollen and more uniformed shape after scouring process. Magnification: X4000; Bar=30μm.

(e) Longitudinal sides of raw buffalo brown cotton fibers. There is natural twist at fiber surface due to spiral secondary walls inside. Magnification: X1000; Bar=100μm.

(f) Longitudinal sides of scoured buffalo brown cotton fibers. There is less twist due to swollen surface after scouring process. Magnification: X1000; Bar=100μm.

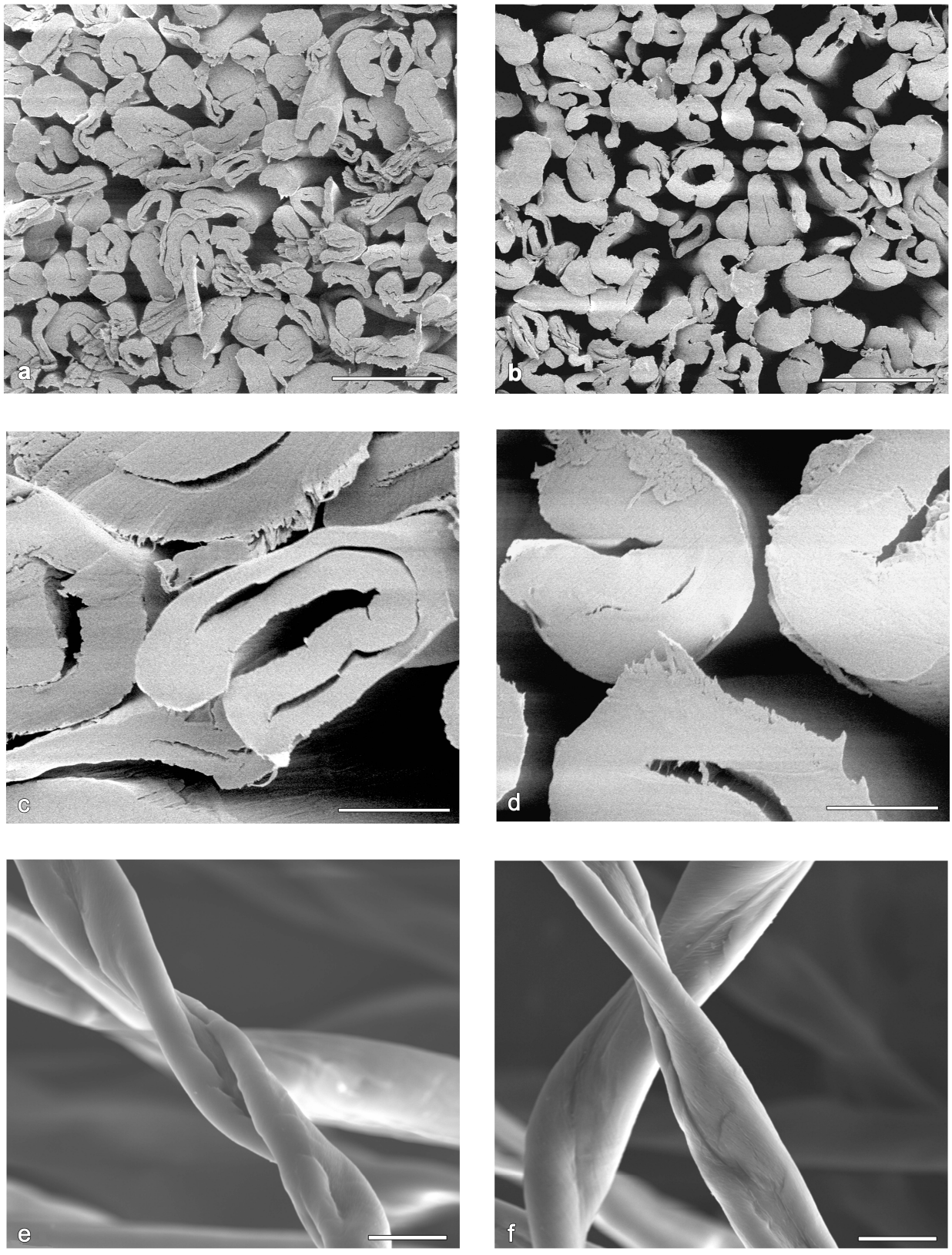

a
b
c
d
e
f

Figure 4-11. SEM images of naturally coyote brown cotton fibers

(a) Cross-sections of raw coyote brown cotton fibers. Magnification: X648; Bar=200μm

(b) Cross-sections of scoured coyote brown cotton fibers with Na_2CO_3. Cross–sections show slightly swollen and more uniformed shape after scouring process. Magnification: X648; Bar=200μm.

(c) Cross-sections of raw coyote brown cotton fibers. Magnification: X4000; Bar=30μm

(d) Cross-sections of scoured coyote brown cotton fibers with Na_2CO_3. Cross–sections show swollen and more uniformed shape after scouring process. Magnification: X4000; Bar=30μm.

(e) Longitudinal sides of raw coyote brown cotton fibers. There is natural twist at fiber surface due to spiral secondary walls inside. Magnification: X1000; Bar=100μm.

(f) Longitudinal sides of scoured coyote brown cotton fibers. There is less twist due to swollen surface after scouring process. Magnification: X1000; Bar=100μm.

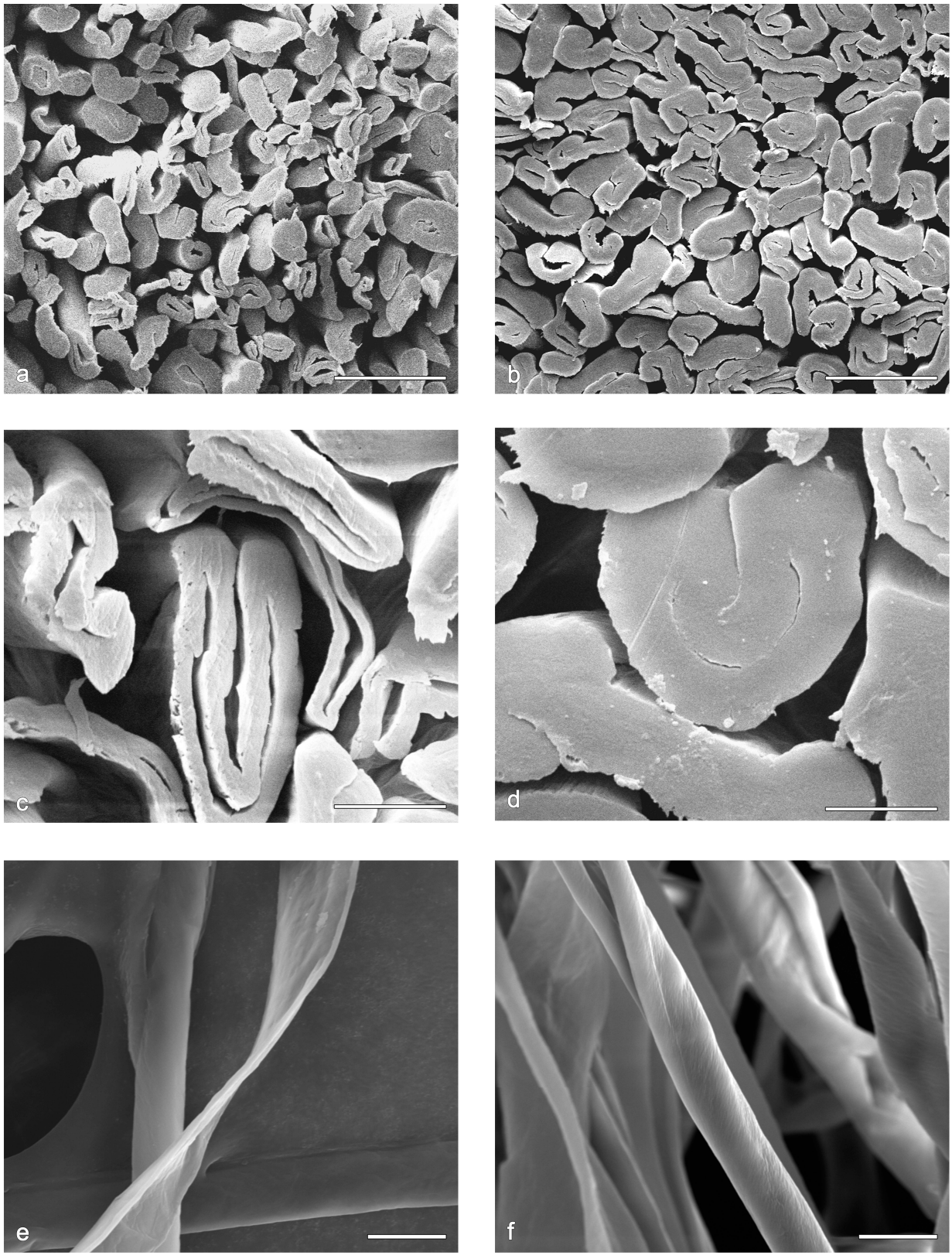

a
b
c
d
e
f

Figure 4-12. SEM images of naturally green cotton fibers

(a) Cross-sections of raw green cotton fibers. Magnification: X648; Bar=200μm

(b) Cross-sections of scoured green cotton fibers with Na_2CO_3. Cross–sections show significantly swollen and more uniformed shape after scouring process. Magnification: X648; Bar=200μm.

(c) Cross-sections of raw green cotton fibers. Large lumen area compressed is observed. Magnification: X4000; Bar=30μm

(d) Cross-sections of scoured green cotton fibers with Na_2CO_3. Cross–sections show swollen a lot and much more uniformed shape after scouring process. Lumen area became smaller due to the swollen. Magnification: X4000; Bar=30μm.

(e) Longitudinal sides of raw green cotton fibers. Fiber has very flat, ribbon like, shape. Magnification: X1000; Bar=100μm.

(f) Longitudinal sides of scoured green cotton fibers. The fiber surface became more round due to swollen surface after scouring process. Magnification: X1000; Bar=100μm.

Fiber surface changes that occur in scouring provide only a partial explanation for color changes of the fiber. Attempts at TEM observations using various resins with and without fixation were inconclusive because the resins did not penetrate the fiber interior well. New methods for fixation or embedding will be tried in the future work.

X-rays surface analysis

Table 4-5 shows potassium (%) that was observed from the surfaces of three naturally colored cottons using X-ray. X-ray detector observed mainly oxygen and carbon, and only a small amount of potassium. However, potassium percentage decreased significantly after alkali scouring.

Table 4-5. Potassium content on surfaces of naturally colored cottons (%)

	Raw	Scoured
Buffalo brown cotton	1.42	0.11
Coyote brown cotton	2.02	0.10
Green cotton	1.33	0.06

ICP (Inductively Coupled Plasma) analysis

Fibers that were dissolved into solutions and the fiber scouring solutions were analyzed using ICP (Inductively Coupled Plasma). Twenty-seven heavy metal concentrations were detected in this analysis (Table 4-6). The three colored cottons showed a similar amount of heavy metal content. The main heavy metals in colored cotton fibers are aluminum, calcium, potassium, magnesium, phosphorus, and antimony and these are different metal contents from those detected from naturally colored cotton in previous research [20, 41]. Previous research used

naturally colored cotton samples from the farms in India and China. Differences between the studies are partially due to different metal content of the soil.

After scouring some heavy metals were released from the fibers, including aluminum, potassium, magnesium, and phosphorus. The three naturally colored cottons contain 0.4 ~ 0.5% of potassium by weight and these amounts decreased significantly after scouring. The scouring solutions contain more potassium than other heavy metals. It was confirmed that scouring process reduced potassium (%) of naturally colored cotton fibers. This is significant since there was no previous research on the relationship between potassium and pigmentation in cotton fibers.

Table 4-6. Weight percentage (%) of heavy metal contents in three naturally colored cotton fibers

	buffalo brown cotton	scoured buffalo brown cotton	buffalo brown cotton scouring solution	coyote brown cotton	scoured coyote brown cotton	coyote brown cotton scouring solution	green cotton	Scoured green cotton	green cotton scouring solution
Ag	.00032	0	.00001	0	0	0	0	.00027	0
Al	.00869	.00062	.00124	.01793	.00530	.00120	.01227	.01105	.00114
As	.00500	.00041	.00014	.00411	.02182	.00012	.00229	.00869	.00018
B	.00230	.00017	.00574	.00200	.00251	.00511	.00173	.00225	.00629
Ba	.00510	.00002	.00001	.00043	.00058	.00001	.00048	.00084	.00001
Be	.00032	.00003	0	.00032	.00041	0	.00030	.00041	0
Ca	.01028	.00793	.00253	.11372	.02964	.00499	.14470	.24824	.00249
Cd	.00015	0	0	.00004	.00058	0	.00004	.00038	0
Co	.00093	.00008	0.0001	.00070	.00067	0	.00052	.00107	0
Cr	.00047	.00002	0.0002	.00011	.00248	.00001	-.00061	.00050	.00002
Cu	.00048	.00008	0.0004	.00037	.00023	.00004	-.00048	.00257	.00018
Fe	.00630	.00036	.00035	.01041	.02260	.00041	.00621	.01225	.00044
K	.43397	.10764	.26450	.53760	.02265	.20100	.54624	.00250	.28913
Mg	.06932	.00565	.00239	.06914	.09469	.00394	.07693	.08961	.00239
Mn	.00063	.00006	.00002	.00094	.00082	.00003	.00114	.00195	.00004
Mo	.00401	.02021	.00006	.00221	.01973	.00004	.00223	.00803	.00109
Na	.01251	.08955	.42163	.00871	.08807	.46563	.00812	.02954	.44350
Ni	.00094	.00008	.00001	.00370	.00068	.00001	.00052	.00104	.00001
P	.05448	.02142	.01663	.05259	.02681	.01601	.06040	.02714	.01328
Pb	.00288	.00019	.00007	.00139	.00204	.00005	0	.00345	.00005
Sb	.02201	.03134	.00092	.01081	.06021	.00076	.01156	.04341	.00337
Se	.01225	.01095	.00011	.00916	.03706	.00009	.00828	.01816	.00029
Si	0	0	.05535	0	0	.04945	0	-.00160	.05870
Sr	.00790	.00005	.00002	.00070	.00109	.00003	.00083	.00104	.00001
Tl	.05115	.00152	.00202	.00260	.00048	.00104	0	.01044	.00073
V	.00300	.00002	.00001	.00013	.00020	.00001	0	.00036	.00001
Zn	.02770	.00019	.00007	.00548	.00282	.00023	.00397	.00608	.00011
Total	.79390	.29800	.67400	.84680	.43680	.75000	.77280	.52960	.73700

Color Changes of Naturally Colored Cottons after Enzyme Treatment and UV Exposure

Color changes of buffalo brown cotton after single enzyme treatment and UV exposure by hours

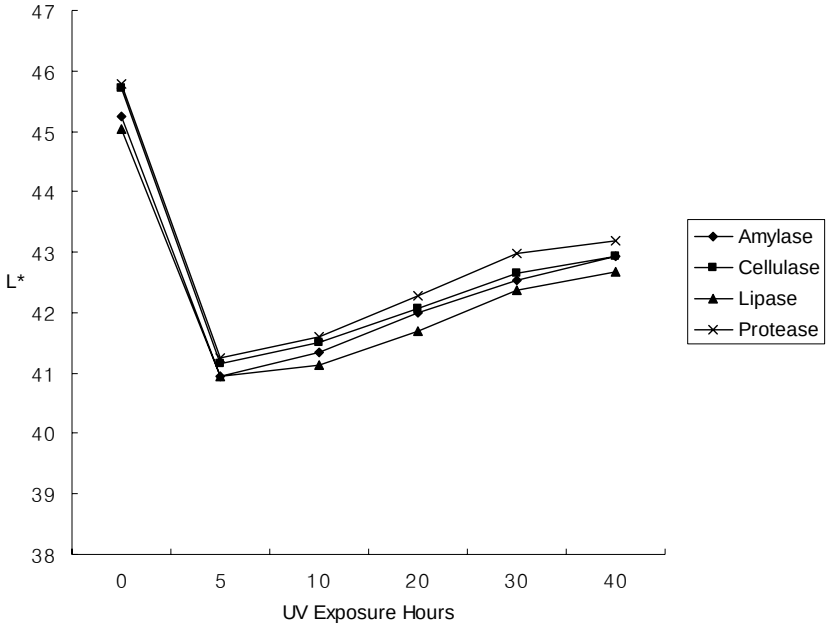


Figure 4-13. Changes of L* of single enzyme treated naturally buffalo brown colored cottons after UV exposure

Lightness of enzyme treated buffalo brown cotton decreased after five hours of UV exposure and then increased slowly after additional UV exposure (figure 4-13). This result was unexpected and different from previous research on raw naturally colored cotton fiber. It was reported that there is a slight increase in lightness of raw colored cotton fiber after UV exposure [21]. To confirm of this result, the same experiment was repeated for a new set of samples and the same decrease in lightness was observed after five hours of UV exposure. There was no significant difference between different enzyme types.

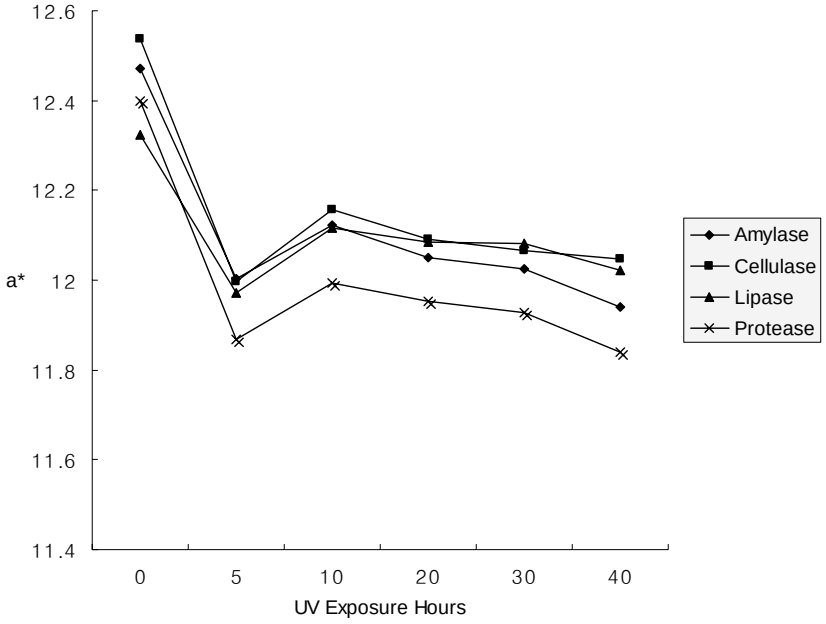


Figure 4-14. Changes of a* of single enzyme treated naturally buffalo brown colored cottons after UV exposure

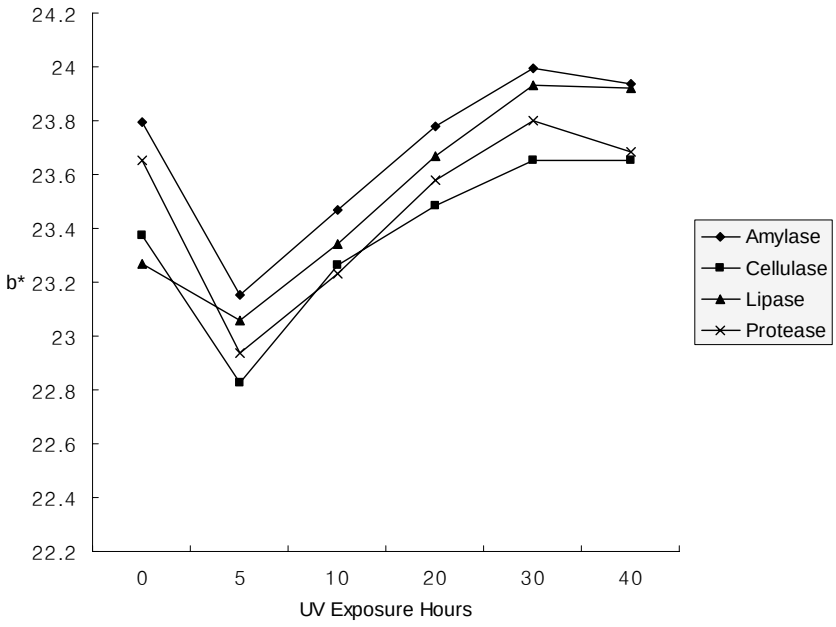


Figure 4-15. Changes of b* of single enzyme treated naturally buffalo brown colored cottons after UV exposure

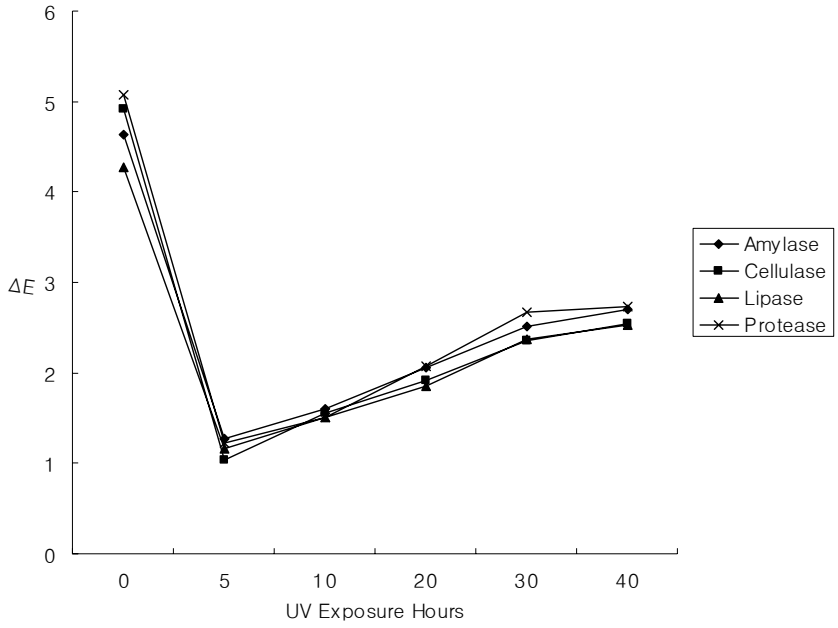


Figure 4-16. Changes of ΔE of single enzyme treated naturally buffalo brown colored cottons after UV exposure

The value a* decreased after UV exposure (figure 4-14). The amount of decrease was large after five hours of UV exposure. This indicates that UV exposure decrease redness in buffalo brown cotton fiber. Protease treated sample showed the lowest a* after 40 hours of UV exposure. Figure 4-15 shows change of b* of buffalo brown cotton. After five hours of UV exposure, b* decreased, but there were large increases after additional UV exposure. After 40 hours of UV exposure buffalo brown color became more yellow. Lipase treatment resulted in a large increase in b* after UV exposure. Cellulase treatment resulted in a lower b* before and after UV exposure. ΔE showed large decrease after five hours of UV exposure and showed a increase after additional UV exposure (figure 4-16). The overall color changes were not significantly different between different enzyme types.

Color changes of coyote brown cotton after single enzyme treatment and UV exposure by hours

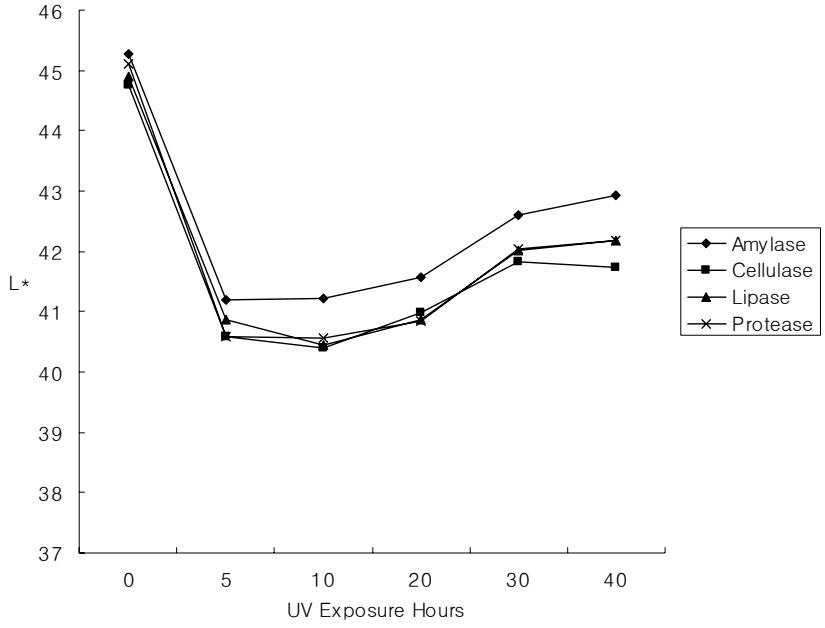


Figure 4-17. Changes of L* of single enzyme treated naturally coyote brown colored cottons after UV exposure

Lightness of enzyme treated coyote brown cotton showed large a decrease after five hours of UV exposure and then increased slowly after more UV exposure (figure 4-17). This result was unexpected and different from previous research with raw naturally colored cotton fiber. The repeated experiment confirmed this decrease after five hours of UV exposure. There was no difference between different enzyme treatments before UV exposure. After 40 hours of UV exposure, the amylase treated samples showed the highest lightness and cellulase treated samples were the darkest.

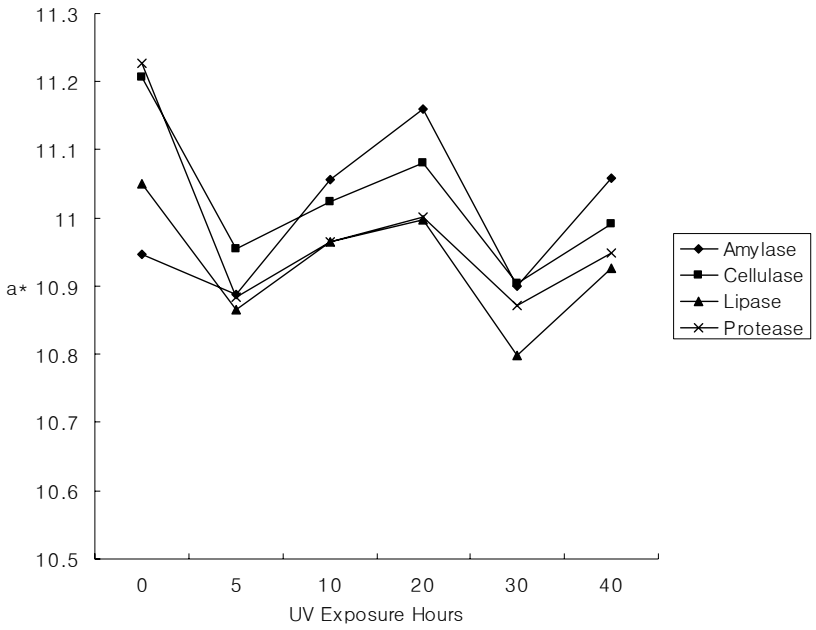


Figure 4-18. Changes of a* of single enzyme treated naturally coyote brown colored cottons after UV exposure

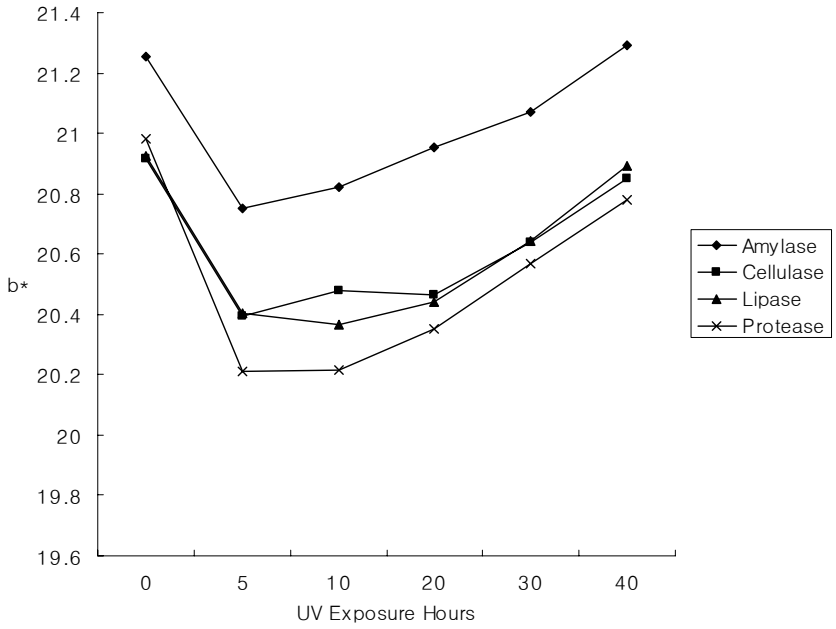


Figure 4-19. Changes of b* of single enzyme treated naturally coyote brown colored cottons after UV exposure

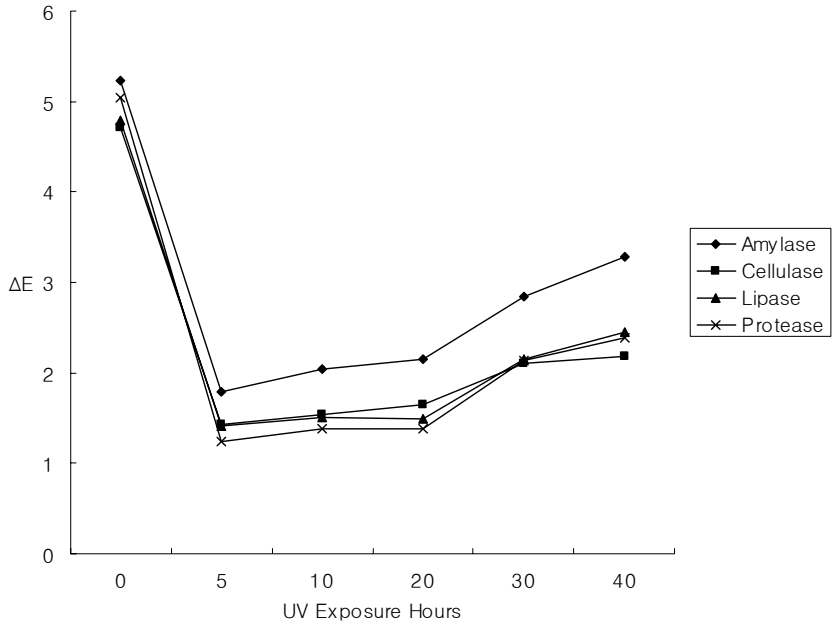


Figure 4-20. Changes of ΔE of single enzyme treated naturally coyote brown colored cottons after UV exposure

The a* value showed changed during UV exposure (figure 4-18). After five hours of UV exposure, the a* value decreased, but increased after more UV exposure. However, there was a large decrease in a* value after 30 hours exposure. Figure 4-19 shows change of b* of coyote brown cotton. The b* decreased after five hours of UV exposure and increased after more UV exposure, which indicates color became more yellow after 40 hours of UV exposure. Amylase showed the largest b* both before and after UV exposure. Protease showed the lowest b* after UV exposure. Overall color change (ΔE) was greatest after five hours of exposure (figure 4-20). The overall color changes were large after amylase treatment, and the other three enzyme treatments showed similar color changes.

Color changes of green cotton after single enzyme treatment and UV exposure by hours

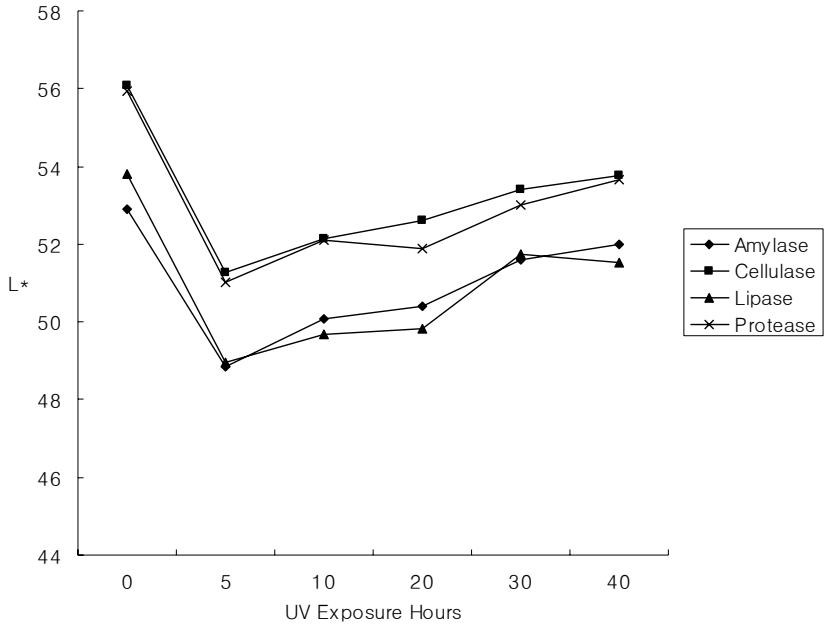


Figure 4-21. Changes of L* of single enzyme treated naturally green colored cottons after UV exposure

Green cotton also showed large decreases in L* after five hours of UV exposure and then increase slowly (figure 4-21). This result was confirmed again after the repeated experiment. Cellulase and protease treated samples showed larger L* than amylase and lipase treated samples before and after UV exposure.

Figure 4-22 shows changes of a* of enzyme treated green cotton after UV exposure. The a* value increase after five hours of UV exposure and increased slightly more after more UV exposure. The increase of a* indicates loss of green color and gain of red color after UV exposure. Amylase treated samples showed larger a* both before and after UV exposure than other samples. The b* of green cotton increased slightly with UV exposure (figure4-23). Cellulase treated samples have the largest b* and amylase treated samples have the lowest b*. The amount of increase was larger for amylase treated samples.

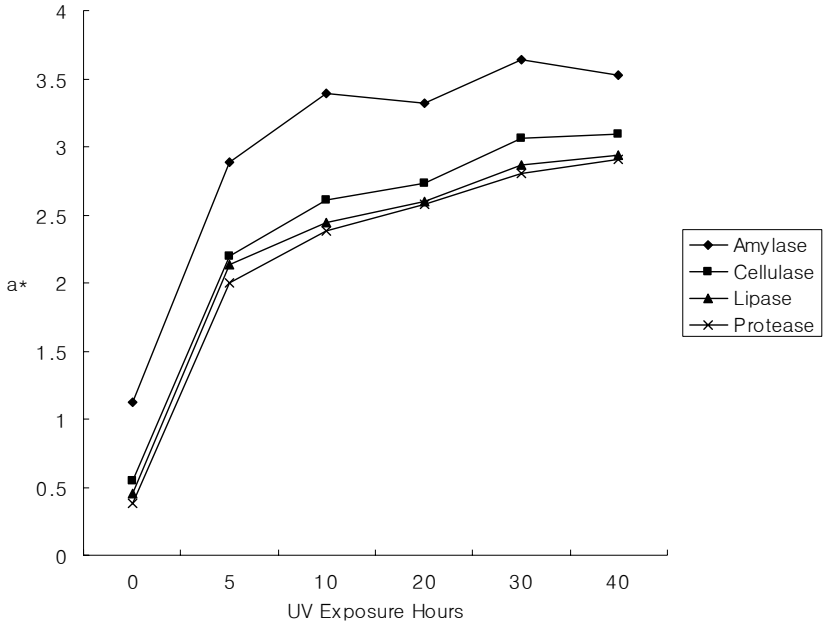


Figure 4-22. Changes of a* of single enzyme treated naturally green colored cottons after UV exposure

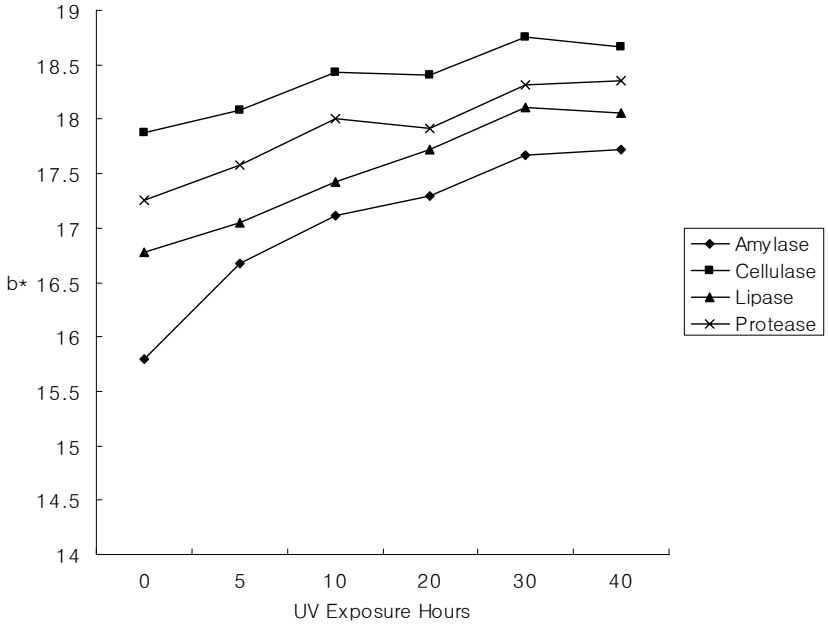


Figure 4-23. Changes of b* of single enzyme treated naturally green colored cottons after UV exposure

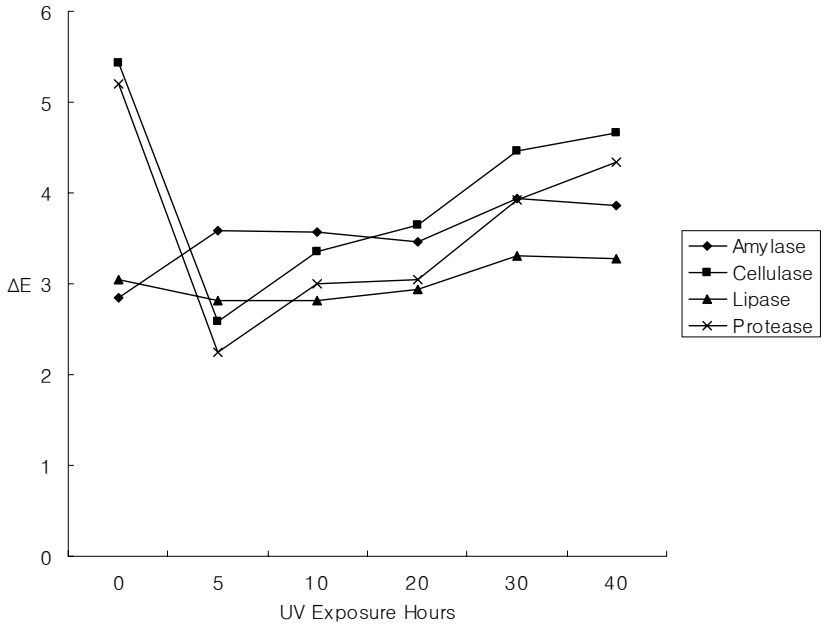


Figure 4-24. Changes of ΔE of Single Enzyme Treated Naturally green Colored Cottons after UV Exposure

The overall result indicates that green cotton became more yellow after UV exposure. Overall color change (ΔE) of green cotton showed inconsistent results due to different effect of L*, a*, and b* (figure 4-24).

After single enzyme treatment, the first five hours of UV exposure resulted in large decreases in lightness for three naturally colored cottons. More UV exposure increased lightness of cotton samples slightly.

Color changes of buffalo brown cotton after multiple enzyme treatment and UV exposure by hours

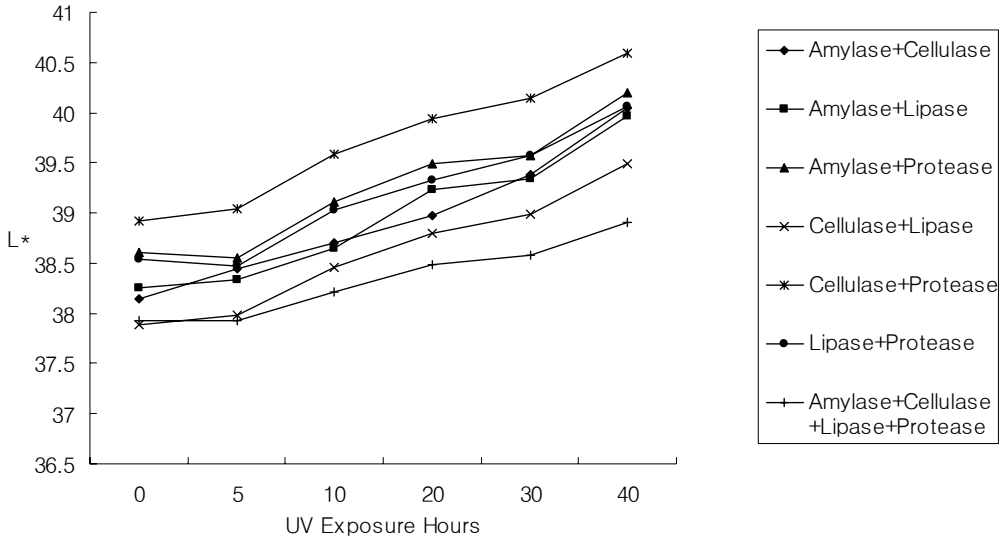


Figure 4-25. Changes of L* of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure

Figure 4-25 shows change of lightness of enzyme treated buffalo brown cotton after UV exposure. Lightness increased slowly with UV exposure. Cellulase and lipase treated samples showed the highest lightness before and after 40 hours of UV exposure. Four enzyme treated samples showed the lowest lightness before and after UV exposure.

The a* value of buffalo brown cotton after enzyme treatment increased after 40 hours of UV exposure (Figure4-26). The increase happened mainly after five hours of exposure. Increase of a* indicates that the color became more red after UV exposure. The a* was higher for cellulase and protease treated samples and very lower for four enzyme treated samples before and after UV exposure. Figure 4-27 shows change of b*.

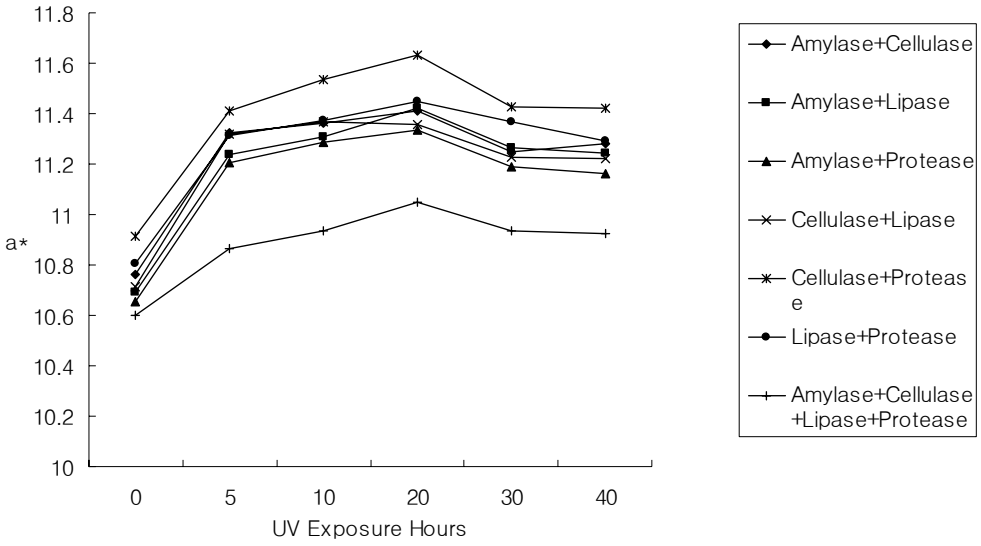


Figure 4-26. Changes of a* of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure

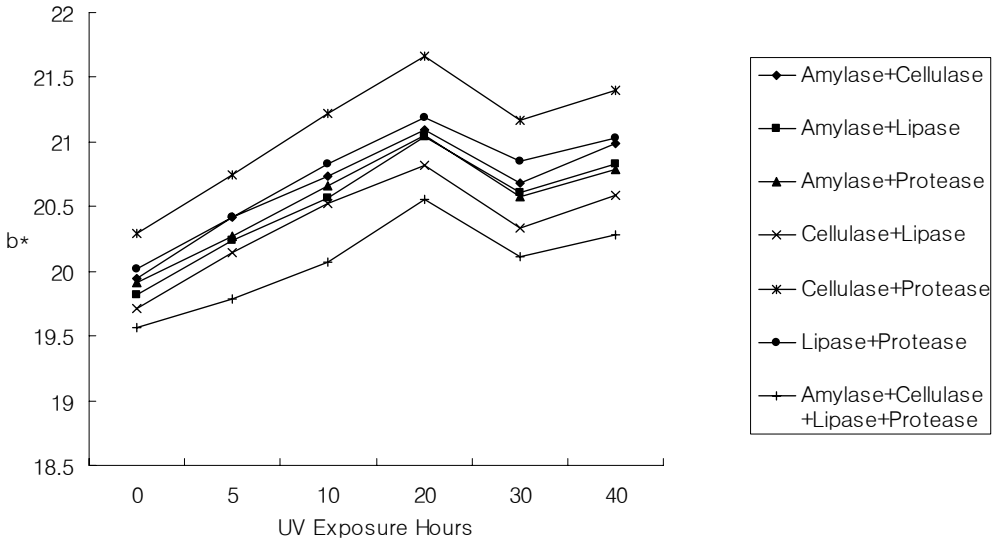


Figure 4-27. Changes of b* of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure

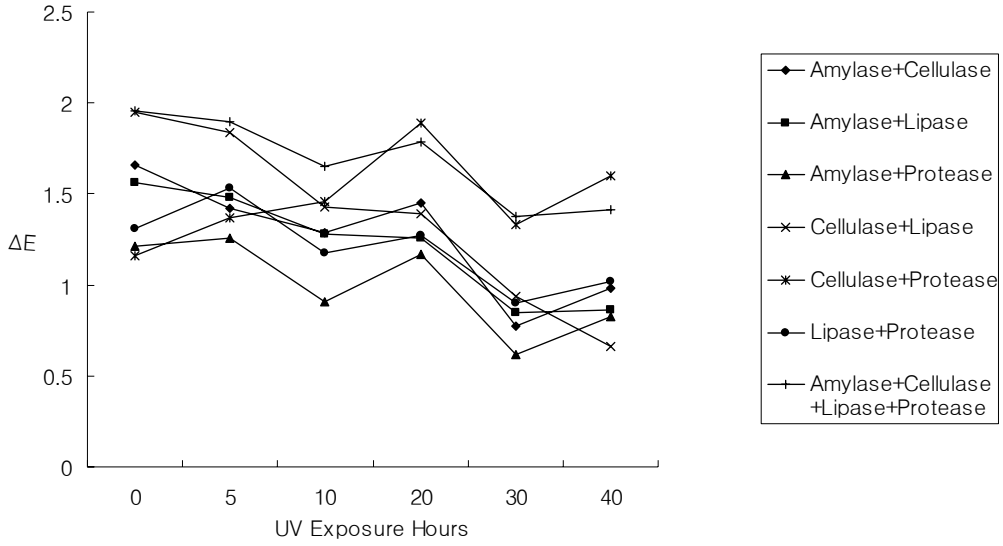


Figure 4-28. Changes of ΔE of multiple enzyme treated naturally buffalo brown colored cottons after UV exposure

All multiple enzyme treated samples showed increase of b* until 20 hours of UV exposure. However there were fluctuations in b* after 20 hours. All samples became more yellow than before UV exposure. The b* was highest for cellulase and protease treated samples and lowest for four enzyme treated samples.

Color changes of coyote brown cotton after multiple enzyme treatment and UV exposure by hours

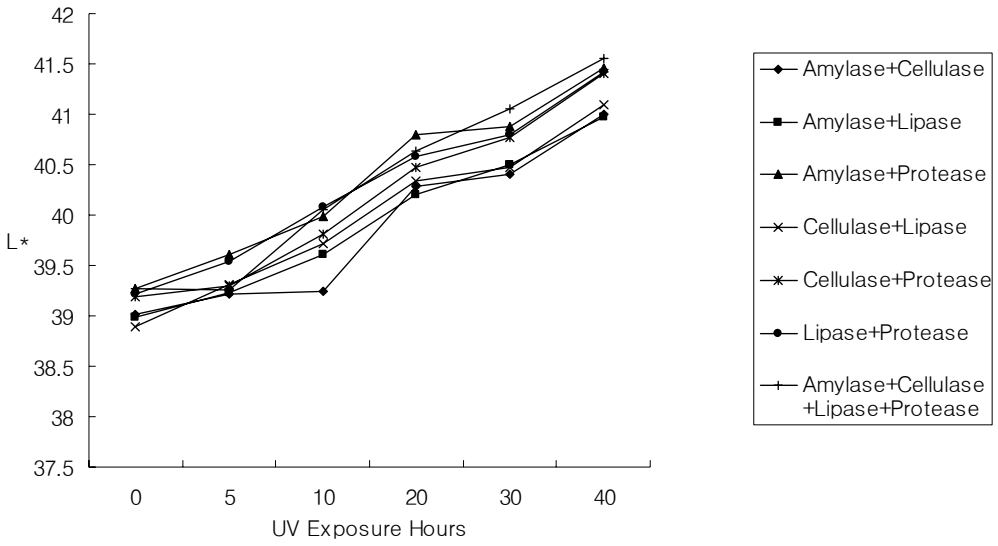


Figure 4-29. Changes of L* of multiple enzyme treated naturally coyote brown colored cottons after UV exposure

Figure 4-29 shows change of lightness of enzyme treated coyote brown cotton after 40 hours of UV exposure. Lightness increased with UV exposure. All multiple enzyme treated samples showed similar increase in L* during UV exposure. The overall increase was similar to the increase in L* of buffalo brown cotton.

The a* value of coyote brown cotton after enzyme treatment increased slightly after 40 hours of UV exposure (Figure 4-30). The increase happened mainly after five hours of exposure. After five hours, the a* was not significantly changed. Increase of a* indicates that the color became more red after UV exposure. The a* was higher for amylase and lipase treated samples than other samples before and after UV exposure.

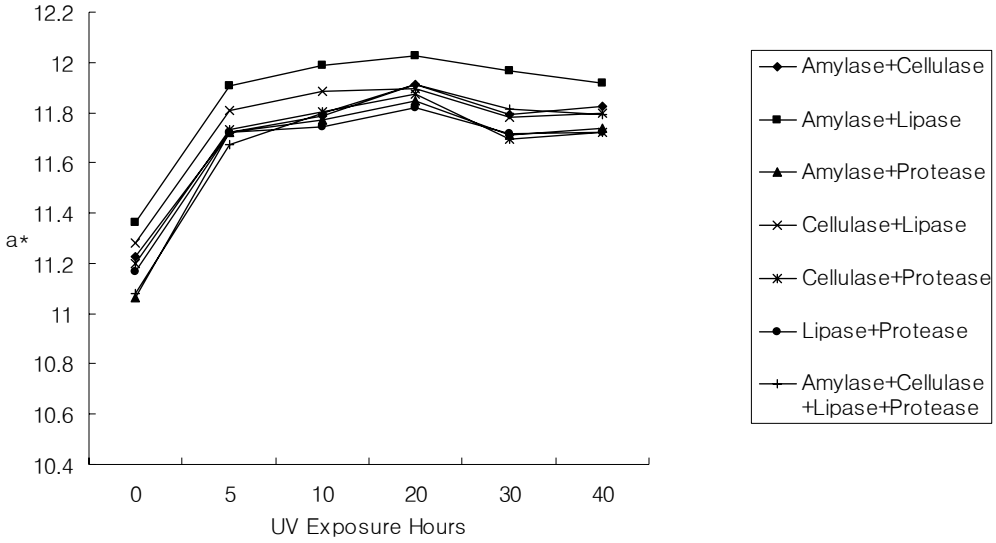


Figure 4-30. Changes of a* of multiple enzyme treated naturally coyote brown colored cottons after UV exposure

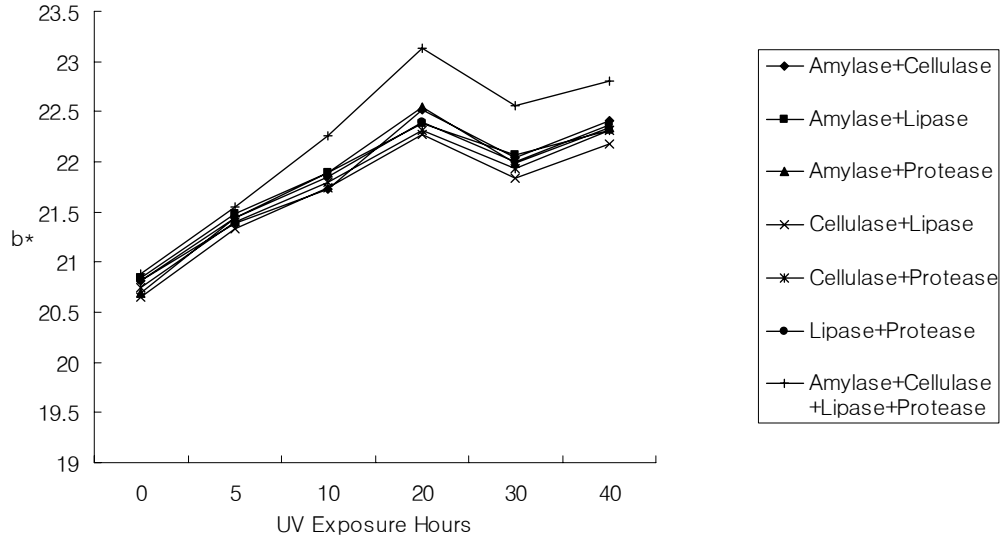


Figure 4-31. Changes of b* of multiple enzyme treated naturally coyote brown colored cottons after UV exposure

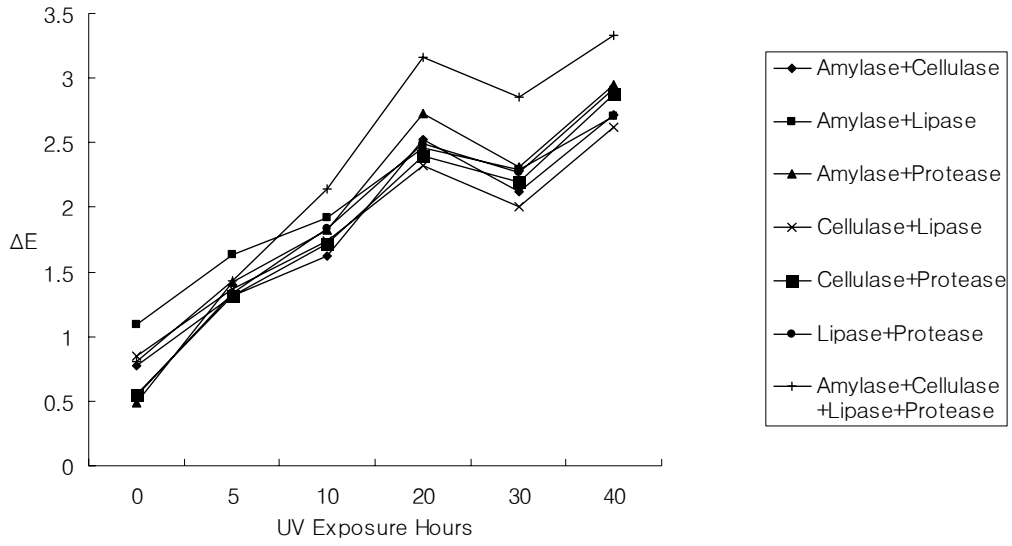


Figure 4-32. Changes of ΔE of multiple enzyme treated naturally coyote brown colored cottons after UV exposure

Figure 4-31 shows change of b* of enzyme treated coyote brown cotton. All multiple enzyme treated samples showed increase of b* through 20 hours of UV exposure. However, after 20 hours, there were fluctuating in b*. Color of all samples became more yellow than before UV exposure. The b* was highest for four enzyme treated samples. The overall color change (ΔE) increased during 40 hours of UV exposure (figure 4-32).

Color changes of green cotton after multiple enzyme treatment and UV exposure by hours

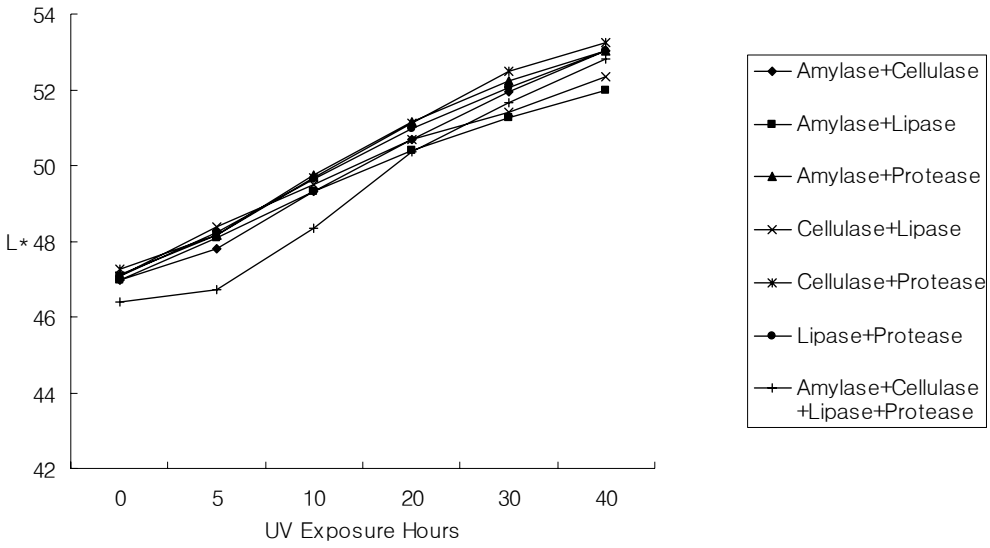


Figure 4-33. Changes of L* of multiple enzyme treated naturally green colored cottons after UV exposure

Figure 4-33 shows increase in lightness of enzyme treated green cotton up to 40 hours of UV exposure. All multiple enzyme treated samples showed similar increases in L* during UV exposure. The overall increase in lightness was much larger for the green cotton than the two brown colored cottons.

The a* of green cotton after enzyme treatment shows a large increase after 40 hours of UV exposure (Figure 4-34). The increase happened mainly after five hours exposure. The a* became the positive after longer exposure. This change of a* indicates that the color was changed from reddish color to greenish color during UV exposure. The increase happened to all multiple enzyme treatment conditions consistently.

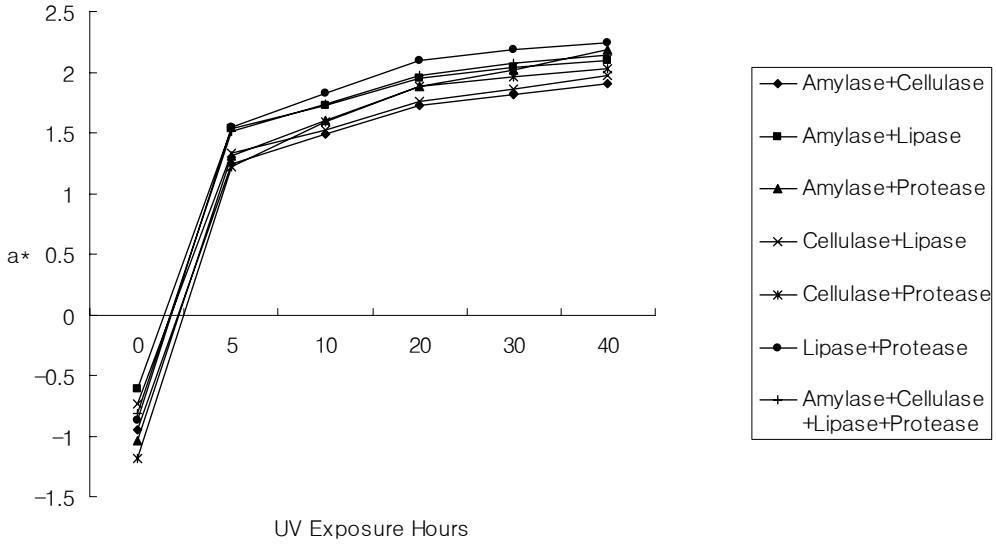


Figure 4-34. Changes of a* of multiple enzyme treated naturally green colored cottons after UV exposure

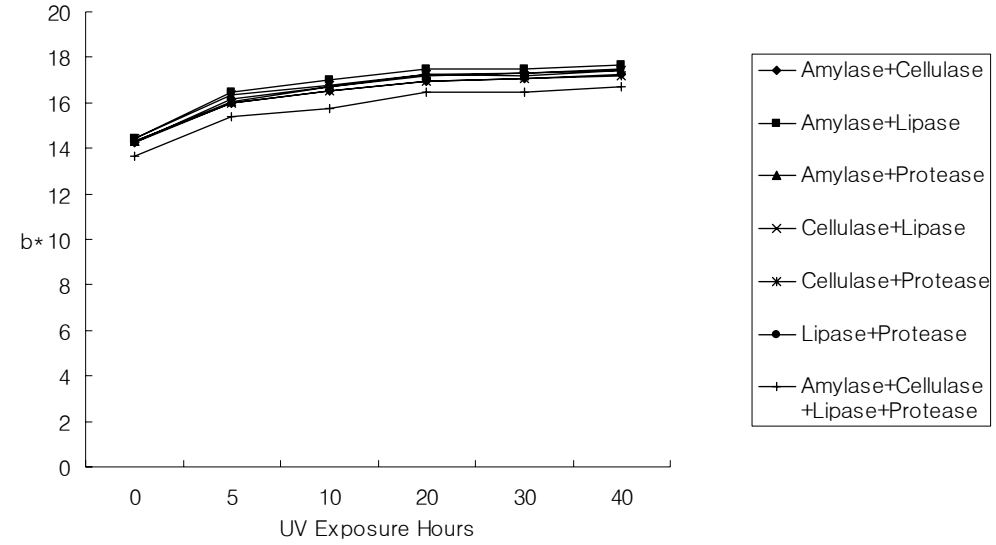


Figure 4-35. Changes of b* of multiple enzyme treated naturally green colored cottons after UV exposure

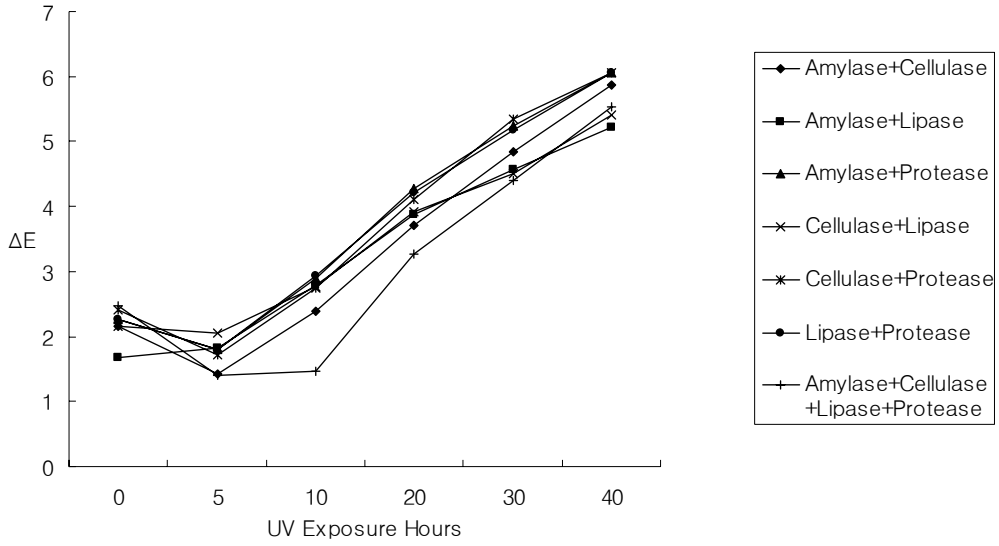


Figure 4-36. Changes of ΔE of multiple enzyme treated naturally green colored cottons after UV exposure

Figure 4-35 shows change of b* of enzyme treated green cotton. Every sample showed slight increases in b*. This indicates that color of every sample became more yellow than before UV exposure. The b* was lowest for four enzyme treated samples. The overall color change (ΔE) increased during 40 hours of UV exposure (figure 4-36).

Effect of single enzyme concentration on color change of naturally colored cotton

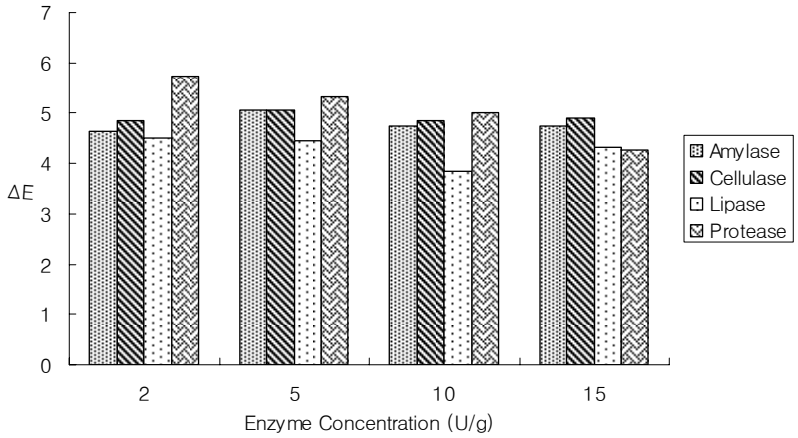


Figure 4-37. Effect of single enzyme treatment concentration on color change (ΔE) of buffalo brown cotton

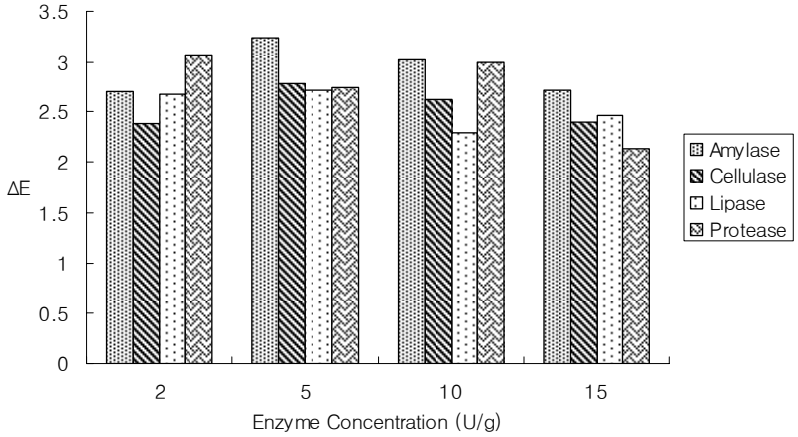


Figure 4-38. Effect of single enzyme treatment concentration on color change (ΔE) of buffalo brown cotton after 40 hours of UV exposure

Figure 4-37 and 438 show the overall color change of buffalo brown cotton with enzyme treatment of different concentrations. Greater color change occurred after lower percentage of enzyme treatment. Color change was affected by concentration of lipase treatment more than other enzyme treatments for buffalo brown cotton. After 40 hours of UV exposure, lower ΔE was obtained than before UV exposure and this is due to the complex effect of L*, a*, b* values.

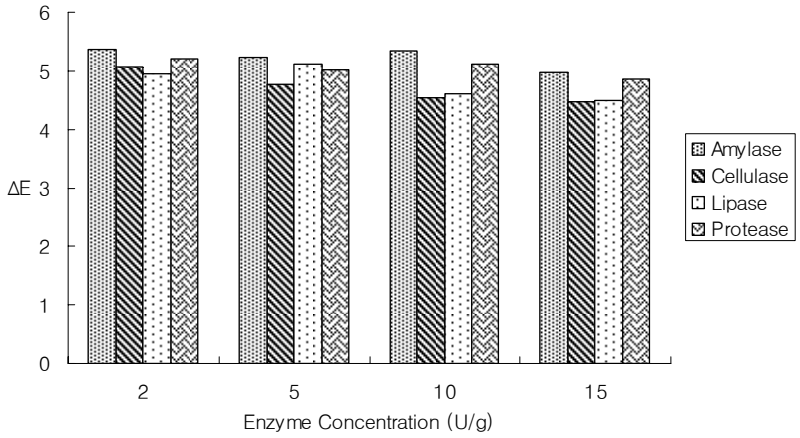


Figure 4-39. Effect of single enzyme treatment concentration on color change (ΔE) of coyote brown cotton

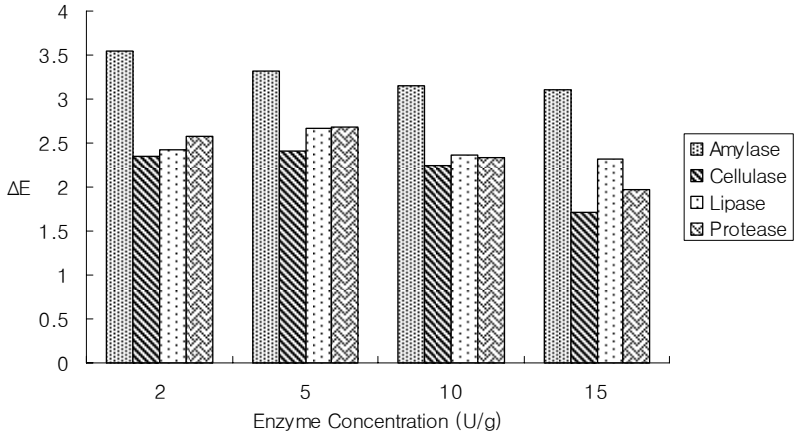


Figure 4-40. Effect of single enzyme treatment concentration on color change (ΔE) of coyote brown cotton after 40 hours of UV exposure

Figure 4-39 and figure 4-40 show the overall color change of coyote brown cotton with enzyme treatment of different concentrations. There was no significant difference between results from different enzyme concentrations. Cellulase treated samples showed more color change at lower enzyme concentration. After 40 hours of UV exposure, lower ΔE was obtained than before UV exposure and this is due to the complex effect of L*, a*, b* values, similar to the results for buffalo brown cotton.

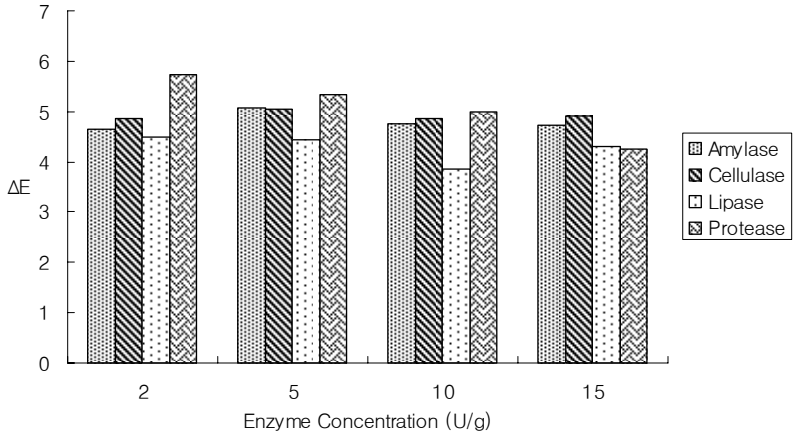


Figure 4-41. Effect of single enzyme treatment concentration on color change (ΔE) of green cotton

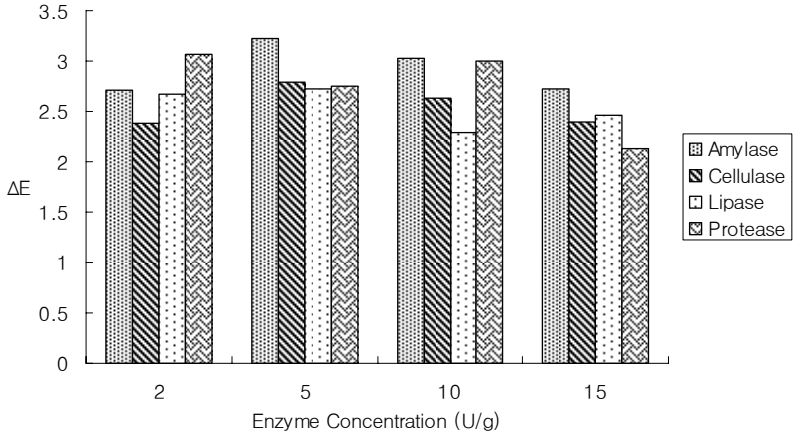


Figure 4-42. Effect of single enzyme treatment concentration on color change (ΔE) of green cotton after 40 hours of UV exposure

Figure 4-41 and figure 4-42 show the overall color change of green cotton with enzyme treatment of different concentrations. Lower enzyme concentration treatments resulted in more color change. Lipase treated samples show the lowest color change after 1% of treatment. Protease treated samples show more color change at lower enzyme concentration. After 40 hours

of UV exposure, lower ΔE was obtained and this is due to the complicated interaction of L*, a*, b* values.

The overall color change (ΔE) did not increase with increase of enzyme amount in the treatment solutions. Each enzyme has its own optimal concentration to affect color of naturally colored cottons and this varied depending on the fiber types. However, lower concentration was more effective on color change for the most cases.

Effect of multiple enzyme concentration on color change of naturally colored cotton

Figure 4-43 and figure 4-44 show the overall color change of buffalo brown cottons with multiple enzyme treatments of different concentrations. Some combinations were not affected by enzyme concentrations such as cellulase and lipase treated samples and all four enzyme treated samples. After 40 hours of UV exposure, cellulase and protease treated samples showed greater color change for 1% enzyme treatment than 0.5% enzyme treatment. Four enzyme treated samples showed more color change for 0.5% enzyme treatment.

Figure 4-45 and figure 4-46 show the overall color change of coyote brown cottons with multiple enzyme treatments of different concentrations. Some combinations were not affected by enzyme concentrations such as amylase and lipase treated samples. Most of the samples showed more color change after 0.5% enzyme treatment before UV exposure. After 40 hours of UV exposure, 1% enzyme treatment showed slightly more color changes.

Figure 4-47 and figure 4-48 show the overall color change of green cottons with multiple enzyme treatments of different concentrations. Four enzymes treatment showed no difference between different concentrations. Most of the samples showed more color change after 0.5% enzyme treatment. After 40 hours of UV exposure, 1% enzyme treatment showed more color

changes.

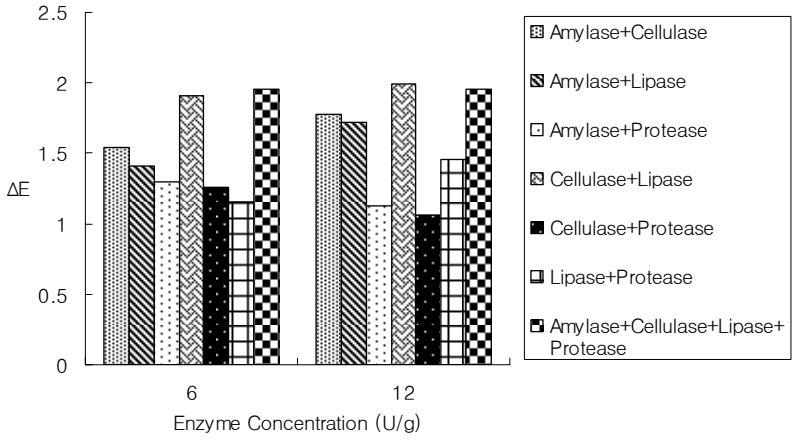


Figure 4-43. Effect of multiple enzyme treatment concentration on color change (ΔE) of buffalo brown cotton

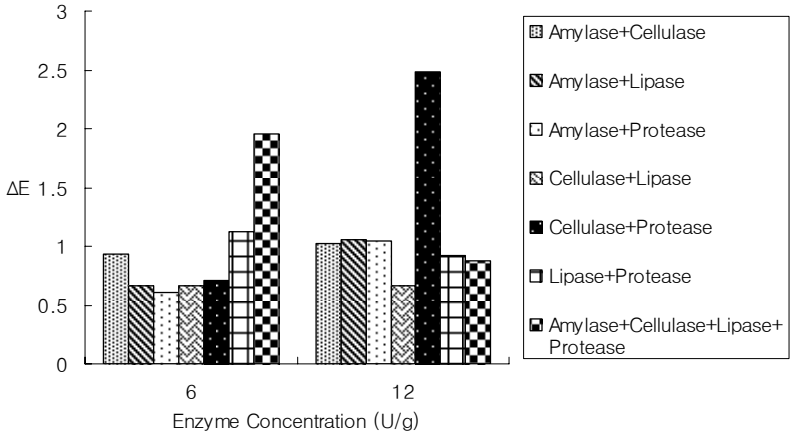


Figure 4-44. Effect of multiple enzyme treatment concentration on color change (ΔE) of buffalo brown cotton after 40 hours of UV exposure

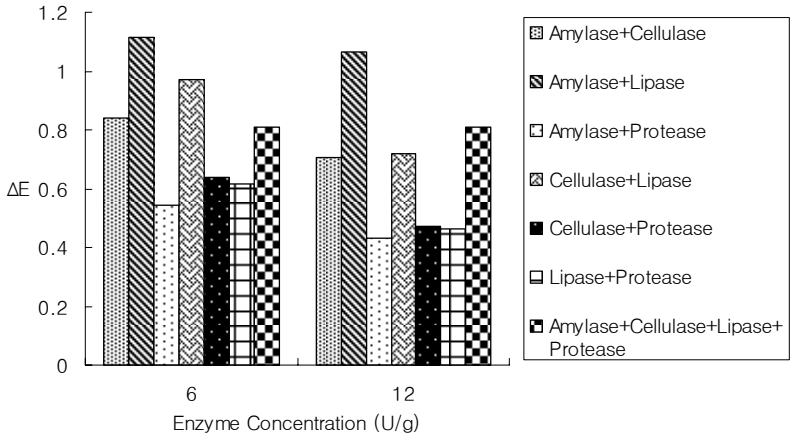


Figure 4-45. Effect of multiple enzyme treatment concentration on color change (ΔE) of coyote brown cotton

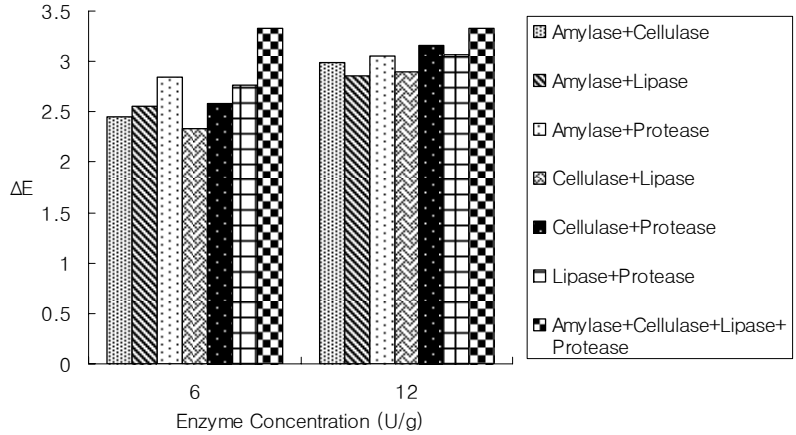


Figure 4-46. Effect of multiple enzyme treatment concentration on color change (ΔE) of coyote brown cotton after 40 hours of UV exposure

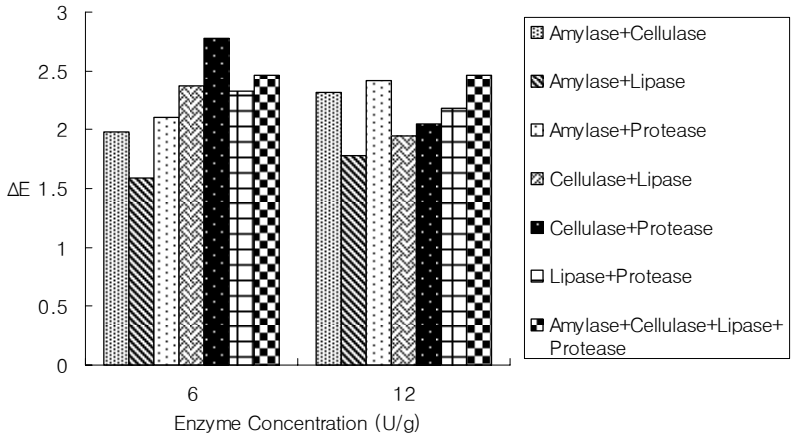


Figure 4-47. Effect of multiple enzyme treatment concentration on color change (ΔE) of green cotton

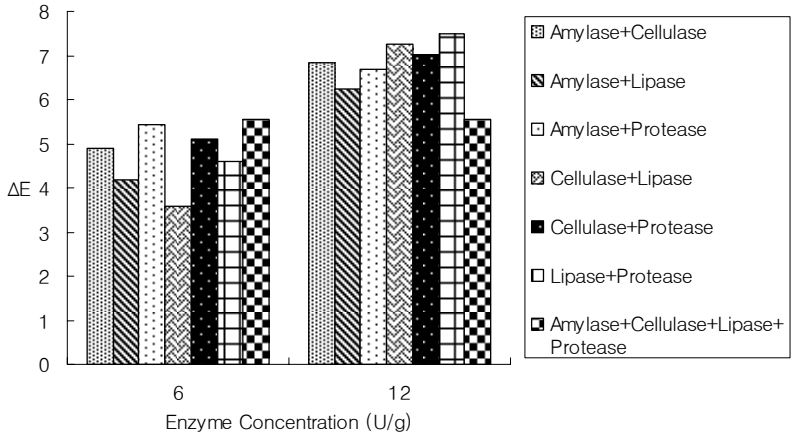


Figure 4-48. Effect of multiple enzyme treatment concentration on color change (ΔE) of green cotton after 40 hours of UV exposure

The overall color change (ΔE) varied for each combination. Each enzyme combination has its own optimal concentration to affect color of naturally colored cottons and this varied

depending on fiber types. However, lower concentration caused more color change for the most cases before UV exposure and higher concentration caused more color change after 40 hours of UV exposure.

Statisitcal Analysis

Mixed model was used to analyze the effect of enzymes on lightfastness of three naturally colored cottons. A SAS program (Appendix A) was developed and used to complete the analysis. The following null hypothesis was tested to study the different effect of enzyme treatment on three colored cottons.

H_0: UV exposure hours, enzymes and enzyme concentrations are significant effects on moisture regain (%).

Table 4-7. Statistical results of lightness of single enzyme treated samples after UV exposure

Cotton	Variables	DF	F value	Pr> F
Buffalo brown	UV exposure hours	5	1674.84	<.0001
	Enzyme types	3	5.40	0.0032
	Enzyme concentration	3	2.07	0.1193
Coyote brown	UV exposure hours	5	1571.50	<.0001
	Enzyme types	3	18.03	<.0001
	Enzyme concentration	3	6.02	0.0017
Green	UV exposure hours	5	341.37	<.0001
	Enzyme types	3	28.45	<.0001
	Enzyme concentration	3	1.08	0.3688

Table 4-7 shows results from single enzyme treated samples (Appendix A). The UV exposure hours variable was significant for three naturally colored cottons (α=0.05). As shown in previous pages, values of lightness were changed as UV exposure hours increased and this

change is significant statistically. Enzyme treatment was also a significant variable for lightness. All three naturally colored cottons showed significantly different changes in lightness depending on different enzyme treatment after UV exposure (α=0.05). For buffalo brown and green cotton, enzyme concentration did not significantly affect lightness. Lightness of coyote brown cotton was significantly affected by enzyme concentration (α=0.05).

Table 4-8. Statistical results of lightness of multiple enzyme treated samples after UV exposure

Cotton	Variables	DF	F value	Pr> F
Buffalo brown	UV exposure hours	5	209.67	<.0001
	Enzyme types	6	5.84	0.0003
	Enzyme concentration	1	5.86	0.0210
Coyote brown	UV exposure hours	5	436.03	<.0001
	Enzyme types	6	1.96	0.0990
	Enzyme concentration	1	2.57	0.1179
Green	UV exposure hours	5	732.36	<.0001
	Enzyme types	6	0.90	0.0866
	Enzyme concentration	1	24.06	0.1179

The variable of UV exposure hours was significant for change in lightness in three naturally colored cottons (α=0.05). In other words, the increases in lightness of colored cottons with increase of UV exposure hours are significant statistically (α=0.05). The enzyme treatment variable was a significant only for buffalo brown cotton. For coyote brown and green cotton, the enzyme treatment did not significantly affect color (α=0.05). For coyote brown and green cotton, enzyme concentration did not significantly affect lightness. Lightness of buffalo brown cotton was significantly affected by enzyme concentration (α=0.05) (Table 4-8).

Change of L*, a* and b* after Scouring, Enzyme Treatment and UV exposure

Figure 4-49 shows overall lightness change after each process. $CaCO_3$ scouring process resulted in a decrease in lightness for three colored cottons. Colored cottons show decreased lightness also after enzyme treatment. Green colored cotton showed larger change in lightness comparing to both brown colored cottons.

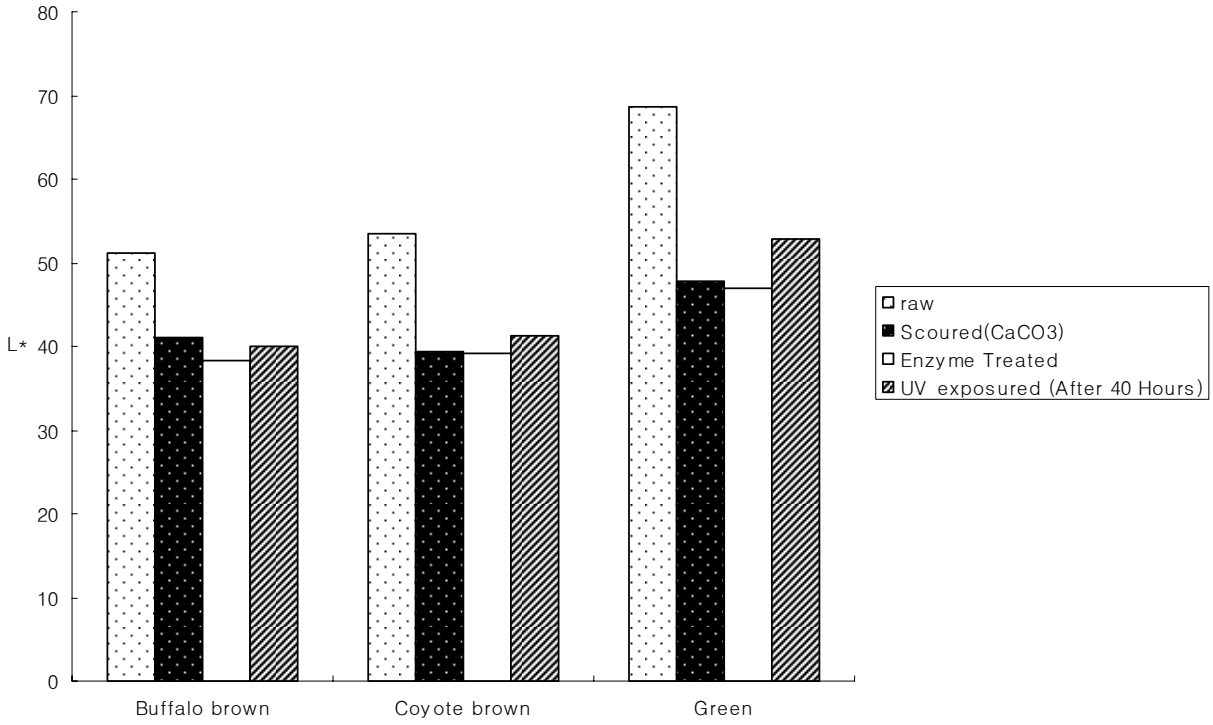


Figure 4-49. Changes of L* of naturally colored cottons after scouring and enzyme treatment

Figure 4-50 shows changes of hue of three colored cottons by a* and b* in the CIELab color map. Most of the colors are located at the first quadrilateral dimension with positive a* and b* values except some of green colored cotton samples after scouring process. Buffalo brown and coyote brown cottons are closely located to each other.

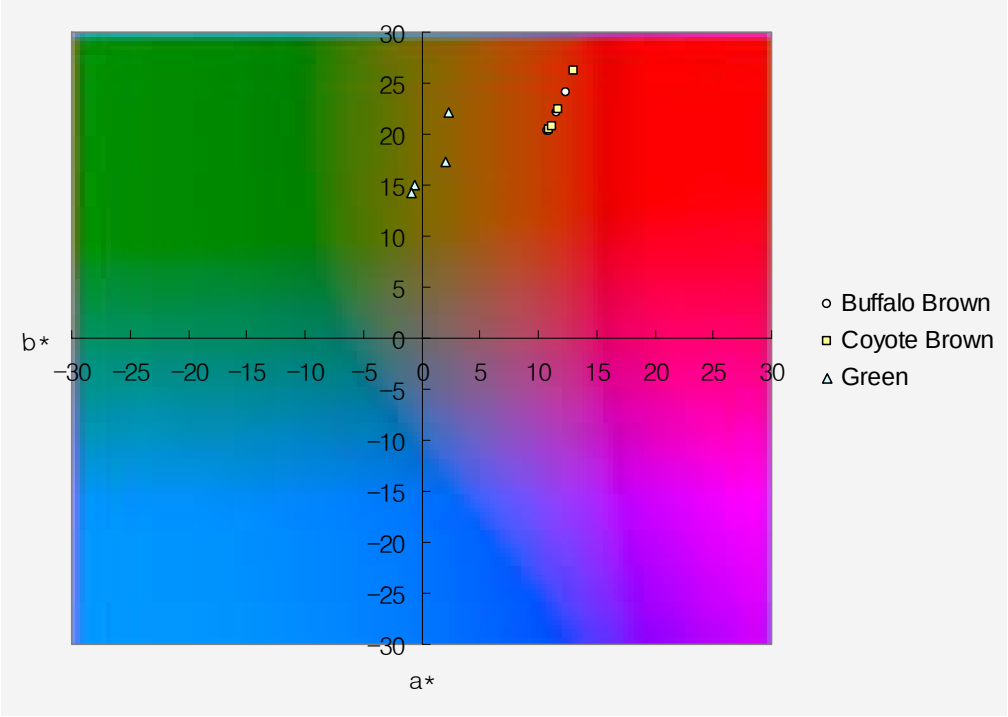


Figure 4-50. Changes of a* and b* of naturally colored cottons after scouring and enzyme treatment

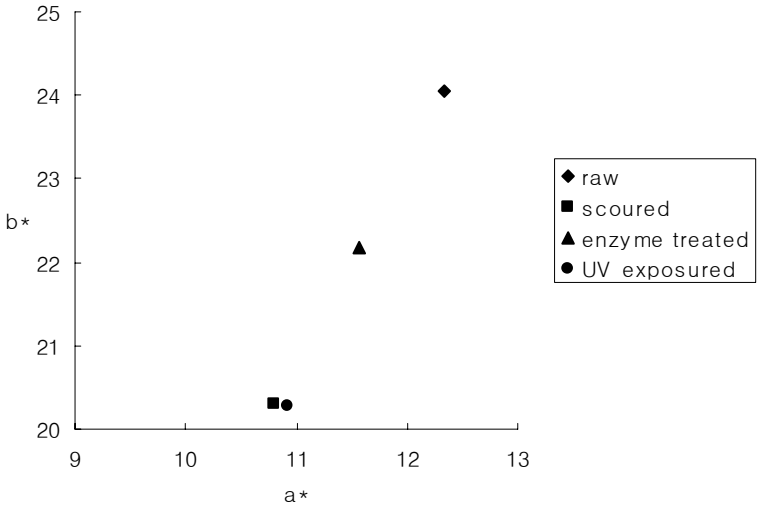


Figure 4-51. Changes of a* and b* of naturally buffalo brown colored cottons after scouring and enzyme treatment

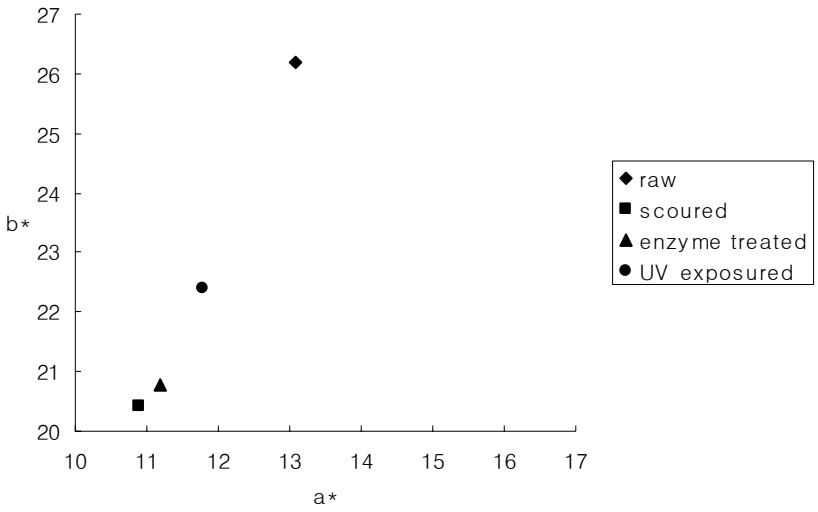


Figure 4-52. Changes of a* and b* of naturally coyote brown colored cottons after scouring and enzyme treatment

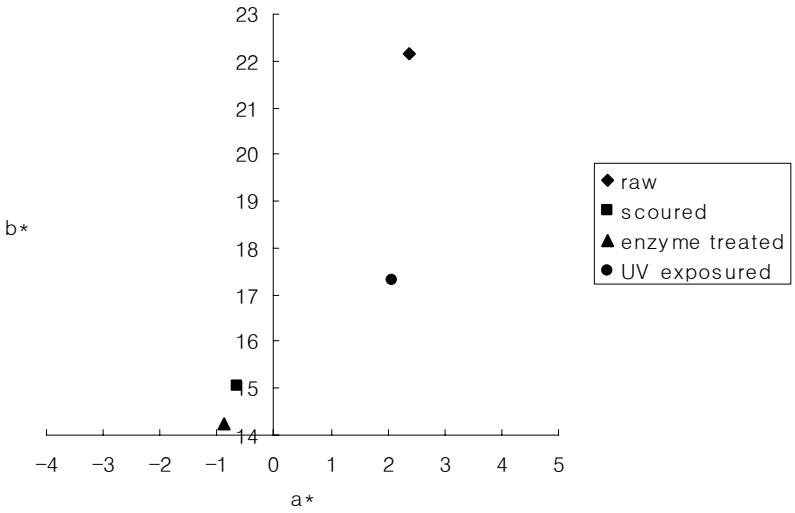


Figure 4-53. Changes of a* and b* of naturally green colored cottons after scouring and enzyme treatment

Figure 4-50 shows change of position of samples at the a* and b* plane as they are processed with different treatment. The detailed movement of each sample according to UV

exposure hours are shown in appendix B. Figure 4-51 indicates how hue of buffalo brown cottons is changed during processes. The raw fiber has the highest a* and b* values, which indicates that buffalo brown cotton has originally more red and yellow color, a higher chroma. After scouring, b* decreased so that yellow color decreased. Enzyme treatment process brought color close to the original color but UV exposure changed this back to the color which was similar to that after scouring process. Coyote brown cotton also has the highest a* and b* when it was raw (figure 4-52). After scouring process, color showed the lowest a* and b* values. The a* and b* value increased slightly after enzyme treatment and increased more increased after UV exposure. Both brown cottons showed the highest a* and b* when they were raw. But after scouring process they became less red and yellow. These changes became moderate after enzyme treatment and UV exposure.

Before scouring process green cotton was located at the positive area of a* and b* (figure 4-53). The green cotton appeared more yellow than green so that a* was very low even though it was positive, and b* was high. After scouring, a* became negative, which indicates color became greener. The b* value also decreased after scouring process. Green color became stronger after enzyme treatment but after 40 hours of UV exposure, the color became more yellow and red.

Moisture Regain (%) Changes of Naturally Colored Cotton after Scouring and Enzyme treatment

Weight Change after Enzyme Treatment

After scouring, the three fibers showed decreases in weight. This is due to the removal of the materials such as fat, lignin and pectin from the fibers so that the larger decrease indicates the higher non-cellulosic materials in the fiber. The weight loss was larger for the green colored cotton than for the two brown colored cottons.

Enzyme treatments were done for the scoured colored cotton samples and these treatments slightly increased weight for all naturally colored cotton samples, which was not expected. The weight of coyote brown cotton was largest, at 101.15% in average, then green cotton increased to 100.72% in average. The buffalo brown cotton showed the smallest changes as 100.50% in average.

Enzyme detergent in commonly used to remove dirt and each enzyme reacts with its own substrates. Cellulase is used to remove surface fuzz of cotton fabrics and give colored fabric a fresh look. Lipase removes oil based stains where as amylase is added to remove starch based stains such as chocolate, gravy, rice pasta, baby food, sauces and potato that act as a glue and binds particulate soil to the surface. Protease removes protein-based laundry stains such as grass, blood, egg and perspiration. Therefore after each enzyme treatment the detachment between fibers and substrates was expected. So it is suggested that the increase in weight is not because of obtaining materials but because of obtaining moisture in the fibers which can be achieved by losing hydrophobic materials. To investigate this assumption, the moisture regain test was done for each sample.

Table 4-9. Weight changes of buffalo brown colored cotton after enzyme treatment, as percentage of raw cotton weight

Raw Cotton	After Scouring	After Enzyme Treatment	
100	94.48	Amylase	100.30
		Cellulase	100.57
		Lipase	100.67
		Protease	100.42

Table 4-10. Weight changes of coyote brown colored cotton after enzyme treatment, as percentage of raw cotton weight

Raw Cotton	After Scouring	After Enzyme Treatment	
100	95.23	Amylase	100.20
		Cellulase	101.29
		Lipase	100.18
		Protease	102.94

Table 4-11. Weight changes of green colored cotton after enzyme treatment, as percentage of raw cotton weight

Raw Cotton	After Scouring	After Enzyme Treatment	
100	92.69	Amylase	100.47
		Cellulase	101.36
		Lipase	100.47
		Protease	100.58

Moisture regain (%) of single enzyme treated naturally colored cotton

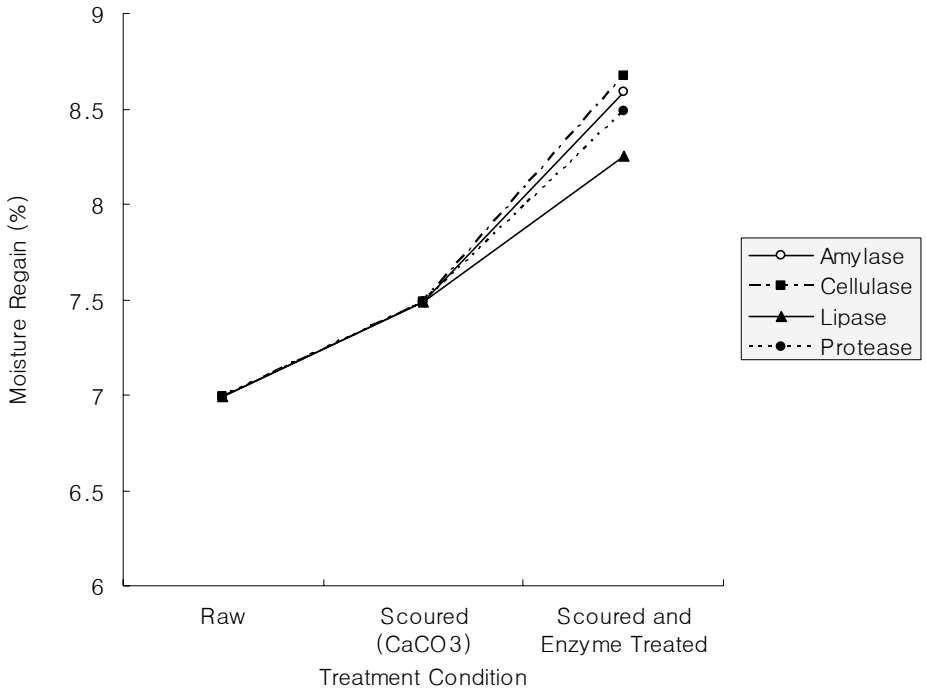


Figure 4-54. Effect of single enzymes on moisture regain (%) changes of buffalo brown naturally colored cotton after scouring and enzyme treatment

Figure 4-59 shows increase of moisture regain (%) of buffalo brown cotton after scouring and enzyme treatment. The moisture regain of raw buffalo brown cotton was 6.991% which was lower than the moisture regain of conventional white cotton. But after scouring process the rate increased to 7.491%. After enzyme treatments the rate increased up to 8.886% which is higher than conventional white cotton. There were different changes in moisture regain depending on enzymes and concentrations applied. After cellulase treatment the moisture regain of buffalo brown cotton increased to 8.7095% in average which is higher than the moisture regain after other treatments (appendix C). The difference in enzyme concentration did not show significant effect.

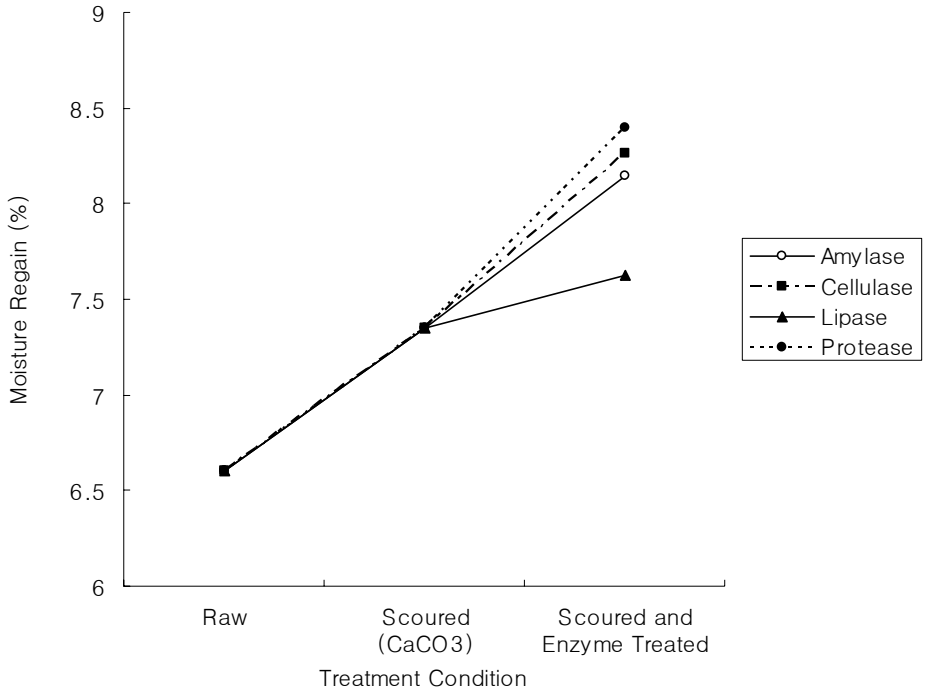


Figure 4-55. Effect of single enzymes on moisture regain (%) changes of coyote brown naturally colored cotton after scouring and enzyme treatment

Continuous increase of moisture regain (%) of coyote brown cotton after treatments is shown in figure 4-60. The moisture regain of raw coyote brown cotton (6.603%) was slightly lower than that of raw buffalo brown cotton. After scouring the moisture regain increased to 7.342% and after enzyme treatment it increased further to 8.401% when protease was applied. The overall increase in moisture regain of the coyote brown colored cotton after enzyme treatments was lower compared to buffalo brown cotton. When lipase was applied the moisture regain showed the lowest value, at 7.624% in average. The effect of protease on the moisture regain was largest at 8.591% in average. Protease showed a large effect and lipase showed a small effect on both brown colored cottons. The slightly different effects of enzymes on the

moisture regain may be due to the different fiber components between buffalo brown cotton and coyote brown cotton (Appendix C).

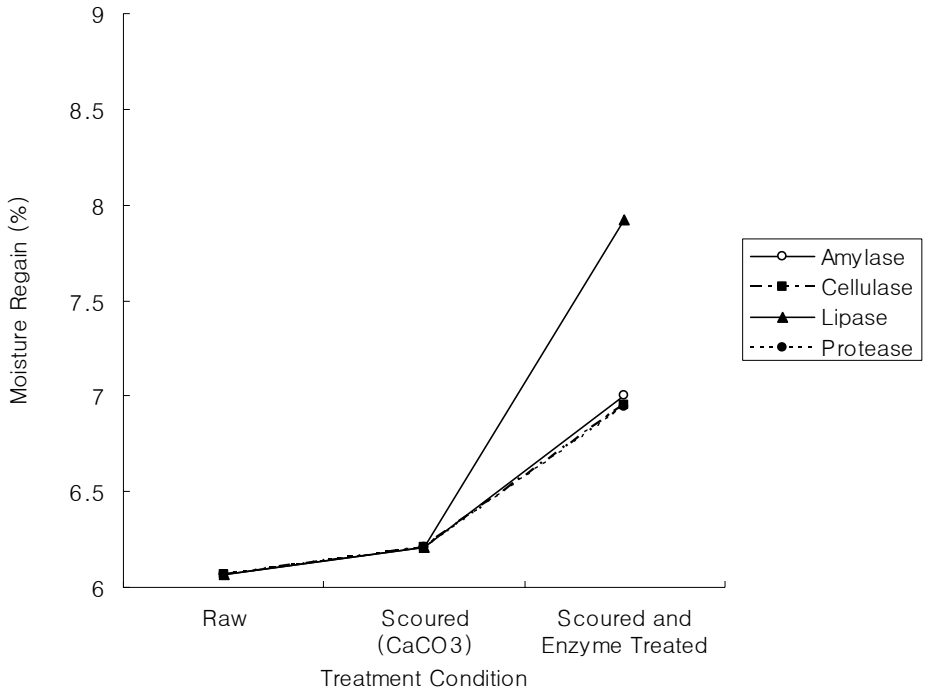


Figure 4-56. Effect of single enzymes on moisture regain (%) changes of green naturally colored cotton after scouring and enzyme treatment

Figure 4-61 shows that the moisture regain of raw green cotton is the lowest among three naturally colored cottons. This is due to the higher hydrophobic content in green colored cotton fibers. The scouring process increased the moisture regain to 6.211%. After enzyme treatment the moisture regain increased to 8.641% by lipase but the overall moisture regains after enzyme treatments were low. The highest rated appeared after lipase treatment, at 7.918% in average, which indicates that the fat in green colored cotton may have been the main cause of the low moisture regain. On the other hand, the protease treatment which was the most effective

enzyme for both brown colored cottons showed the lowest increase in the moisture regain, as 6.944% in average. This means that green colored cotton has different constituents than the both brown colored cottons (Appendix C).

Statistical analysis of moisture regain (%) after single enzyme treatment

ANOVA was used to analyze the effect of enzyme on moisture regain change of three naturally colored cottons because the responses, moisture regain (%), are continuous and the predictor variables, enzymes and fibers, are categorical. A SAS program (Appendix C) was developed and used to complete the analysis. The following null hypothesis was tested to study the different effects of enzyme treatment on three colored cottons.

H_0: Enzymes, fiber types and interaction between enzymes and fiber types are significant effects on moisture regain (%).

Table 4-12 shows Duncan grouping result. Means in the same group are not significantly different (α=0.05). The order of the moisture regain (%) between fiber types was same after enzyme treatment. Buffalo brown cotton has highest moisture regain (%) in average and green cotton has the lowest moisture regain (%) in average after enzyme treatment. After each enzyme treatment, the moisture regain (%) of each enzyme treated buffalo brown cotton was not significantly different from each other (α=0.05). Only no-enzyme treated fibers showed significantly low moisture regain (%). Coyote brown cotton showed significantly lower moisture regain (%) when treated by Lipase compared to other enzyme treatments. No-enzyme treated fibers have significantly lower moisture regain (%) than lipase treated fibers as well as other

Table 4-12. Duncan grouping of means of moisture regain (%) from single enzyme treatment on naturally colored cotton

Buffalo Brown	Coyote Brown	Green
Cellulase	Protease	Lipase
Amylase	Cellulase	Amylase
Protease	Amylase	Cellulase
Lipase	Lipase	Protease
No treated (Scoured Only)	No treated (Scoured Only)	No treated (Scoured Only)

*Upper position group in the table indicates higher moisture regain (%) in means.

enzyme treated fibers. Green fiber showed significantly higher moisture regain after lipase treatment than with other enzyme treatment. Other enzyme treatments on green fiber resulted lower moisture regain (%) than two brown cottons (Appendix C) and no-enzyme treated green fibers showed even lowest moisture regain (%) among all combinations of fiber types and enzyme treatment.

Cellulase treatment was associated with higher moisture regain (%) of both brown cottons and lipase treatment has the smallest effect. On the other hand, green cotton was affected by lipase treatment more than other three enzyme treatments.

Moisture regain (%) of multiple enzyme treated naturally colored cotton

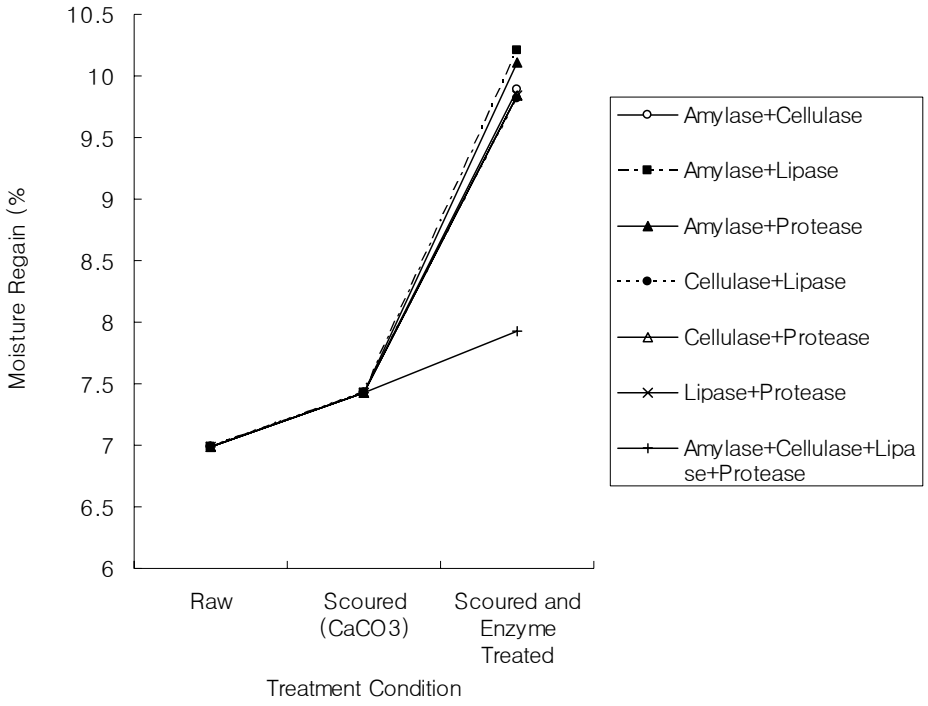


Figure 4-57. Effect of multiple enzymes on moisture regain (%) changes of buffalo brown naturally colored cotton after scouring and enzyme treatment

Figure 4-62 shows increase of moisture regain (%) of buffalo brown cotton after scouring and multiple enzyme treatment. After enzyme treatments the moisture regain increased further up to 10.432% when treated by cellulase and lipase together. There were different changes in moisture regain depending on enzymes and concentrations applied. Amylase and cellulose mixture increased moisture regain (%) of buffalo brown cotton up to 10.207 %. Four enzyme mixture treatments showed only small moisture regains (%). Enzyme concentrations had no significant effect on moisture regain (Appendix C).

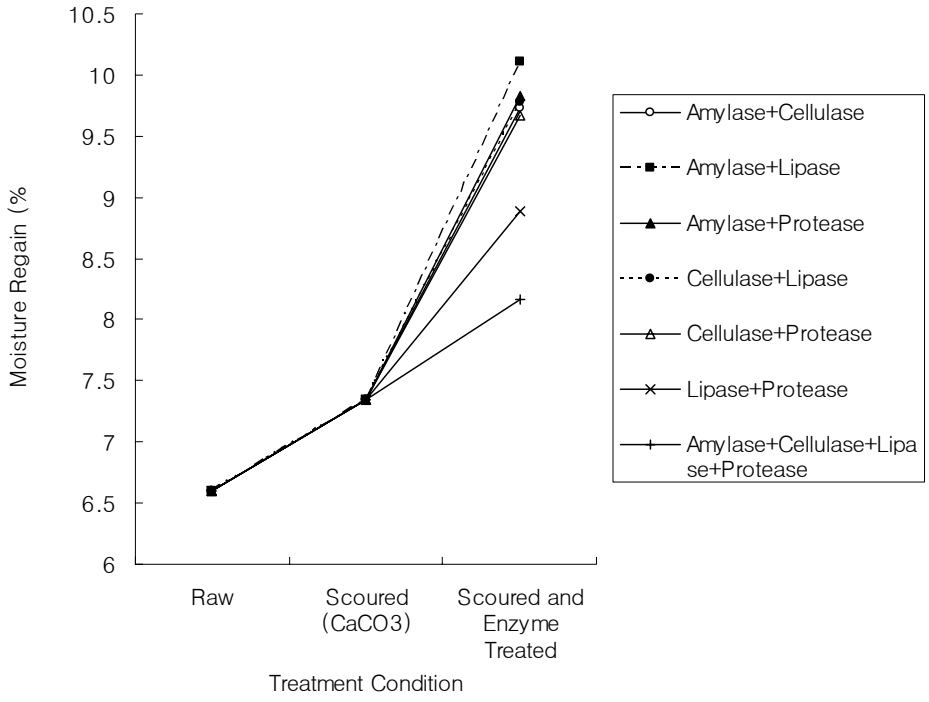


Figure 4-58. Effect of multiple enzymes on moisture regain (%) changes of coyote brown naturally colored cotton after scouring and enzyme treatment

Increase of moisture regain (%) of coyote brown cotton after scouring and multiple enzyme treatments is shown in figure 4-63. After enzyme treatment the moisture regain increased up to 10.171% when amylase and lipase were applied together. The overall increase in moisture regain of the coyote brown colored cotton after multiple enzyme treatments was lower compared to buffalo brown cotton. When lipase and protease were applied together the moisture regain was lower (8.882 %) than other combinations. Four enzymes mixture resulted in the lowest moisture regain (8.158 %) which was higher than that of buffalo brown cotton after same treatment. The effect of amylase on the moisture regain was largest and lipase showed a small effect on both brown colored cottons in common when used with other enzymes (Appendix C).

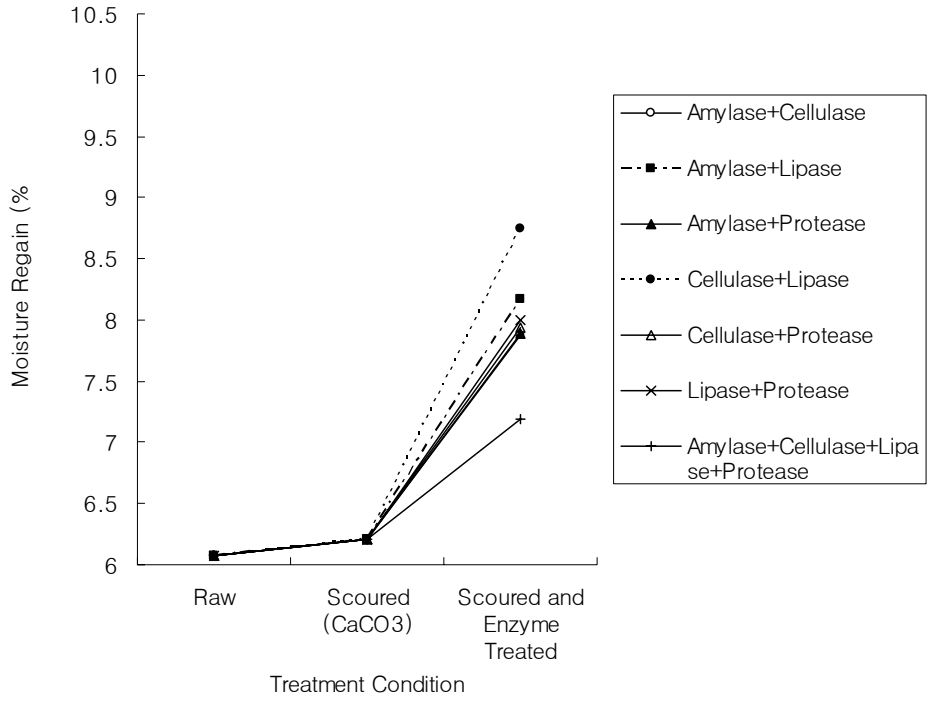


Figure 4-59. Effect of multiple enzymes on moisture regain (%) changes of green naturally colored cotton after scouring and enzyme treatment

Figure 4-64 shows that moisture regain of raw green cotton is the lowest among three naturally colored cottons and has less increase after scouring. After enzyme treatment the moisture regain increased to 9.326% by cellulase and lipase together. The second highest moisture regain (%) occured after amylase and lipase treatment. Therefore lipase was also the most effective enzyme to increase moisture regain (%) when multiple enzymes were applied. But overall moisture regains of green cotton after enzyme treatments were lower than two brown colored cottons. Four enzymes mixture showed the lowest moisture regain (%) among all combinations, which is the same result as those from two brown colored cottons (Appendix C).

Statistical analysis of moisture regain (%) after multiple enzyme treatment

ANOVA was used to analyze the effect of enzyme on moisture regain change of three naturally colored cottons because the responses, moisture regain (%), are continuous and the predictor variables, enzymes and fibers, are categorical. A SAS program (Appendix C) was developed and used to complete the analysis. The following null hypothesis was tested to study the different effect of multiple enzyme treatment on three colored cottons.

H_0: Enzymes, fiber types and interaction between enzymes and fiber types are significant effects on moisture regain (%).

Table 4-13 shows Duncan grouping result. Means with the same letter group are not significantly different (α=0.05) (Appendix C).

Table 4-13. Duncan grouping of means of moisture regain (%) from multiple enzyme treatment on naturally colored cotton

Buffalo Brown	Coyote Brown	Green
Amylase + Lipase	Amylase + Lipase	Cellulase + Lipase
Amylase + Protease	Amylase + Protease	Amylase + Lipase
Amylase + Cellulase	Cellulase + Lipase	Lipase + Protease
Lipase + Protease	Amylase + Cellulase	Cellulase + Protease
Cellulase + Protease	Lipase + Protease	Amylase + Protease
Cellulase + Lipase	Cellulase + Protease	Amylase + Cellulase
Amylase + Cellulase + Lipase + Protease	Amylase + Cellulase + Lipase + Protease	Amylase + Cellulase + Lipase + Protease
No treated (Scoured Only)	No treated (Scoured Only)	No treated (Scoured Only)

*Upper position group in the table indicates higher moisture regain (%) in means.

The order of the moisture regain (%) between fiber types was same as after single enzyme treatment. Buffalo brown cotton has the highest moisture regain (%) in average and green cotton has the lowest moisture regain (%) in average after multiple enzyme treatment. After each multiple enzyme treatment, cellulase and lipase mixed enzyme treatment resulted in lower moisture regain (%) then other combinations. The lowest moisture regain (%) was gained after all four mixed enzyme treatments but it was significantly higher than no-enzyme treated cotton. Coyote brown cotton showed an increase after multiple enzyme treatment, similar to the result of buffalo brown cotton. All paired enzyme treatments showed significant increases in moisture regain (%) and four mixed enzyme treatment showed lower moisture regain (%) after the treatment than other multiple enzyme treatments. No-enzyme treatment showed significantly lowest moisture regain (%) of coyote brown cotton. Green fiber showed good moisture regain only after amylase and lipase mixed treatment and cellulose and lipase mixed treatment. Other enzyme treatments resulted in lower moisture regain (%) than two brown cottons (Appendix C) and no-enzyme treated green fibers showed lowest moisture regain (%) among all combinations of fiber types and enzyme treatment.

Amylase has larger effect on moisture regain increase of two naturally brown cottons than other enzymes when the enzymes are used as mixed. For green fiber, lipase was the most effective enzyme on moisture regain (%) increase when used as mixed with other enzymes.

Overall change in moisture regain (%)

The scouring and enzyme treatment changed weights of the three naturally colored cotton: buffalo brown, coyote brown, and green colored cotton. The scouring process decreased weights of the three naturally colored cottons and the enzyme treatment after scouring process

increased weight of the three naturally colored cottons. Moisture regain tests showed that the increase in weight of the three naturally colored cottons result from the increase of moisture regain after the enzyme treatment. Figure 4-65 shows overall change of moisture regain (%). Buffalo brown cotton shows the highest moisture regain (%) and green shows the lowest moisture regain (%) at every condition. Multiple enzyme treatment shows more increase in moisture regain (%).

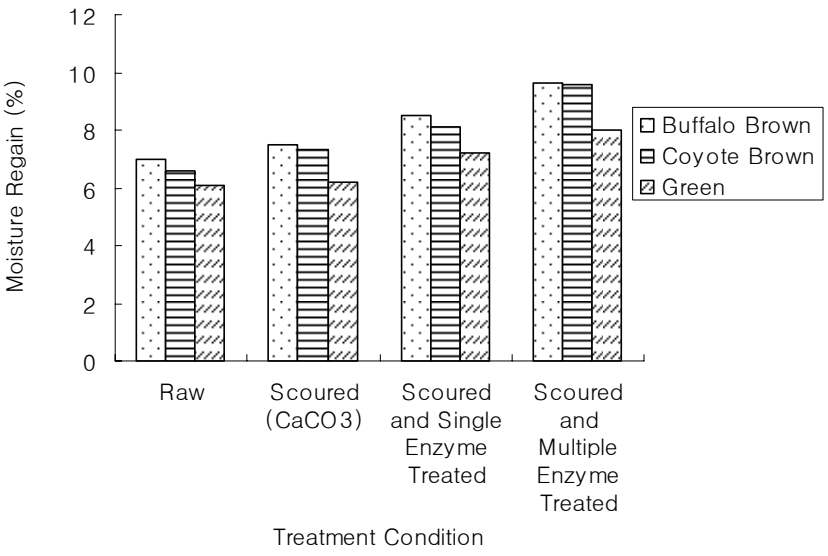


Figure 4-60. Moisture Regain (%) Changes of Naturally Colored Cottons after Scouring and Enzyme treatment

The amount of change of the moisture regain varied depending on the fiber types and enzyme types. Buffalo brown cotton has a good moisture regain (%) when treated by cellulase and coyote brown has good moisture regain (%) after treatment by protease and amylase. Both brown cottons show their lowest increases in moisture regain (%) after lipase treatment. However, lipase was most effective in increasing moisture regain (%) of green cotton. This could be due to

the different components of the three naturally colored cottons. When all four enzymes were applied together, the increase of moisture regain (%) was low for three naturally colored cottons.

CHAPTER 5

CONCLUSIONS AND FUTURE WORK

Conclusions

The primary objective of this research was to study the character of three separate naturally colored cottons. The research focused on the color and moisture regain (%) of buffalo brown, coyote brown and green cotton fibers. Color of naturally colored cottons was measured after scouring process, enzyme treatment as laundering detergent and UV exposure. Moisture regain (%) change was observed after scouring process and enzyme treatment. There were three scouring processes using NaOH, CaCO3 and two enzymes of pectinase and cellulase. Investigations on fiber were tried using wax extraction, light microscopy, scanning electron microscopy, X-ray and inductively coupled plasma to determine main causes of color change after scouring. Amylase, cellulase, lipase and protease were used for enzyme treatment process. UV exposure was done using weathering machine up to 40 hours.

After three scouring processes colors of each sample were measured. Enzymatic scouring did not change colors of three naturally colored cottons significantly. For comparison, colored cottons were treated with only water with the same condition as scouring. The result from water treatment was similar to the result from enzymatic scouring process. After both alkali process, three naturally colored cotton showed decrease of L*, a*, b*. Among three fibers green fiber showed the largest change in color as ΔE of 18.705. Three naturally colored cottons showed larger decrease in L*, a*, b* when treated by $CaCO_3$ rather than by NaOH. When color of fiber

was darker, remain solution in the scouring bath also colored deeply. Various experiments were tried to determine cause of darker color of both fiber and scouring solution after scouring. Wax extraction using hexane did not change color of colored cottons significantly, which indicates higher wax content of naturally colored cottons than conventional white cotton did not affect the unique color change after scouring. Movement of pigments toward outer part of colored cottons was observed through light microscopy. SEM images showed swollen and more uniform fiber cross-sections than those before scouring. Rounder cross-section can effect deeper color after scouring. Potassium was detected from both X-ray and ICP analysis. ICP analysis showed potassium decreased significantly after scouring solution and scouring bath contained potassium after the process.

Enzyme was applied as single treatments and multiple treatments with two or four enzymes. Lightness of three naturally colored cottons decreased slightly. However, decreased values of a* and b* of both brown colored cottons after scouring recovered toward the values of raw cottons. Green cotton showed more decreased values of a* and b* than values after scouring. Green cotton lost its yellow color and became green after scouring and enzyme treatment.

UV exposure was done for 40 hours and color was measured every 10 hours mostly. Dyes cotton fabric generally show colorfastness after UV exposure. Scoured and single enzyme treated three naturally colored cottons showed significant decrease in lightness after five hours of UV exposure. Repeated experiments confirmed this result. After five hours lightness increased slowly as color was faded. Multiple enzymes treated three naturally colored cottons showed slight increase while UV was exposed. After UV exposure buffalo brown showed decreased in value of a* and b*. Therefore after enzyme treatment color became similar to the color after scouring. Coyote brown cotton and green cotton showed increase in value of a* and b*. Green

cotton showed great change after 40 hours of UV exposure especially. Statistical result showed that ‘UV exposure hours’ and ‘enzyme types’ were significant variables for lightness of three naturally colored cottons. When mutiple enzyme treatments were done, ‘UV exposure hours’ was the only significant variable for lightness of three naturally colored cottons in common.

Weight of naturally colored cottons was measured after scouring and enzyme treatment. After scouring weight total decreased as impurities removed from fiber. However, enzyme treatment increased weight of three naturally colored cottons. To determine the cause of this increase, moisture regain (%) test was done. Results showed increase in moisture regain (%) after enzyme treatment. Naturally colored cottons have lower moisture regain (%) than conventional white cotton due to higher hydrophobic contents. Improvement in moisture regain (%) after enzyme treatment is a desirable result. Statistical results showed that enzyme treated samples have higher moisture regain than untreated samples. Duncan grouping showed that lipase treatment affected green cotton more than brown cottons.

Future work

1. Previous research [45, 101] showed TEM (Transmission Electron Microscopy) images of green colored cottons. TEM preparations were not successful for dried and scoured colored cotton fibers. However, TEM image for freshly cut brown colored cotton needs to be observed to determine location of pigment of naturally brown cotton.

2. ICP analysis showed loss of potassium during scouring process. The relationship between potassium and fiber character such as color should be pursued.

3. Pigment moved when fiber was treated by alkali and the fiber samples became darker. Detailed chemical analysis should be done for more pigment characterization to explain this

observation.

4. Unexpected decrease in lightness after five hours of UV exposure of single enzyme treated cottons occurred. Determination of the cause may be helpful to understand unique character of pigment.

5. Physical and chemical analysis to determine the change of color after enzyme treatment can be done.

REFERENCES

1. Dimitri, C. and Greene, C. (2002). Recent growth patterns in the US organic foods industry. US Department of Agriculture, Economic Research Service. Agricultural Information Bulletin Number 777.

2. Govindasamy, R. and Italia, J. (1998) A Willingness-to-purchase comparison of integrated pest management and conventional produce. Agribuisiness, 14, 403-414.

3. Tchiadje, N. (2007). Strategies to reduce the impact of salt on crops (rice, cotton and chilli) production: A Case Study of the Tsunami-affected area on India. Desalination, 206, 524-530.

4. Lohr, L. (1998). Implications of organic certification for market structure and trade. American Journal of Agricultural Economics, 80, 1125-1133.

5. Klonsky, K. and Tourte, L. (1998). Organic agricultural production in the United States: debates and directions. American Journal of Agricultural Economics, 80, 1119-1124.

7. Thompson, G. T. (1998). Consumer demand for organic foods: What we know and what se need to know. American Journal of Agricultural Economics, 80, 1113-1118.

8. Guerena, M. and Sullivan, P. (2003). Organic cotton production. Appropriate Technology Transfer for Rural Areas, July.

9. Ton, P. (2002). The international market for organic cotton and eco-textiles. Proceedings of the 14th IFOAM Organic World Congress. Canadian Organic Growers, Ottawa, Ontario, Canada.

10. Lazarov, A., Trattner A., David M. and Ingber A. (2000). Textile dermatitis in Israel: A retrospective study. American Journal of Contact Dermatitis, 11(1), 26-29.

11. An, D. (1992). Textile dye dermatitis. Contact Dermatitis, 27(5), 321-323.

12. Oei, H., Kartanegara, W. and Oei, R. (2003). Textile-dye allergic contact dermatitis. Journal of Allergy and Clinical Immunology, 111 (2) 155.

13. Ferreira, J., Costa, J., Castro, E., and Vaz, M. (2002). Assessment of Atopy in textile industry workers: comparison of two methods. Journal of Allergy and Clinical Immunology, 109 (1) 325.

14. Vreeland, J. and James, M. (1999). The revival of colored cotton. Scientific America, 280 (4), 112-119.

15. Lee, J. (1996). A new spin on naturally colored cotton. Agricultural Research 44 (4), 20-21.

16. Mohammadioun, M., Gallaway, M. and Apodaca, J. K. (1994). An economic analysis of organic cotton as a niche crop in texas. University of Texas at Austin, Bureau of Business Research, Austin, Texas.

17. Gu, H. (2005). Research on the improvement of the moisture absorbency of naturally self-coloured cotton. The Journal of the Textile Institute, 96 (4), 247-250.

18. Naturally Colored Cotton Market in China WEb Resource: http://service.china.org.cn/link/wcm/Show_Text?info_id=153331&p_qry=colored%20and%20cotton

19. Naturally Colored Cotton Market in China WEb Resource: http://www.china.org.cn/english/China/182518.htm

20. Parmar, M. S. and Chakraborty, M. (2001). Thermal and burning behavior of naturally colored cotton. Textile Research Journal 71 (12), 1099-1102.

21. Hustvedt, G. and Crews, C. (2005). The Ultraviolet protection factor of naturally-pigmented cotton. The Journal of Cotton science 49, 47-55.

22. Bowerman, E. H. (1996). Organic cottons. California State University, Fresno Personal

Communication, 6-10-96.

23. Lee, J. (1996). A new spin on naturally colored cottons. Agricultural Research Magazine. 44 (4), 18-19.
24. Duckett, K. E. (1975). Surface properties of cotton fibers. surface characteristics of fibers and textiles. edited by M. J. Schick. Fiber Science Series, Marcel Dekker, Inc., p 67.
25. Dickerson D. K., Lane, E. K., and Rodriguez, D. F., (1996) Evaluation of selected performance characteristics of naturally colored cotton knit fabrics. California Agricultural Technology Institute Research Publication, #96100332.
26. http://www.engr.utk.edu/mse/pages/Textiles/Cotton%20fibers.htm
27. Hollen, N. and Saddler, J. (1968). Natural fibers. Textiles: Third edition. The Macmillan Company. p 31-33.
28. deGruy, I., Carra, J. and Goynes, W. (1973). The fine structure of cotton: An atlas of cotton microscopy. Marcel Dekker, Inc., NY, xii.
29. Guthrie, J. D. (1955). The Chemistry of lint cotton. The chemistry of lint cotton. edited by J. Kyle Ward, Ed. NY, Interscience Publishers, p 121.
30. Tejada, M., Garcia C., Gonzalez, J.L. and Hernandez, M.T. (2006). Use of organic amendment as a strategy for saline soil remediation: Influence on the physical, chemical and biological properties of soil. Soil Biology & Biochemistry, 38, 1413-1421.
31. Ramey Jr., H.H. (1986). Stress influences on fiber development. Cotton Physiology. edited by Mauney, J.R., Stewart, J. McD. Memphis, TN, The Cotton Foundation, p 315-359.
32. Reddy, K. R., Davidonis, G. H. and Johnson, A. S. (1999) Temperature regime and carbon dioxide enrichment alter cotton ball development and fiber properties. Agronomy Journal, 91, 851-858.

33. Pettigrew, W. T., Heitholt J.J. and Meredith Jr., W. R. (1996). Genotype interactions with potassium and nitrogen in cotton of varied maturity. Agronomy Journal, 88, 89-93.

34. Dastur, R. H., Kanwar, S. and Kaiwar, S. A. (1952). Investigations on the red leaf disease in american cottons. II. Red leaf disease in American cottons in Malwa and Bombay-Karnatak. Indian Cotton Growing Revolution, 6, 193-204.

35. Sanchez, P. A. and Salinas, J. G. (1981). Low input technology for managing Oxisols and Ultisols in tropical America. Advances in Agronomy, 34, 280-298.

36. Constable, G. A. and Hodgson, A. S. (1990). A Comparison of drip and furrow irrigated cotton on a cracking clay soil. 3. Yield and Quality of 4 Cultivars. Irrigation Science, 11, 137-142.

37. Constable, G. A., Rochester, I. J. and Hodgson, A. S. (1990). A comparison of drip and furrow irrigated cotton on a cracking clay soil. 1. Growth and nitrogen uptake. Irrigation Science, 11, 137-142.

38. Qui, P. F., Tie, B. Q., Zhou, X. H., Zeng, Q. R. and Zhou, X. S. (2000), Effect of cadmuim and lead in soil on the germination and growth of rice and cotton. Journal of Human and Agricultural Universities, 26, 205-207.

39. Alloway, B. J. (1995). Heavy metals in soils. Blackies, London, England.

40. Kabata-Pendias, A. and Pendias, H. (1992). Trace element in soil and plants, Second Edition. Boca Raton, FL, CRC Press.

41. Angelova, V., Ivanova, R., Delibaltova, V. and Ivanov, K. (2004). Bio-accumulation and distribution of heavy metals in fibre crops (Flax, Cotton and Hemp). Industrial Crops and Products, 19, 197-205.

42. Zhang, W., Wu, H., Ma, C. and Hu, B. (2002). Research on microstructure and main

chemical compositions of the color cotton. Printing and Dyeing, 27, 1-5. (Chinese)

43. Schmutz, A., Jenny, T. and Ryser, U. (1994). A Caffeoyl fatty-acid glycerol ester from wax associated with green cotton fiber suberin. Phytochemistry, 36 (6), 1343-1346.
44. Valko, E. I. (1955). Bleaching. Chemistry and chemical technology of cotton. edited by J. Kyle Ward, Ed. NY, Interscience Publishers, p 121, 152-156.
45. Yatsu, L. Y., Espelie, K. E. and Kolattukudy, P. E. (1983). Ultrastructural and chemical evidence that the cell wall of green cotton fiber is suberized. Plant Physiology, 73, 521-524.
46. Lewin, A. H. and Carroll, F. I. (1981). Separation and identification of the major componenets of cotton fiber wax. Report to Cotton, Incorporated.
47. Campbell, K. S. (1965). Bleaching and process preparatory to dyeing. The American Cotton Handbook, Vol. II. edited by D. S. Hamby. Interscience Publishers, p 768, 769-774.
48. Schmutz, A., Jenny, T., Amrhein, N. and Ryser, U. (1993). Caffeic acid and glycerol are constituents of the suberin layers in green cotton fibres. Planta, 189, 453-460.
49. Ryser, U. and Holloway, P. J. (1985). Ultrastructure and chemistry of soluble and polymeric lipids in cell walls from seed coats and fibers of *Gossypium* species. Planta, 163 (2), 151-163.
50. Graf, E. (1992). Antioxidant potential of ferulic acid. Free Radical Biology and Medicine, 13 (4), 435-448.
51. Halloin, J. M. (1982). Localization and changes in catechin and tannins during development and ripening of cottonseed. New Phytologist, 90 (4), 651-657.
52. Ryser, U. (1999). Cotton fiber irritation and histodifferentiation. Cotton fibers: Developmental biology, quality improvement, and textile processing. edited by A. S. Barsra. Binghamton, NY, Haworth Press, p 46.
53. Tannin in Tea Plant Web Resource:

http://www.ansci.cornell.edu/plants/toxicagents/tannin

54. Caffeic Acid Chemical Structure Web Resource: http://www.chemicalland21.com/lifescience/phar/CAFFEIC%20ACID.htm
55. Cinnamic Acid Chemical Structure Web Resource: http://www.flavornet.org/info/140-10-3.html
56. Lue, BM, Karboune, S, Yeboah, and FK, Kermasha, S (2005) Lipase catalyzed esterification of cinnamic acid and oleyl alcohol in organic solvent media. Journal of Chemical Technology and Biotechnology, 80, 462-468.
57. Aparna, K., Sundar, B. S. and Radhakrishnamurti, P. S. (2003) Kinetics and mechanism of oxidation/addition of cinnamic acid and ethyl cinnamate by trichloroisocyanuric acid. Oxidation Communications, 26(3), 398-402.
58. Ying, C. and Xinyuan, S. (2006). Effect of coloring materials on microstructure of naturally colored cotton. Colourage, 53, 55-58.
59. Richards, A. F., Rowe, T. and Elesini, U. S. (1999). Structure of naturally coloured cottons. Journal of the Textile Institute, 90 (4), 493-499.
60. Chen, H. L. and Yokochi, A. (2000). X-ray diffractometric study of microcrystallite size of naturally colored cottons . Journal of Applied Polymer Science, 76 (9), 1466-1471.
61. Kimmel, L. (2002).Naturally colored cottons for specialty textile products. Journal of American Association of Textile Chemists and Colorists, 2 (5), 25-29.
62. Meredith, R. (1952). Properties of textile materials,I: Tensile strength, breaking extension, and stress-strain relations of fibres. Journal of Textile Institute, 43, 755.
63. Karmakar, S. R. (1999). Application in the pre-treatment processes of textiles. Colourage Annual, 75-84.

64. Li, Y. H. and Hardin, I. R. (1998). Enzymatic scouring of cotton - surfactants, agitation, and selection of enzymes. Textile Chemist and Colorist, 30 (9), 23-29.

65. Li, Y. H. and Hardin, I. R. (1998). Treating cotton with cellulases and pectinases: Effects on cuticle and fiber properties. Textile Research Journal, 68 (9), 671-679.

66. Li, Y. H. and Hardin, I. R. (1997). Enzymatic scouring of cotton: Effects on structure and properties. Textile Chemist and Colorist, 29 (8), 71-76.

67. Lin, C. H. and Hsieh, Y. L. (2001). Direct scouring of greige cotton fabrics with proteases. Textile Research Journal, 71 (5), 425-434.

68. Hartzell, M. and Durrant, S. K. (2000). Enzymatic scouring with agitation to improve cotton fabric wettability. Textile Chemist and Colorist &American Dyestuff Reporter, 32 (8), 86-90.

69. Etters, J. N. (1999). Cotton preparation with alkaline pectinase: An environmental advance. Textile Chemist and Colorist & American Dyestuff Reporter, 1 (3), 33-36.

70. Buschle-Diller, G., El Mogahzy, Y., Inglesby, M. K. and Zeronian, S. H. (1998). Effects of scouring with enzymes, organic solvents, and caustic soda on the properties of hydrogen peroxide bleached cotton yarn. Textile Research Journal, 68 (12), 920-929.

71. Etters, J. N., Husain, P. A. and Lange, N. K. (1999). Alkaline pectinase: An eco friendly approach to cotton preparation. Textile Asia, 5, 83-85.

72. Wang, Q., Fan, X., Hua, Z., Gao, W. and chen, j. (2006). Degradation kinetics of pectins by an Alkaline Pectinase in bioscouring of cotton fabrics. Carbohydrate Polymers, doi:10.1016/j.carbpol.2006.06.031.

73. McNeil, M., Darvill, A. G., Fry, S. C. and Albersheim, P. (1984). Structure and function of the primary cell walls of plants. Annual Review of Biochemistry, 53, 625-663.

74. Tzanov, T., Calafell, M., Guebitz, G. M. and Cavaco-Paulo, A. (2001). Bio-preparation of

cotton fabrics. Enzyme and microbial technology, 29, 357-362.

75. Freytag, R. and J.J., D. (1983). Fundamentals and preparation Part A, Handbook of Fiber Science and Technology:Vol I. Chemical processing of Fibers and Fabrics. edited by M. Lewin and S. B. Sello. N, Marcel Dekker, p 111.
76. Buschle-Diller, G., Person, A., Knight, C. and Fox, S. V. (1999). Naturally colored cotton-shade changes upon wet treatments. AATCC conference book.
77. Lyle, D. S. (1977). Performance of textiles. NY, John Wiley & Sons, p 302-309.
78. Home Washing Products-The Technology of Home Laundry. Association for textile technology, Inc. Monograph No. 108.
79. Jakobi, G. and Lohr, A. (1987). Detergents and textile washing. Detergents and textile washing. NY, VCH Publishers, p 87.
80. Chaplin, M. and Bucke C. (1990). The use of enzymes in detergents. Enzyme Technology. Cambridge University Press, p 89-93.
81. Cavaco-Paulo, A. and Gubitz, G. M. (2003). Catalysis and processing. Textile processing with enzymes. edited by A. Cavaco-Paulo and G. M. Gubitz. Boca Raton, CRC Press LLC, p 90-93, 96.
82. Mitidieri, S., Martinelli, A. H. S., Schrank, A. and Vainstein, M. H. (2006). Enzymatic detergent formulation containing amylase from *Aspergillus niger*: A comparative study with commercial detergent formulations. Bioresource Technology, 97 (10), 1217-1224.
83. Andree, H., Muller, W.-R. and Schmid, R. D. (1980). Lipases as detergent components. Journal of Applied Biochemistry, 2, 218-229.
84. Maurer, K.-H. (2004). Detergent proteases. Current Opinion in Biotechnology, 15, 330-334.
85. Cayle, T. (1975). Enzymes. Detergency: Theory and test methods, part II edited by W. G.

Cutler and R. C. Davis. NY, Marcel Dekker Inc., p 665-678.

86. Joo, H.-S. and Chang, C.-S. (2005). Production of an oxidant and SDS-stable alkaline protease from an alkaophilic *Bacillus claussi* I-52 by submerged fermentation: Feasibility as a laundry detergent additive. Enzyme and Microbial Technology, doi: 10.1016/j.enzmictec.2005.05.008.
87. Dickerson, D. K., Lane, E. F. and Rodriguez, D. F. (1999). Naturally colored cotton : resistance to changes in color and durability when refurbished with selected laundry aids. California Agricultural Technology Institute, #990901.
88. Williams, B. L. and Horridge, P. (1996). Effects of selected laundering and drycleaning pretreatments on the colors of naturally colored cotton. Family and Consumer Sciences Research Journal, 25 (2), 137-158.
89. Giles, C. H. (1965). The fading of colouring matters. Journal of Applied Chemistry 15, 541-550.
90. Wagner, R. D. and Leslei, R. C. (1985). Test methods for determining colorfastness to light. Textile chemist and colorist & American dyestuff reporter, 17 (2), 17-24.
91. Cristea, D. and Vilarem, G. (2006). Improving light fastness of natural dyes on cotton yarn. Dyes and Pigments, 71, 39-46.
92. Van Beek, H. (1983). Light-induced colour changes in dyes and materials. Color Research and Application, 8, 176-181.
93. Giles, C., H, Haslam, R. and Duff, D. G. (1976). An examination of the cause of the humidity sensitivity of the light fastness of dyed materials. Textile Research Journal, 46, 51-54.
94. Jahagirdar, C. J., Srivastava, Y. and Venkatakrishnan, S. (2000). Light fastness of naturally colored cotton germplasm of *Gossypium Hirsutum L.* Journal of the Textile Association, 61

(1), 27-33.

95. Parmar, M. S. and Sharma, R. P. (2000). Development of various colours and shades in naturally coloured cotton fabrics. Indian Journal of Fiber and Textile research, 27, 397-407.

96. CIELAB Coordinate System Web Resource: http://www.specialchem4coatings.com/tc/color-handbook/index.aspx?id=cielab

97. Conrad, C. (1944). Determination of wax in cotton fiber: A new alcohol extraction method. Industrial and Engineering Chemistry, 16 (12), 745-748.

98. http://ral.coafes.umn.edu/icp.htm

99. Bozzola, J. and Russell, L. (1999). Electron microscopy: principles and techniques for biologists. MA, Jones and Bartlett Publishers Inc., p56-58.

100. Miller, G. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar, Analytical Chemistry, 31(3), 426-428.

101. Rajasekaran, K., Muir, A.J., Ingber, B. F. and French, A. D. (2006). A dehydration methods for immature or wet cotton fibers for light and electron microscopy. Textile Research Journal, 76(6), 514-518.

Appendix A

Statistical Analysis of Lightness Change of Naturally Colored Cotton after UV Exposure

SAS code for buffalo brown cotton of single enzyme treatment

```
proc import datafile="D:\cc2\uv exposure data\singlebu.xls" out=singlebu dbms=excel;
getnames=yes;
run;
proc mixed data=singlebu;
class sample uvtime econc etype;
model l=uvtime etype econc;
repeated /subject=sample type=cs;
run;
```

SAS output

```
Model Information
                Data Set                  WORK.SINGLEBU
                Dependent Variable          l
                Covariance Structure        Compound Symmetry
                Subject Effect             sample
                Estimation Method           REML
                Residual Variance Method    Profile
                Fixed Effects SE Method     Model-Based
                Degrees of Freedom Method   Between-Within

                          Class Level Information
                 Class    Levels   Values
                 sample       48   1 2 3 4 5 6 7 8 9 10 11 12 13
                                   14 15 16 17 18 19 20 21 22 23
                                   24 25 26 27 28 29 30 31 32 33
                                   34 35 36 37 38 39 40 41 42 43
                                   44 45 46 47 48
                 uvtime        6   0 5 10 20 30 40
                 econc         4   1 2 3 4
                 etype         4   am cs ls pr

                                Dimensions
                       Covariance Parameters           2
```

Columns in X	15
Columns in Z	0
Subjects	48
Max Obs Per Subject	6
Observations Used	288
Observations Not Used	0
Total Observations	288

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	326.85978467	
1	1	191.70167827	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	sample	0.1022
Residual		0.07085

Fit Statistics

-2 Res Log Likelihood	191.7
AIC (smaller is better)	195.7
AICC (smaller is better)	195.7
BIC (smaller is better)	199.4

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	135.16	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
uvtime	5	235	1674.84	<.0001
etype	3	41	5.40	0.0032
econc	3	41	2.07	0.1193

SAS code for coyote brown cotton of single enzyme treatment

```
proc import datafile="D:\cc2\uv exposure data\singleco.xls" out=singleco dbms=excel;
getnames=yes;
run;
proc mixed data=singleco;
class sample uvtime econc etype;
model l=uvtime etype econc;
repeated /subject=sample type=cs;
```

```
run;
```

SAS output

```
                    Model Information

 Data Set                     WORK.SINGLECO
 Dependent Variable           l
 Covariance Structure         Compound Symmetry
 Subject Effect               sample
 Estimation Method            REML
 Residual Variance Method     Profile
 Fixed Effects SE Method      Model-Based
 Degrees of Freedom Method    Between-Within

              Class Level Information
  Class     Levels    Values
  sample        48    1 2 3 4 5 6 7 8 9 10 11 12 13
                      14 15 16 17 18 19 20 21 22 23
                      24 25 26 27 28 29 30 31 32 33
                      34 35 36 37 38 39 40 41 42 43
                      44 45 46 47 48
  uvtime         6    0 5 10 20 30 40
  econc          4    1 2 3 4
  etype          4    a c l p

                     Dimensions
          Covariance Parameters            2
          Columns in X                    15
          Columns in Z                     0
          Subjects                        48
          Max Obs Per Subject              6
          Observations Used              288
          Observations Not Used            0
          Total Observations             288

                   Iteration History

Iteration    Evaluations    -2 Res Log Like      Criterion
       0              1      274.36358774
       1              1      204.72417912     0.00000000

              Convergence criteria met.
            Covariance Parameter Estimates
```

Cov Parm	Subject	Estimate
CS	sample	0.05935
Residual		0.08097

Fit Statistics

-2 Res Log Likelihood	204.7
AIC (smaller is better)	208.7
AICC (smaller is better)	208.8
BIC (smaller is better)	212.5

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	69.64	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
uvtime	5	235	1571.50	<.0001
etype	3	41	18.03	<.0001
econc	3	41	6.02	0.0017

SAS code for green cotton of single enzyme treatment

```
proc import datafile="D:\cc2\uv exposure data\singlegr.xls" out=singlegr dbms=excel;
getnames=yes;
run;
proc mixed data=singlegr;
class sample uvtime econc etype;
model l=uvtime etype econc;
repeated /subject=sample type=cs;
run;
```

SAS output

Model Information

Data Set	WORK.SINGLEGR
Dependent Variable	l
Covariance Structure	Compound Symmetry
Subject Effect	sample
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based

Degrees of Freedom Method Between-Within

Class Level Information

Class	Levels	Values
sample	48	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
uvtime	6	0 5 10 20 30 40
econc	4	1 2 3 4
etype	4	a c l p

Dimensions

Covariance Parameters	2
Columns in X	15
Columns in Z	0
Subjects	48
Max Obs Per Subject	6
Observations Used	288
Observations Not Used	0
Total Observations	288

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	797.69351153	
1	1	657.31787507	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	sample	0.5733
Residual		0.3806

Fit Statistics

-2 Res Log Likelihood	657.3
AIC (smaller is better)	661.3
AICC (smaller is better)	661.4
BIC (smaller is better)	665.1

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	140.38	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
uvtime	5	235	341.37	<.0001
etype	3	41	28.45	<.0001
econc	3	41	1.08	0.3688

SAS code for buffalo brown cotton of multiple enzyme treatment

```
proc import datafile="D:\cc2\uv exposure data\multiplebu.xls" out=multiplebu
dbms=excel;
getnames=yes;
run;
proc mixed data=multiplebu;
class sample uvtime econc etype;
model l=uvtime etype econc;
repeated /subject=sample type=cs;
run;
```

SAS output

Model Information

Data Set	WORK.MULTIPLEBU
Dependent Variable	l
Covariance Structure	Compound Symmetry
Subject Effect	sample
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
sample	42	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
uvtime	6	0 5 10 20 30 40
econc	2	0.4 0.8
etype	7	ac aclp al ap cl cp lp

Dimensions

Covariance Parameters	2
Columns in X	16

Columns in Z	0
Subjects	42
Max Obs Per Subject	6
Observations Used	252
Observations Not Used	4
Total Observations	256

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	380.55663165	
1	1	194.93473633	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	sample	0.1924
Residual		0.07305

Fit Statistics

-2 Res Log Likelihood	194.9
AIC (smaller is better)	198.9
AICC (smaller is better)	199.0
BIC (smaller is better)	202.4

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	185.62	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
uvtime	5	205	209.67	<.0001
etype	6	34	5.84	0.0003
econc	1	34	5.86	0.0210

SAS code for coyote brown cotton of multiple enzyme treatment

```
proc import datafile="D:\cc2\uv exposure data\multipleco.xls" out=multipleco
dbms=excel;
getnames=yes;
run;
proc mixed data=multipleco;
class sample uvtime econc etype;
model l=uvtime etype econc;
repeated /subject=sample type=cs;
```

```
run;
```

SAS output

```
                Model Information
Data Set                     WORK.MULTIPLECO
Dependent Variable           l
Covariance Structure         Compound Symmetry
Subject Effect               sample
Estimation Method            REML
Residual Variance Method     Profile
Fixed Effects SE Method      Model-Based
Degrees of Freedom Method    Between-Within

               Class Level Information
  Class     Levels    Values
  sample        42    1 2 3 4 5 6 7 8 9 10 11 12 13
                      14 15 16 17 18 19 20 21 22 23
                      24 25 26 27 28 29 30 31 32 33
                      34 35 36 37 38 39 40 41 42
  uvtime         6    0 5 10 20 30 40
  econc          2    0.4 0.8
  etype          7    ac aclp al ap cl cp lp

                    Dimensions
          Covariance Parameters           2
          Columns in X                   16
          Columns in Z                    0
          Subjects                       42
          Max Obs Per Subject             6
          Observations Used             252
          Observations Not Used           0
          Total Observations            252

                  Iteration History
Iteration    Evaluations    -2 Res Log Like      Criterion
       0              1      279.56814240
       1              1      157.84772014     0.00000000

               Convergence criteria met.

             Covariance Parameter Estimates
            Cov Parm    Subject    Estimate
            CS          sample      0.1036
```

Residual	0.06709

Fit Statistics

-2 Res Log Likelihood	157.8
AIC (smaller is better)	161.8
AICC (smaller is better)	161.9
BIC (smaller is better)	165.3

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	121.72	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
uvtime	5	205	436.03	<.0001
etype	6	34	2.04	0.0866
econc	1	34	2.57	0.1179

SAS code for green cotton of multiple enzyme treatment

```
proc import datafile="D:\cc2\uv exposure data\multiplegr.xls" out=multiplegr
dbms=excel;
getnames=yes;
run;
proc mixed data=multiplegr;
class sample uvtime econc etype;
model l=uvtime etype econc;
repeated /subject=sample type=cs;
run;
```

SAS output

Model Information

Data Set	WORK.MULTIPLECO
Dependent Variable	l
Covariance Structure	Compound Symmetry
Subject Effect	sample
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
sample	42	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
uvtime	6	0 5 10 20 30 40
econc	2	0.4 0.8
etype	7	ac aclp al ap cl cp lp

Dimensions

Covariance Parameters	2
Columns in X	16
Columns in Z	0
Subjects	42
Max Obs Per Subject	6
Observations Used	252
Observations Not Used	0
Total Observations	252

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	279.56814240	
1	1	157.84772014	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	sample	0.1036
Residual		0.06709

Fit Statistics

-2 Res Log Likelihood	157.8
AIC (smaller is better)	161.8
AICC (smaller is better)	161.9
BIC (smaller is better)	165.3

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	121.72	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
uvtime	5	205	436.03	<.0001
etype	6	34	2.04	0.0866
econc	1	34	2.57	0.1179

Appendix B

Change of a* and b* of Naturally Colored Cotton after UV Exposure

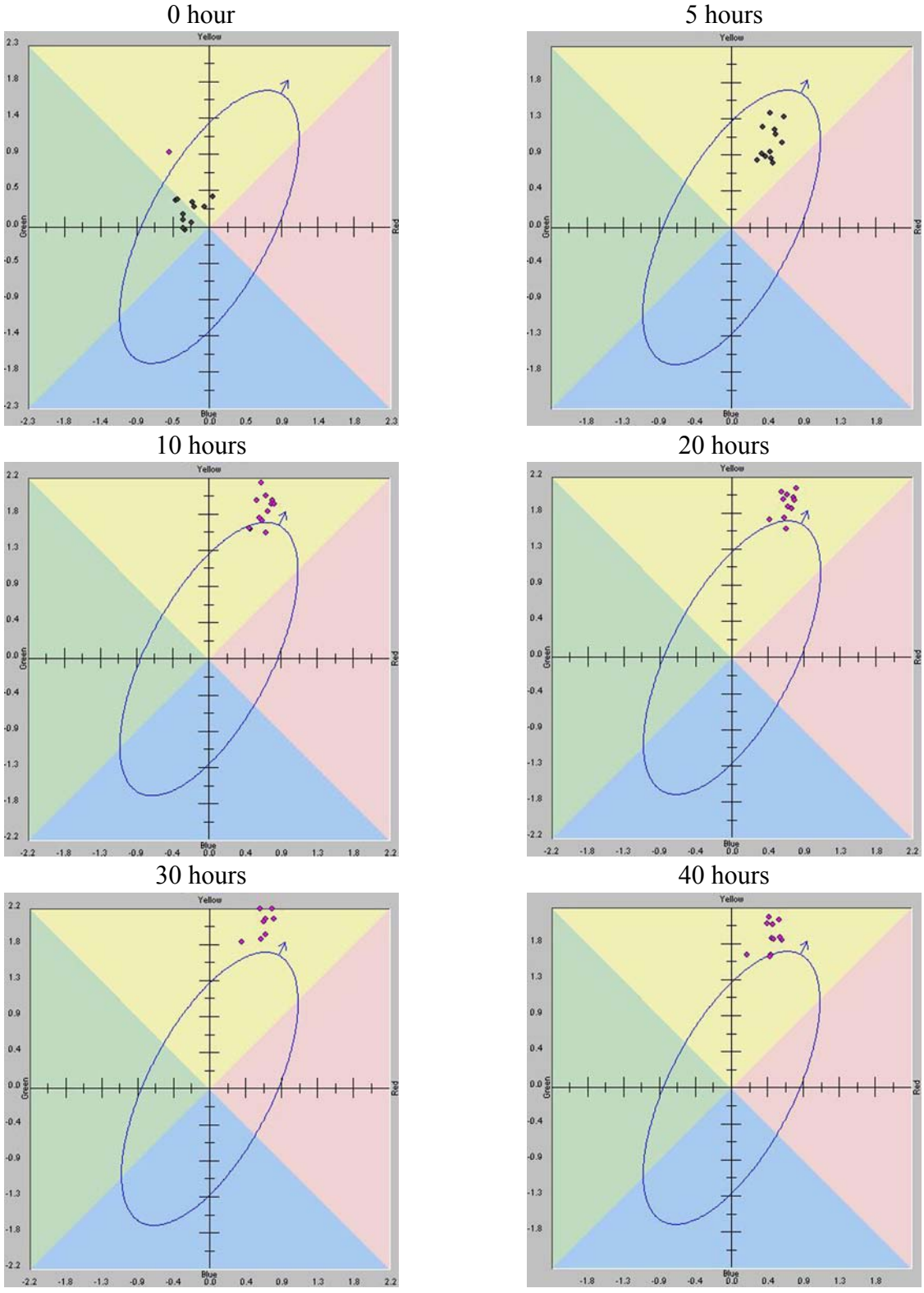


Figure B-1. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (amylase treated)

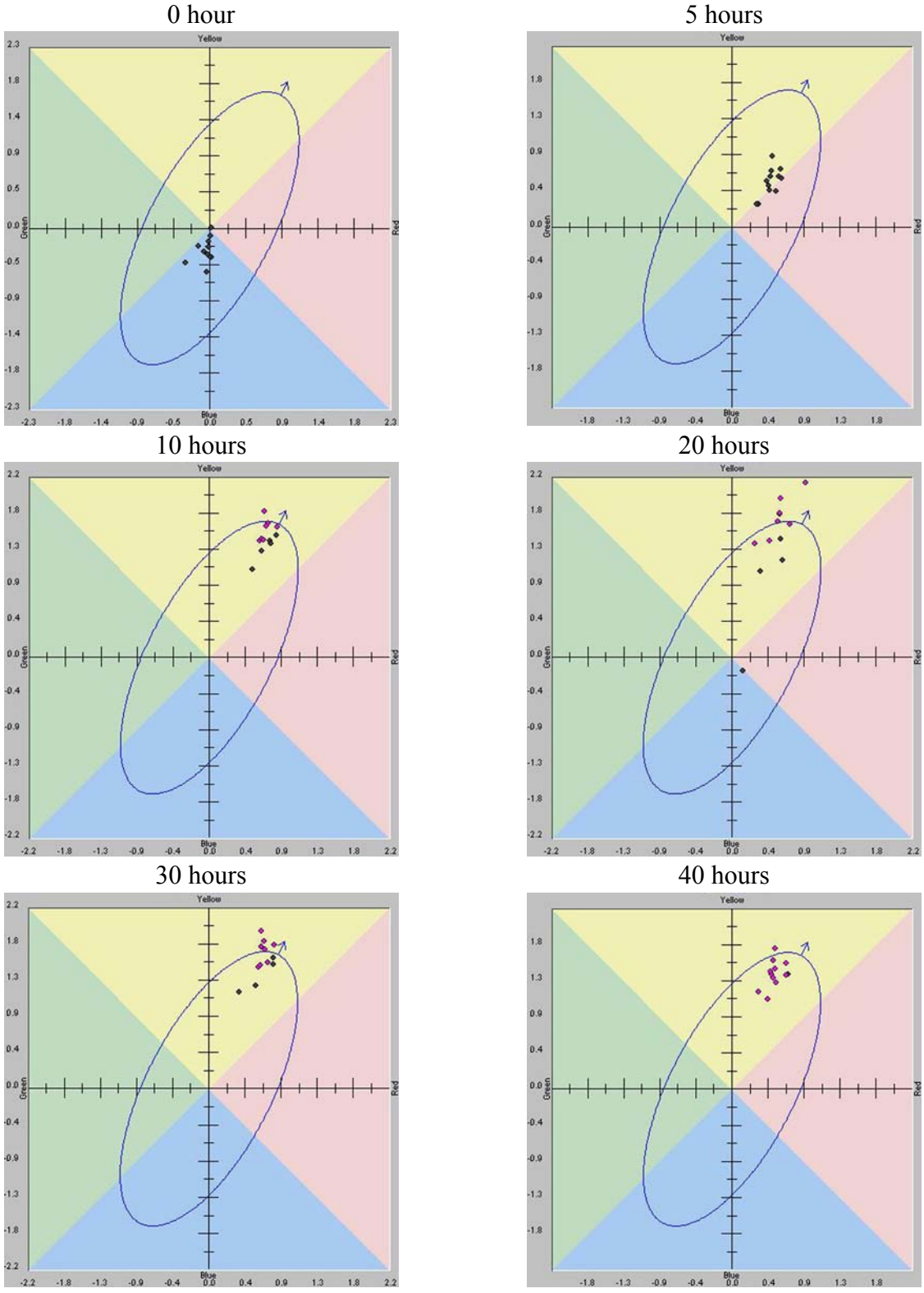


Figure B-2. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (cellulase treated)

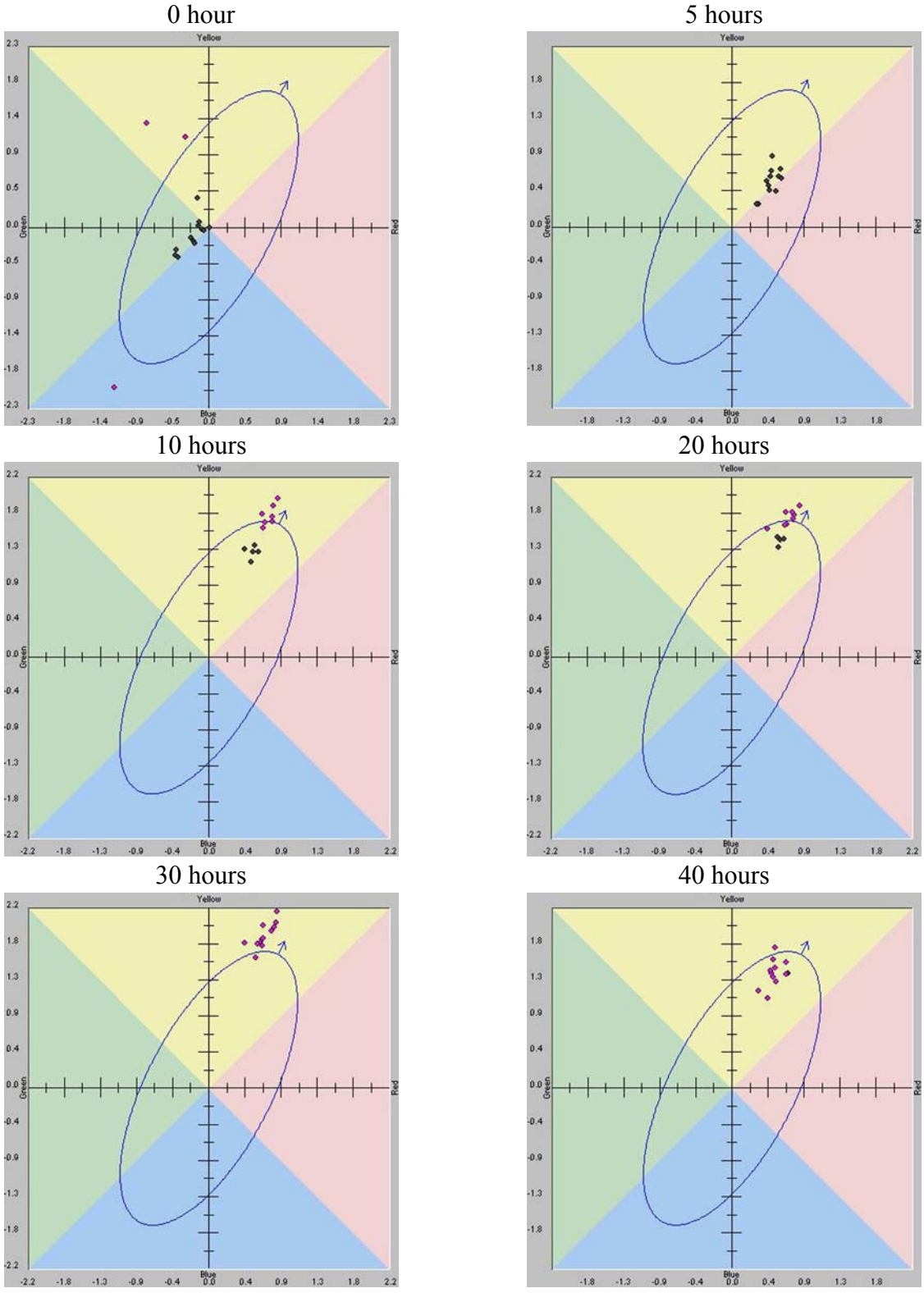


Figure B-3. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (lipase treated)

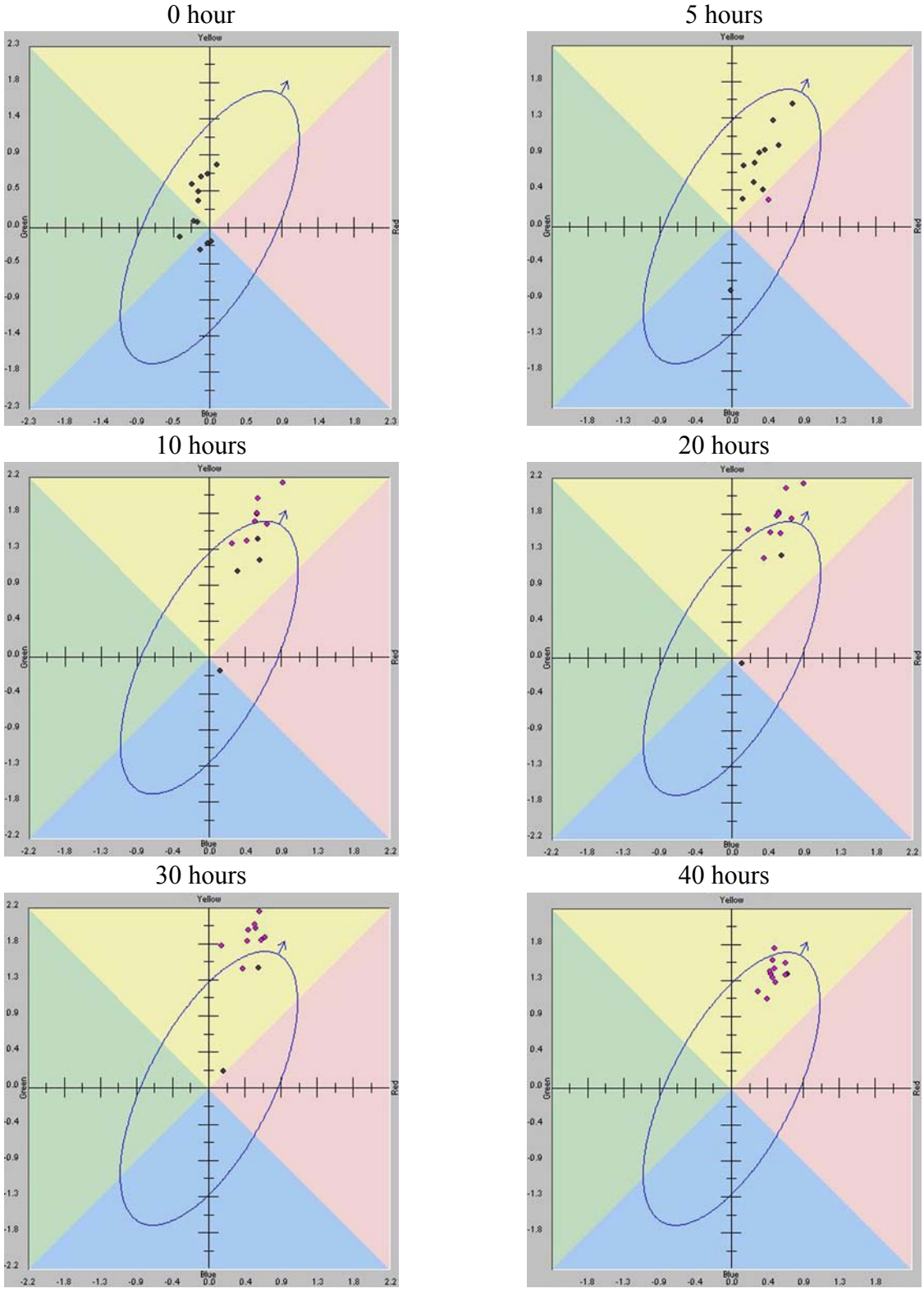


Figure B-4. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (protease treated)

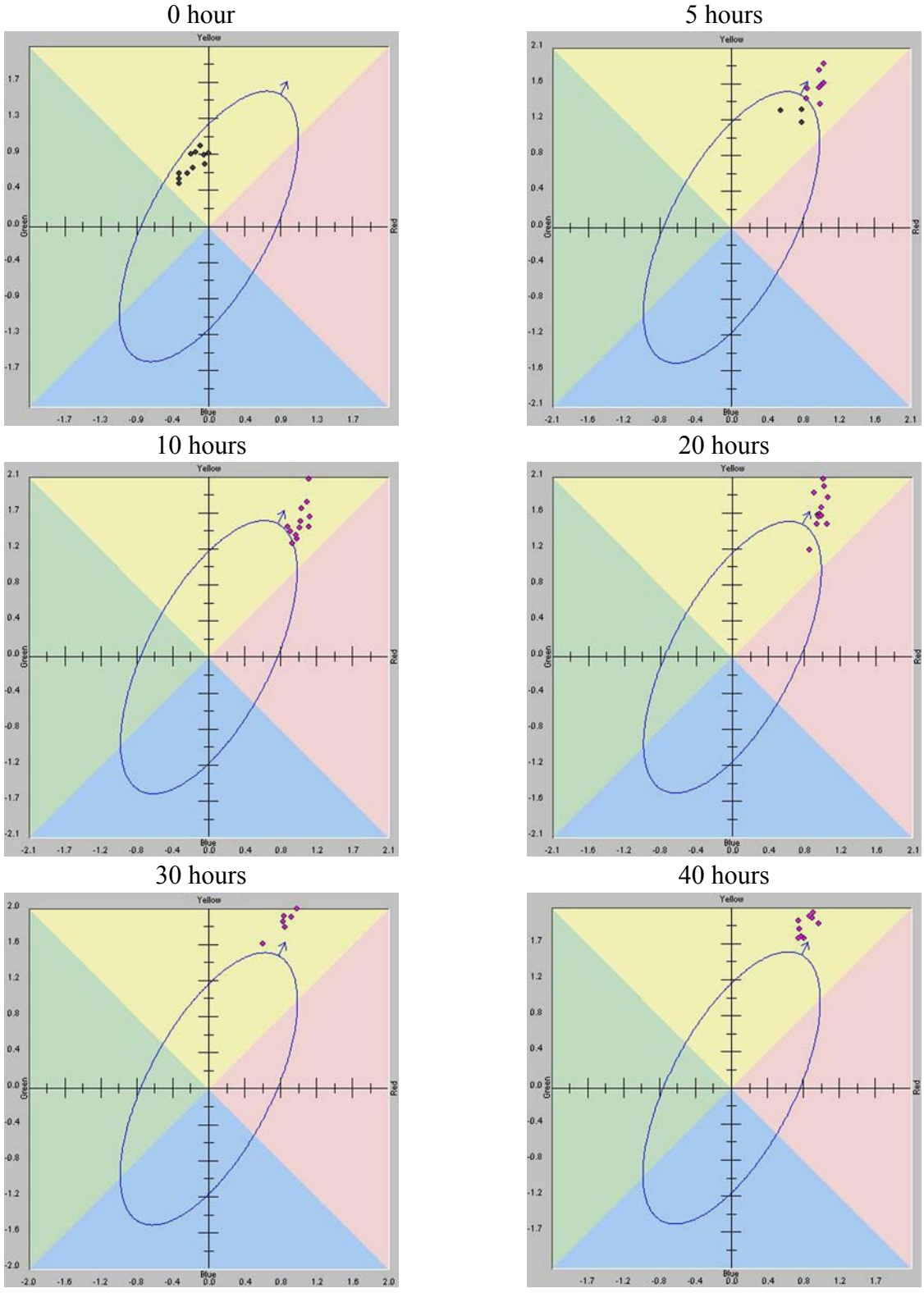


Figure B-5. Effect of UV exposure hours on hue differences of coyote brown colored cotton (amylase treated)

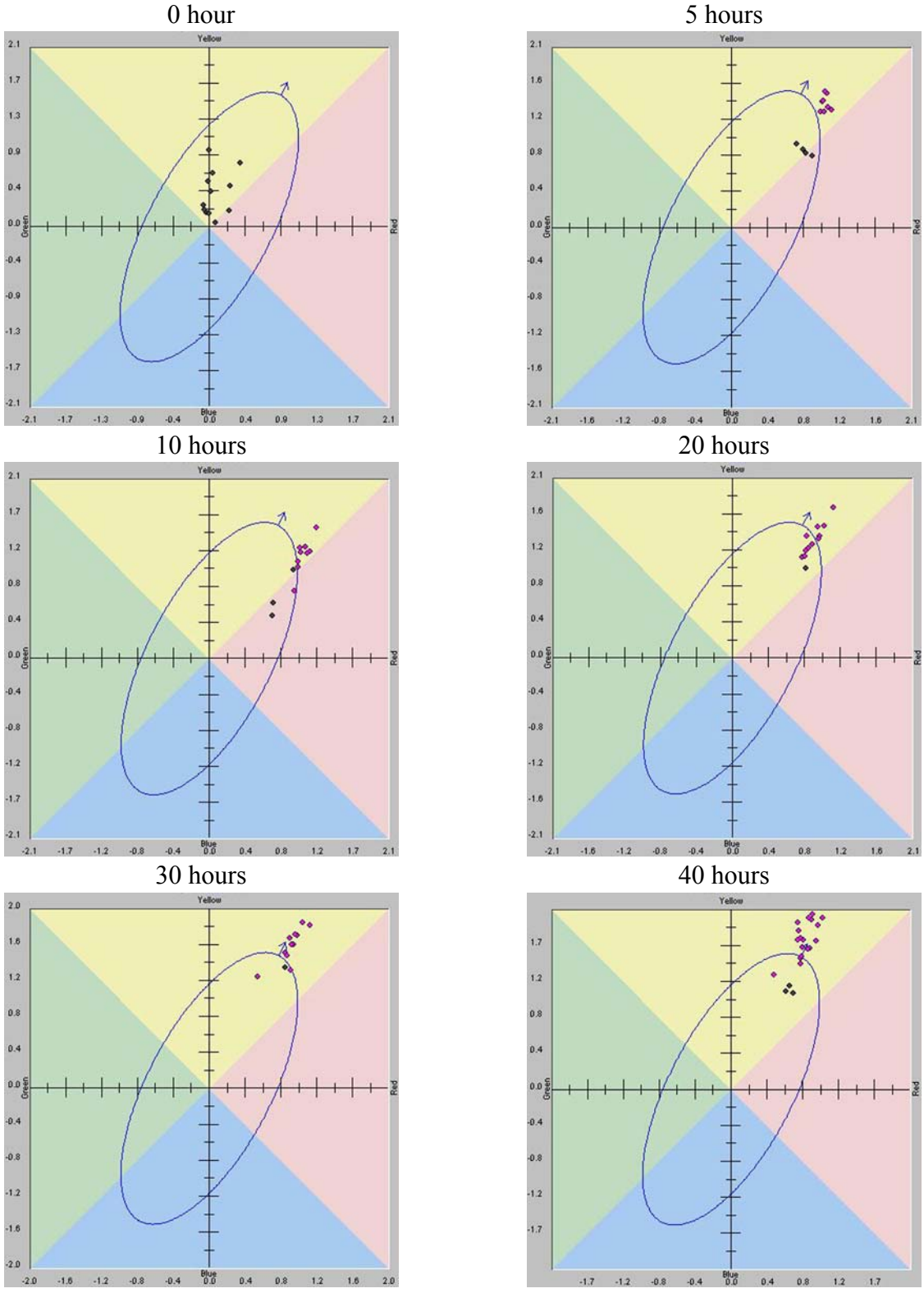


Figure B-6. Effect of UV exposure hours on hue differences of coyote brown colored cotton (cellulase treated)

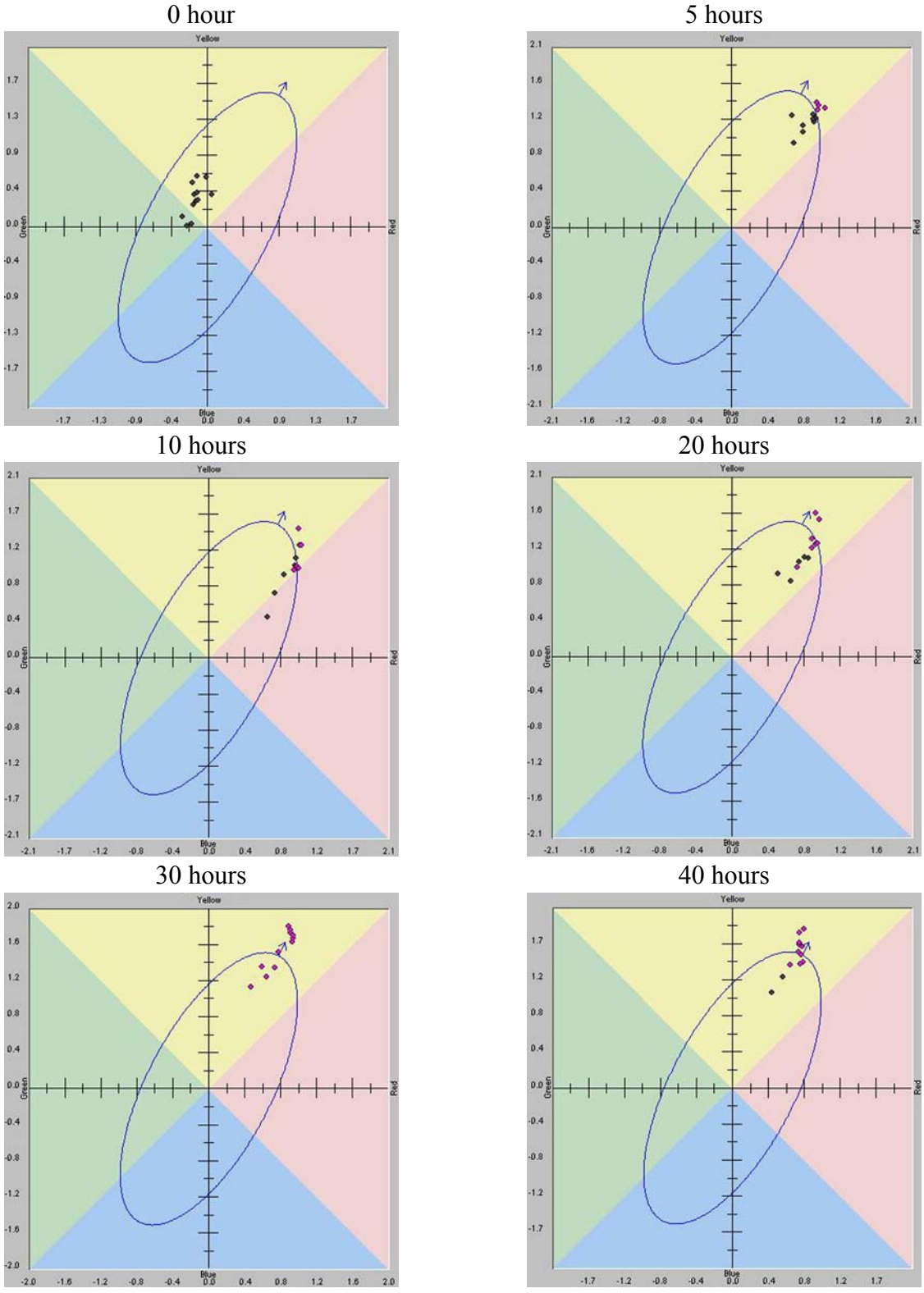


Figure B-7. Effect of UV exposure hours on hue differences of coyote brown colored cotton (lipase treated)

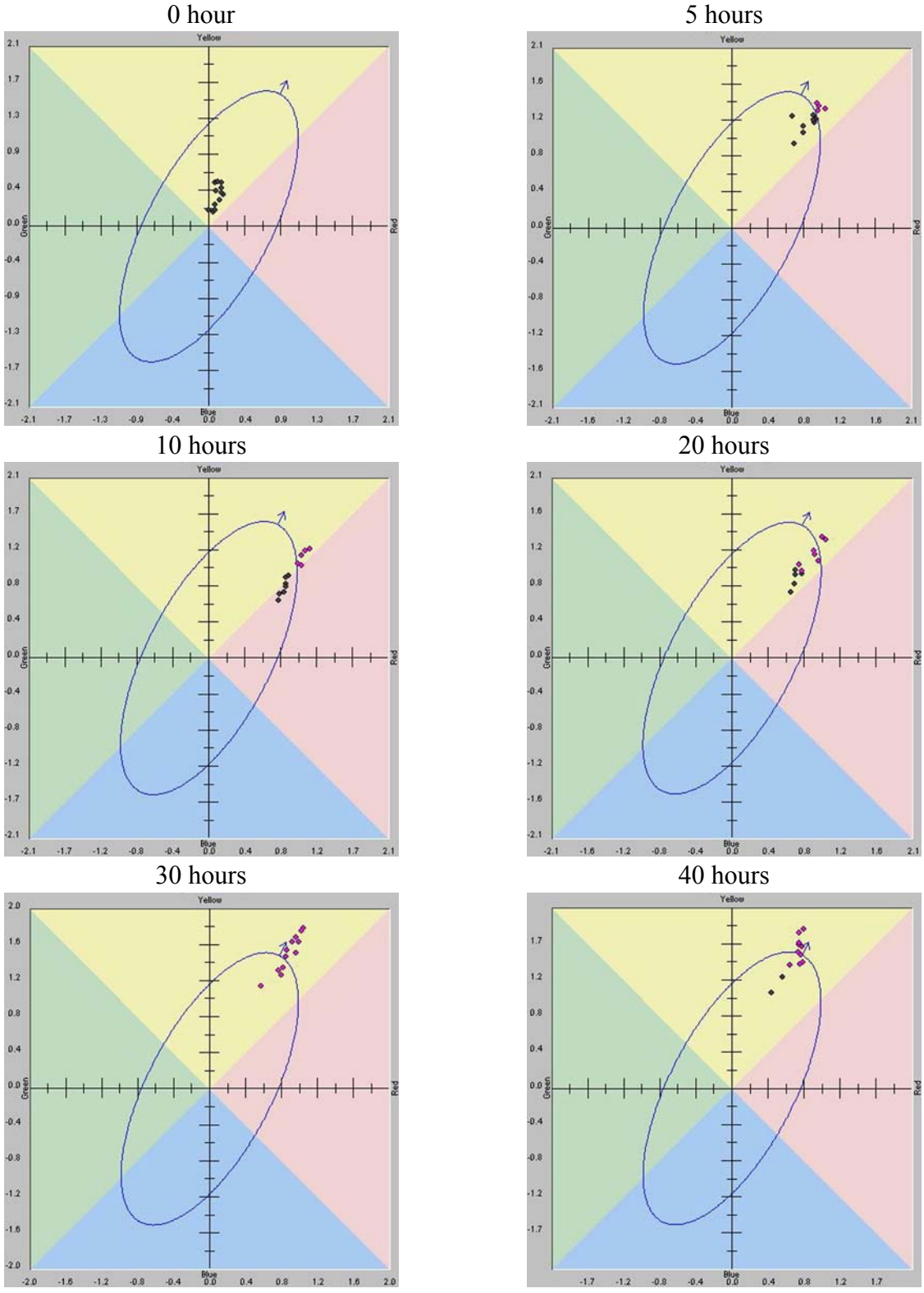


Figure B-8. Effect of UV exposure hours on hue differences of coyote brown colored cotton (protease treated)

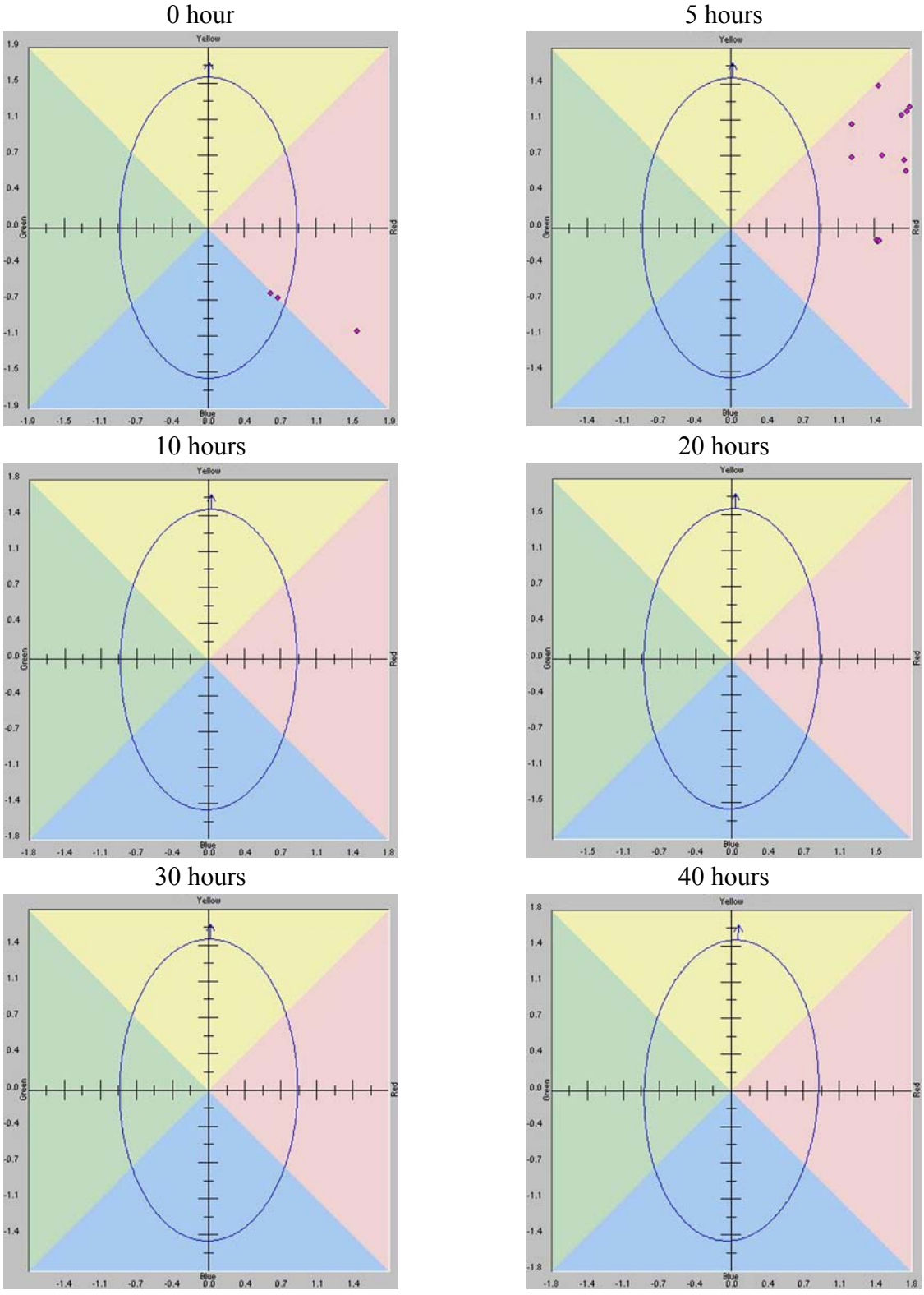


Figure B-9. Effect of UV exposure hours on hue differences of green colored cotton (amylase treated)

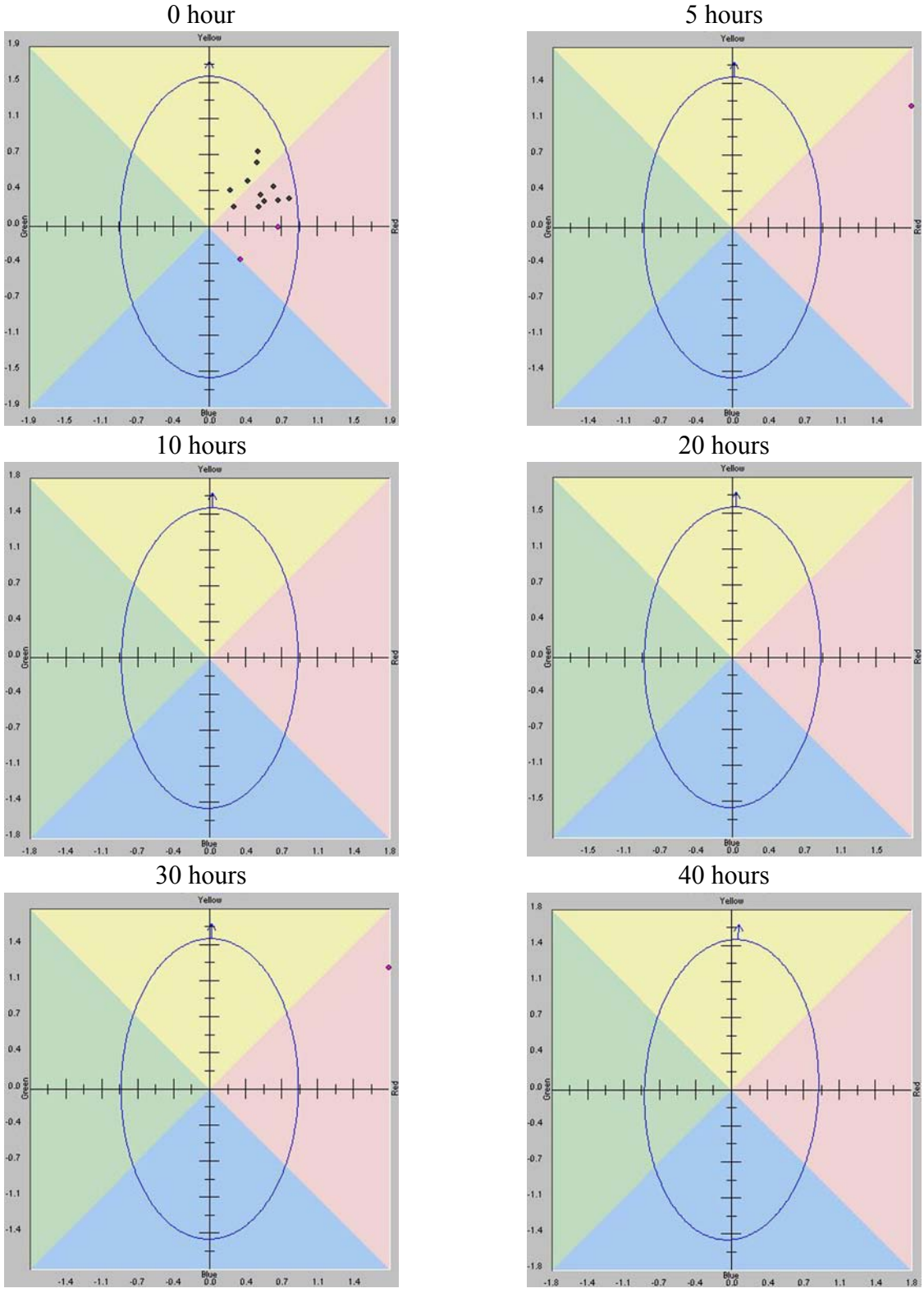


Figure B-10. Effect of UV exposure hours on hue differences of green colored cotton (cellulase treated)

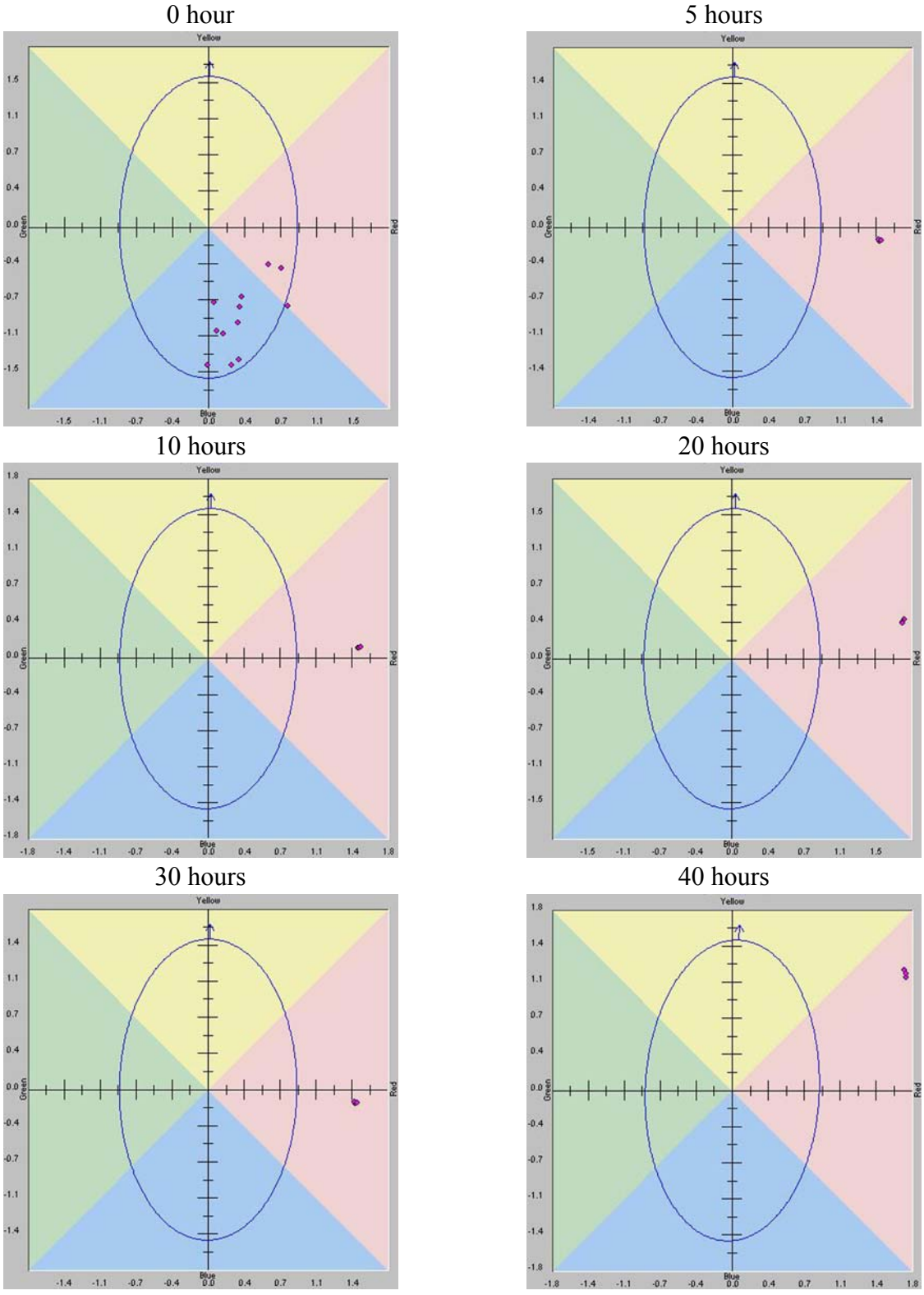


Figure B-11. Effect of UV exposure hours on hue differences of green colored cotton (lipase treated)

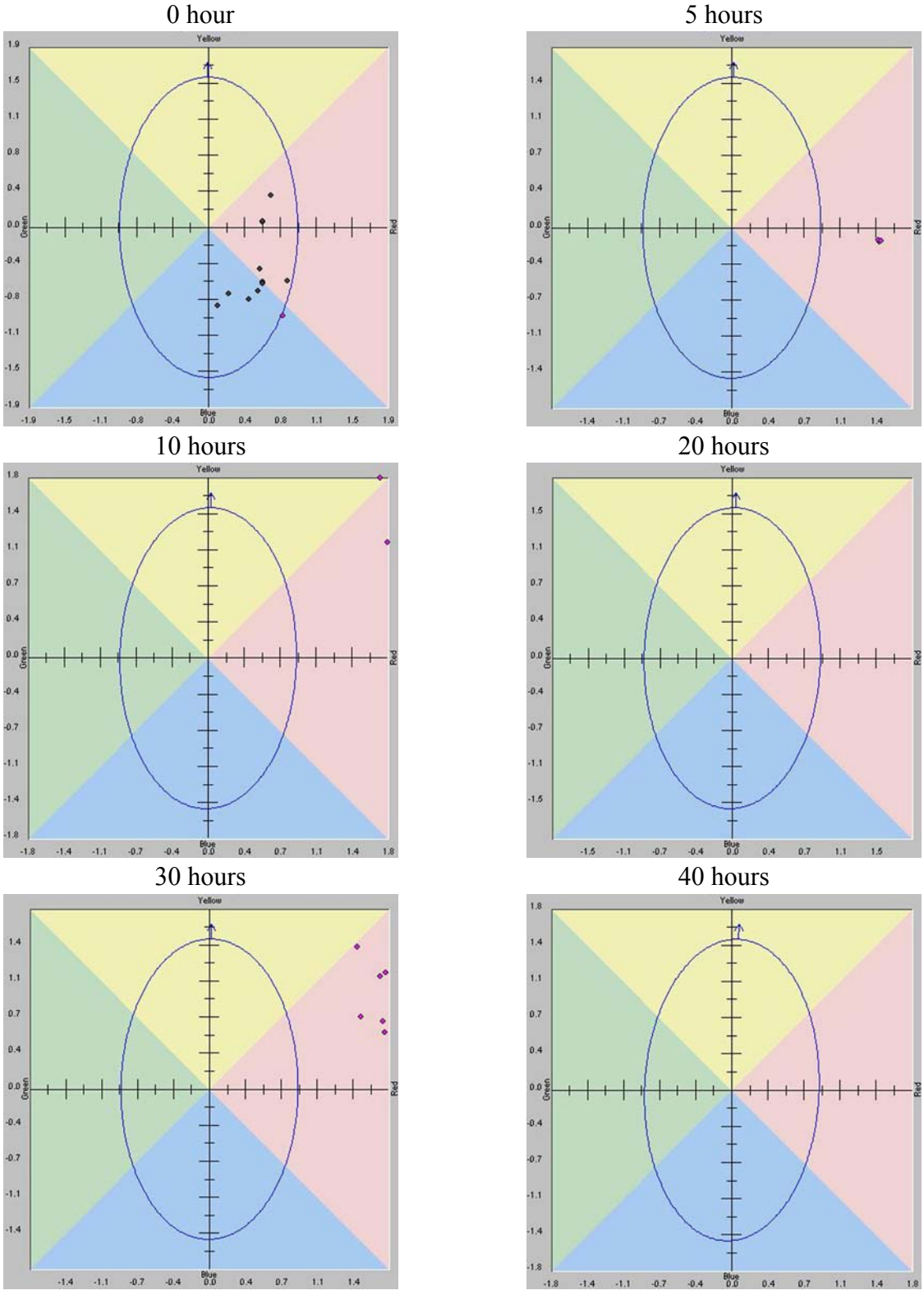


Figure B-12. Effect of UV exposure hours on hue differences of green colored cotton (protease treated)

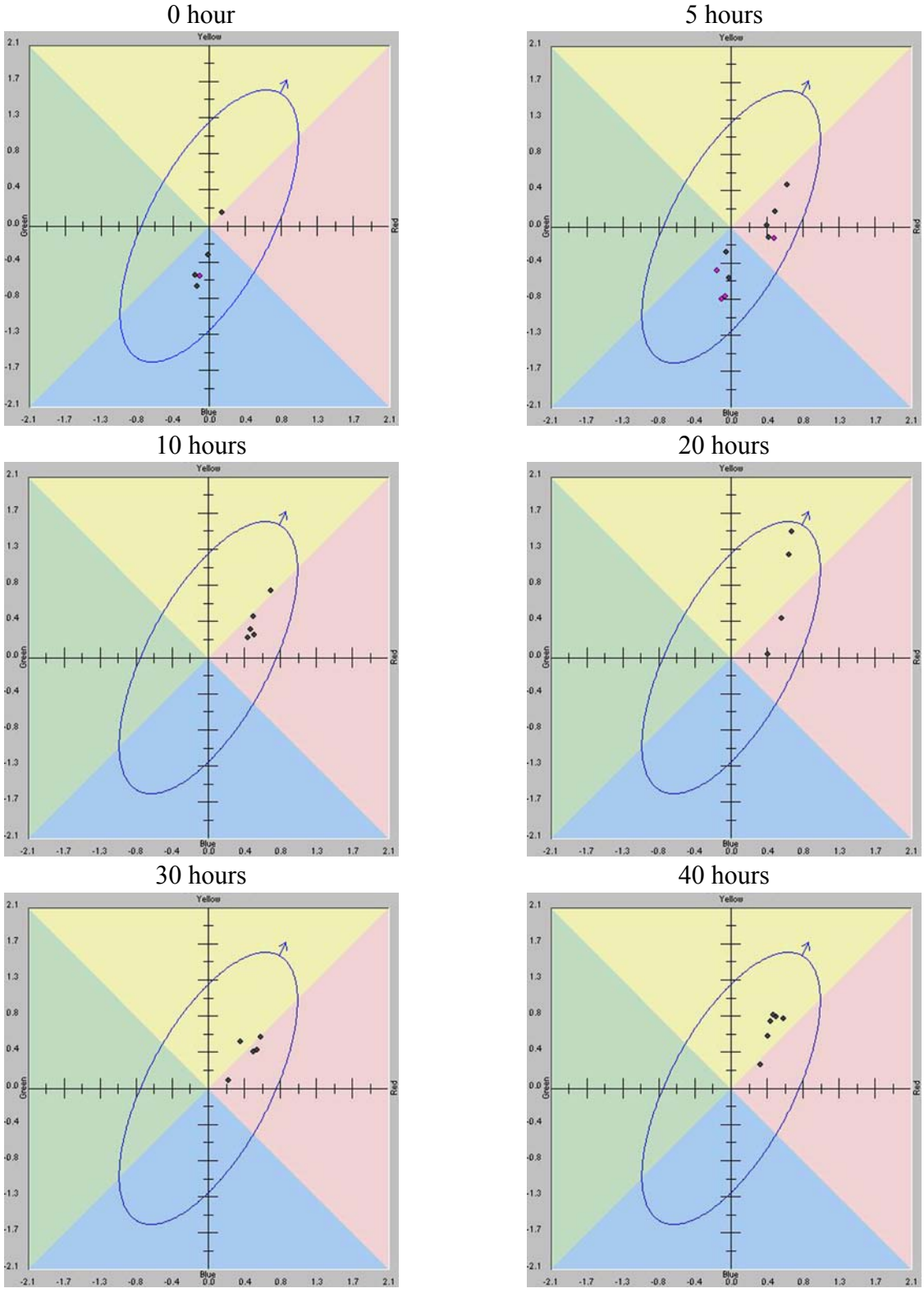


Figure B-13. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (amylase + cellulase treated)

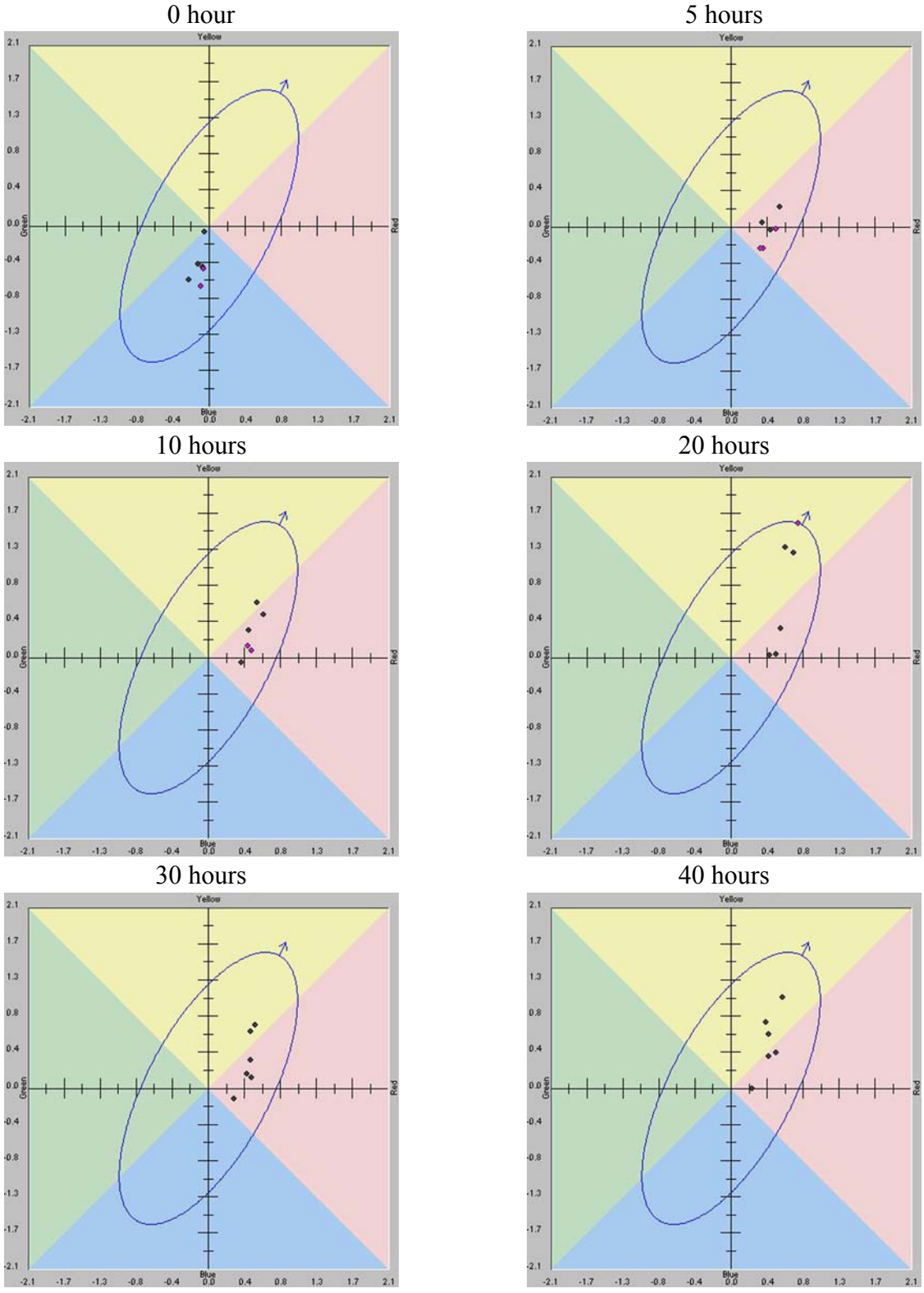


Figure B-14. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (amylase + lipase treated)

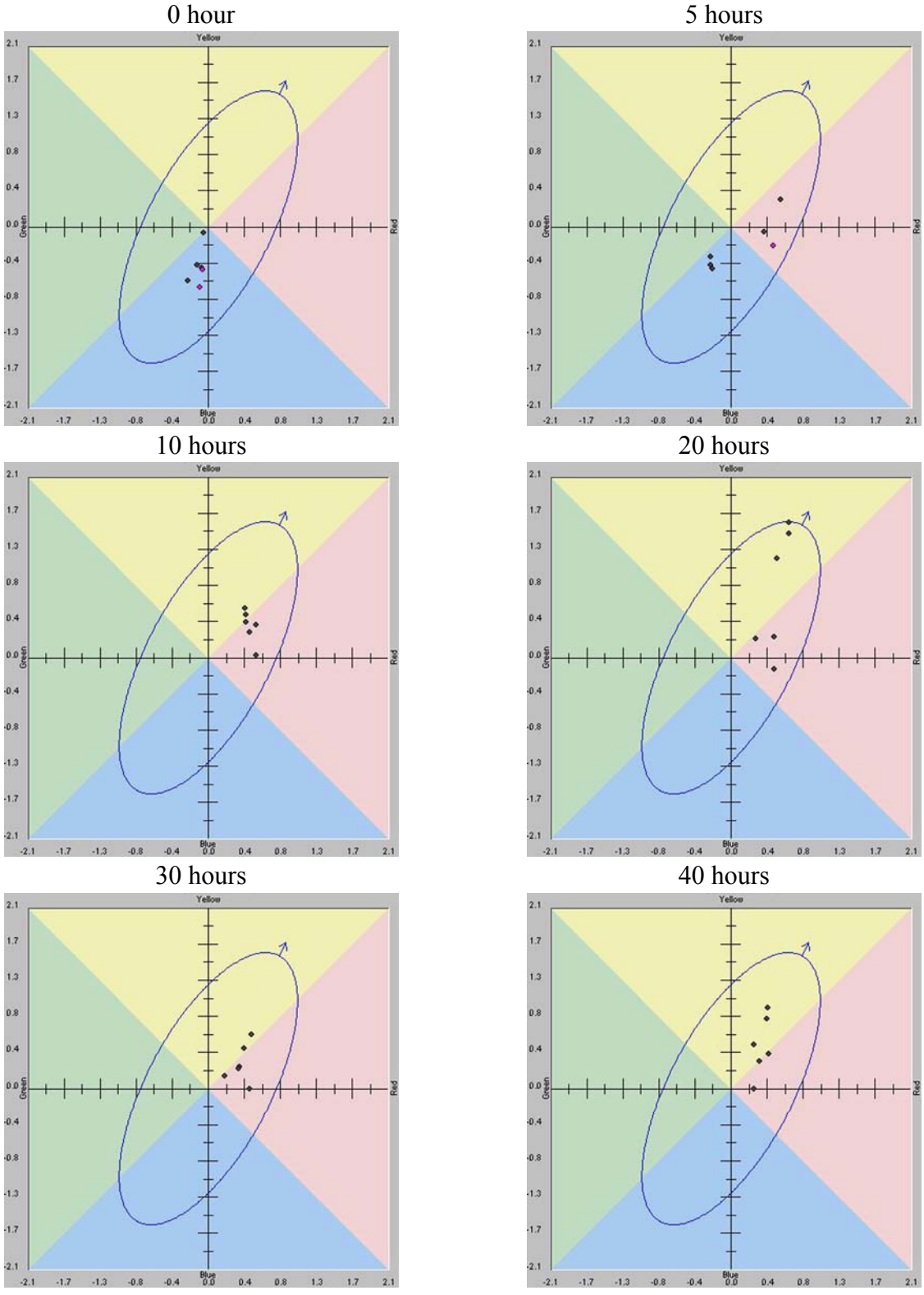


Figure B-15. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (amylase + protease treated)

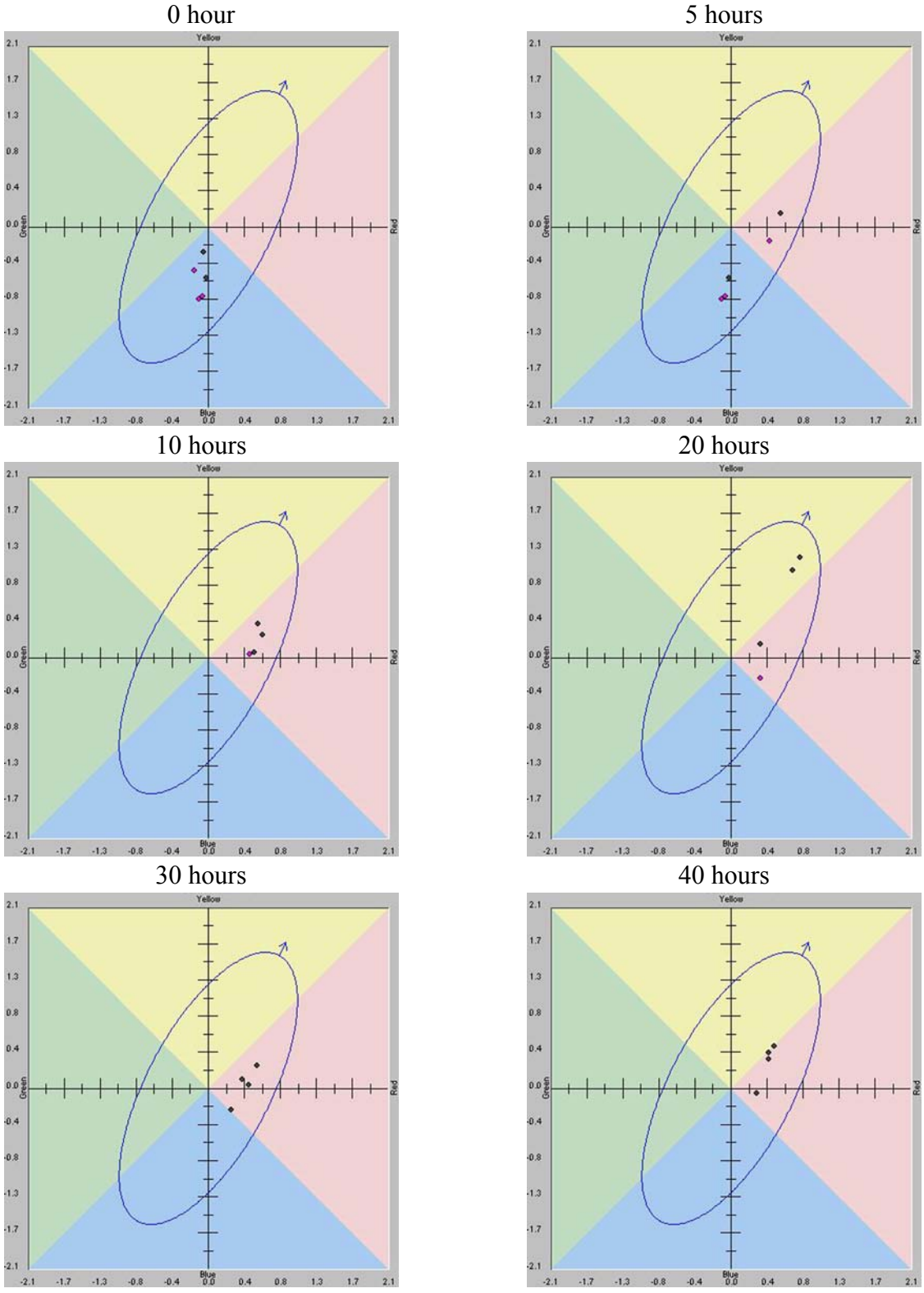


Figure B-16. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (cellulase + lipase treated)

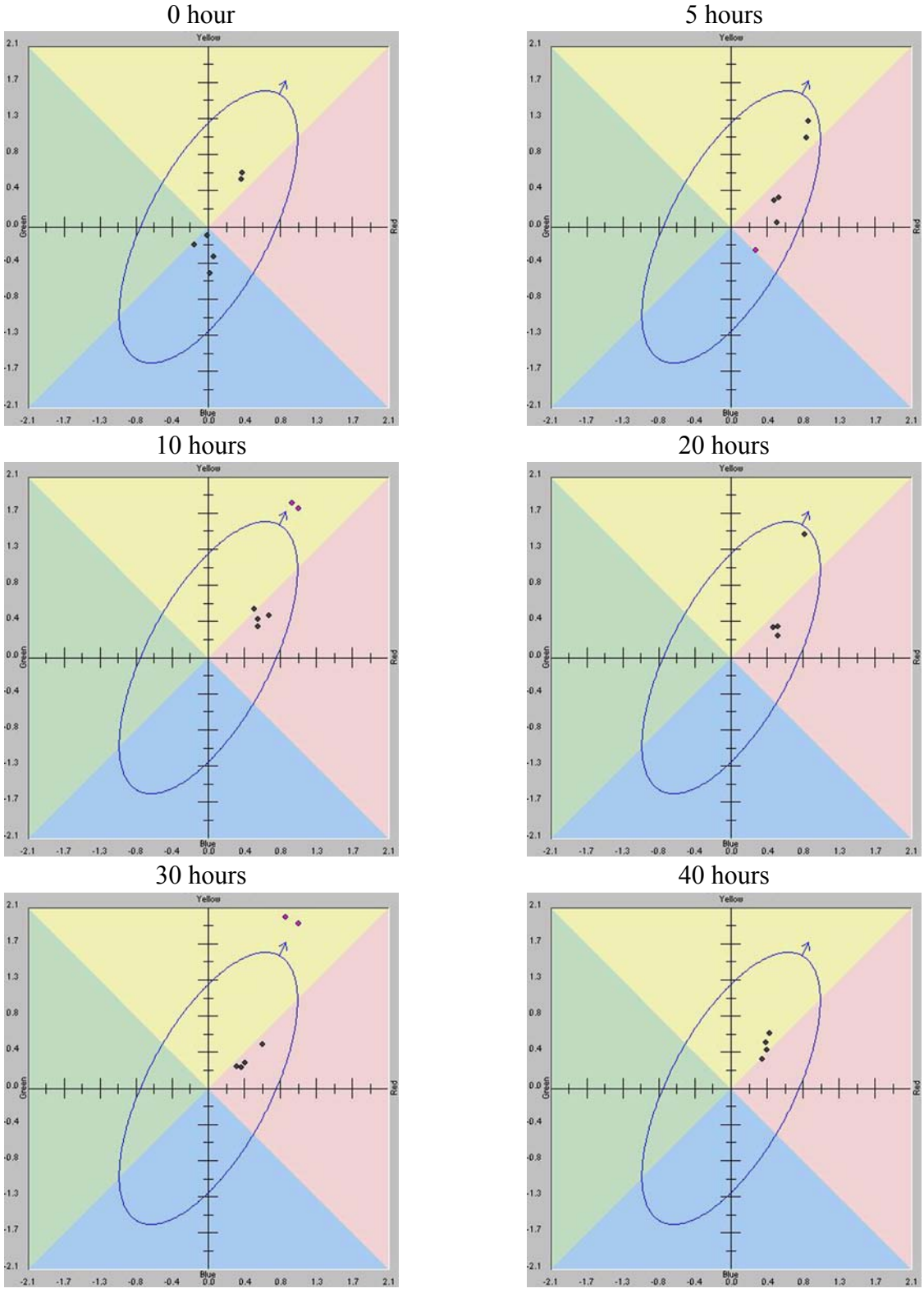


Figure B-17. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (cellulase + protease treated)

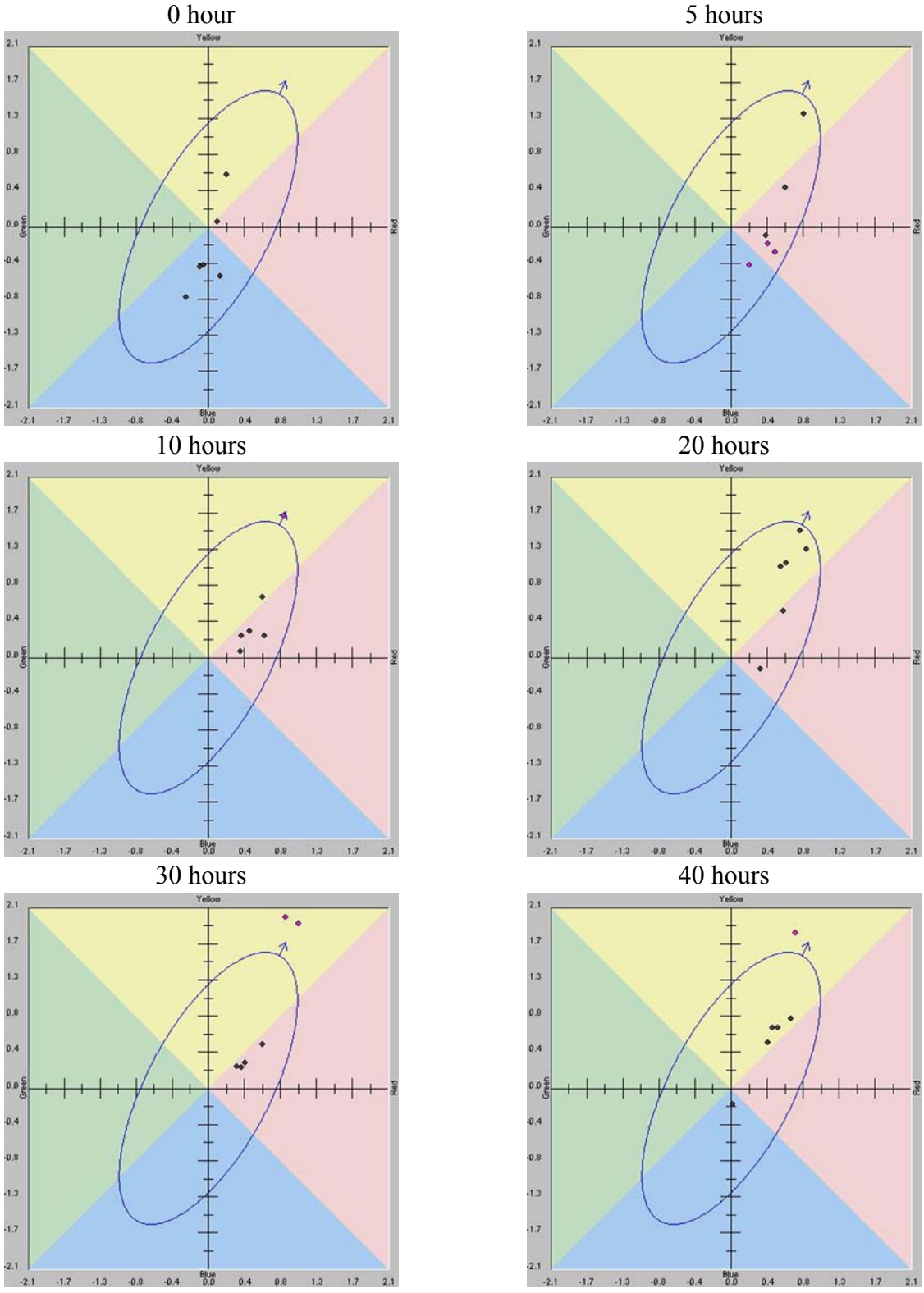


Figure B-18. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (lipase + protease treated)

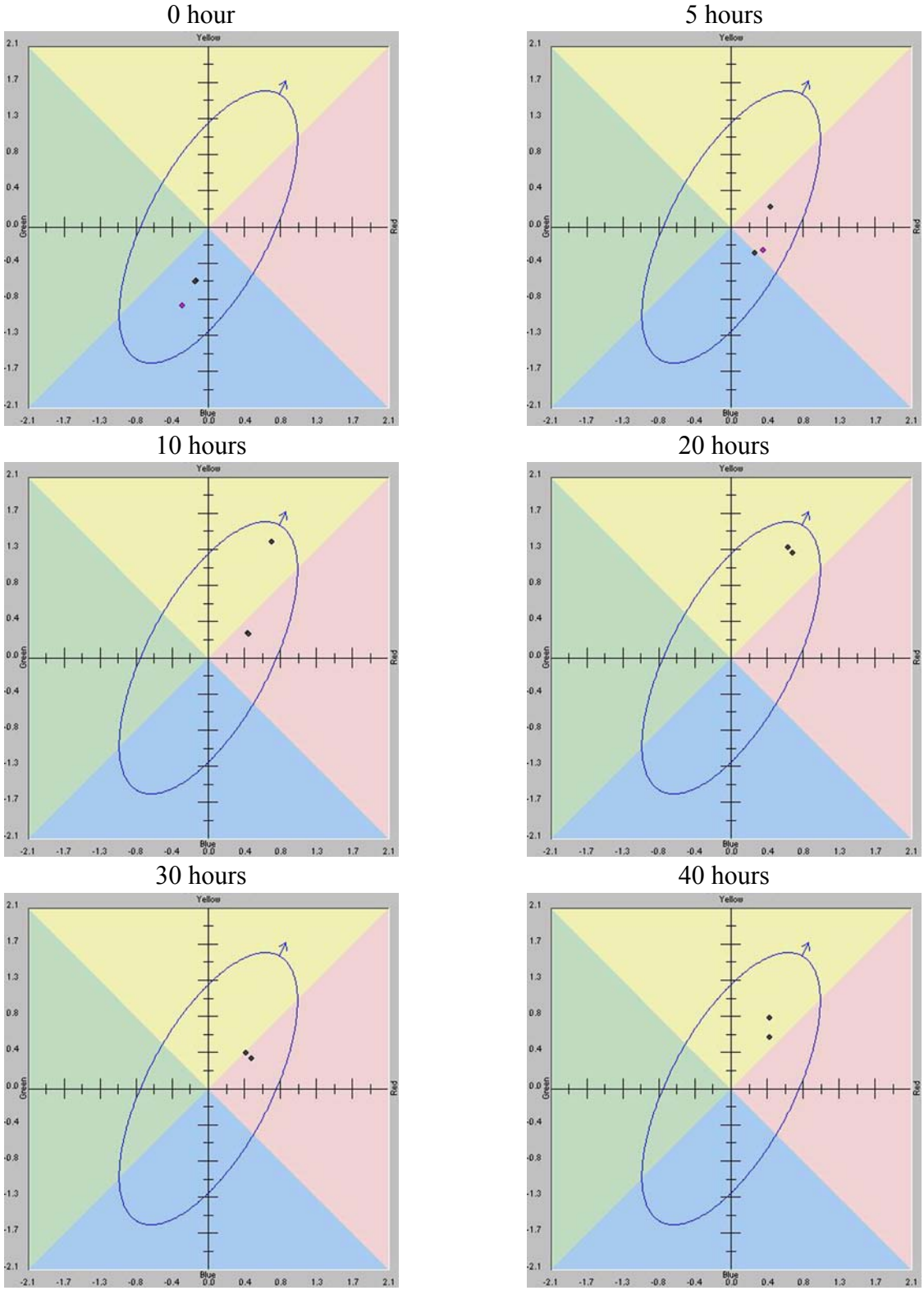


Figure B-19. Effect of UV exposure hours on hue differences of buffalo brown colored cotton (amylase + cellulase + lipase + protease treated)

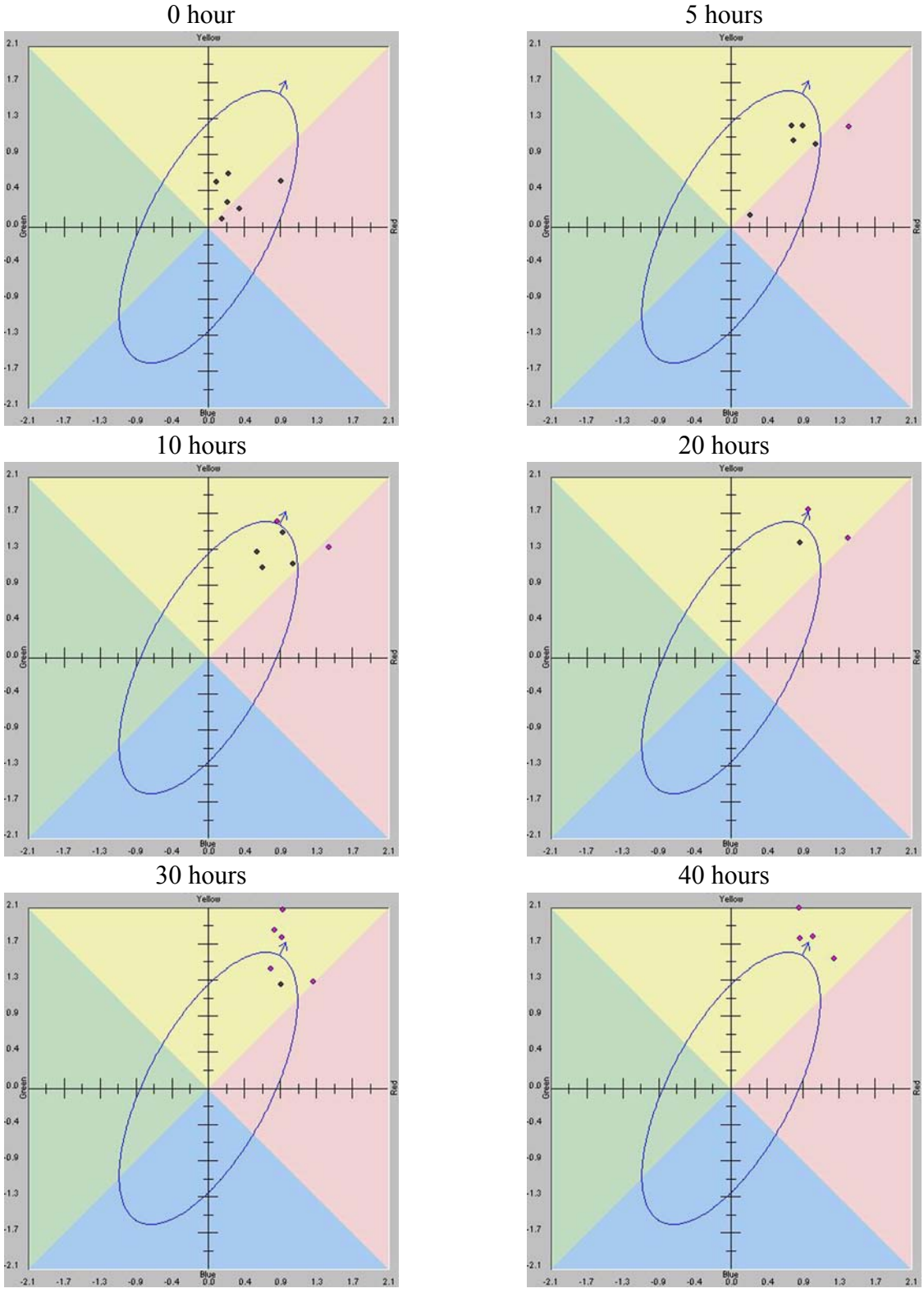


Figure B-20. Effect of UV exposure hours on hue differences of coyote brown colored cotton (amylase + cellulase treated)

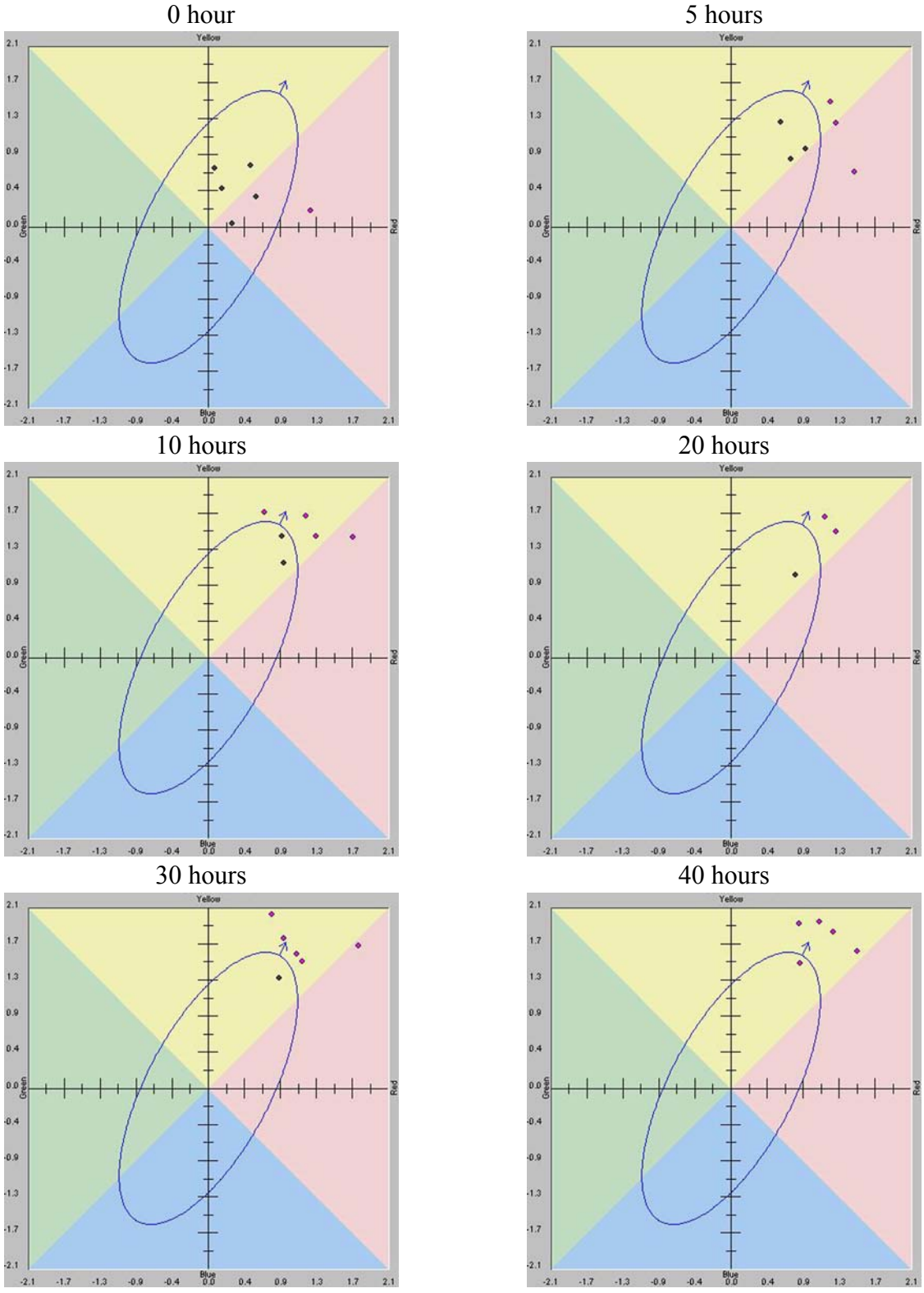


Figure B-21. Effect of UV exposure hours on hue differences of coyote brown colored cotton (amylase + lipase treated)

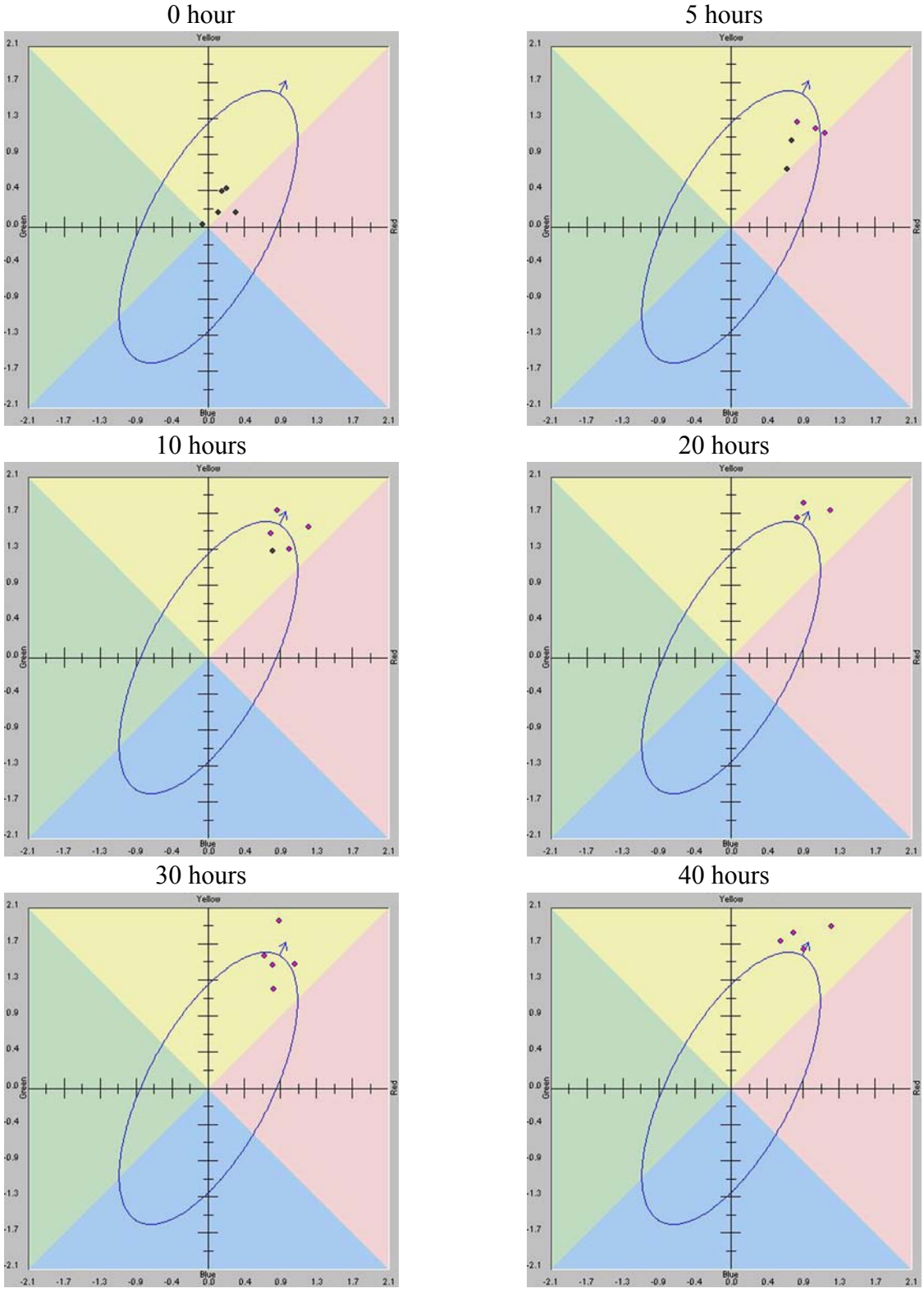


Figure B-22. Effect of UV exposure hours on hue differences of coyote brown colored cotton (amylase + protease treated)

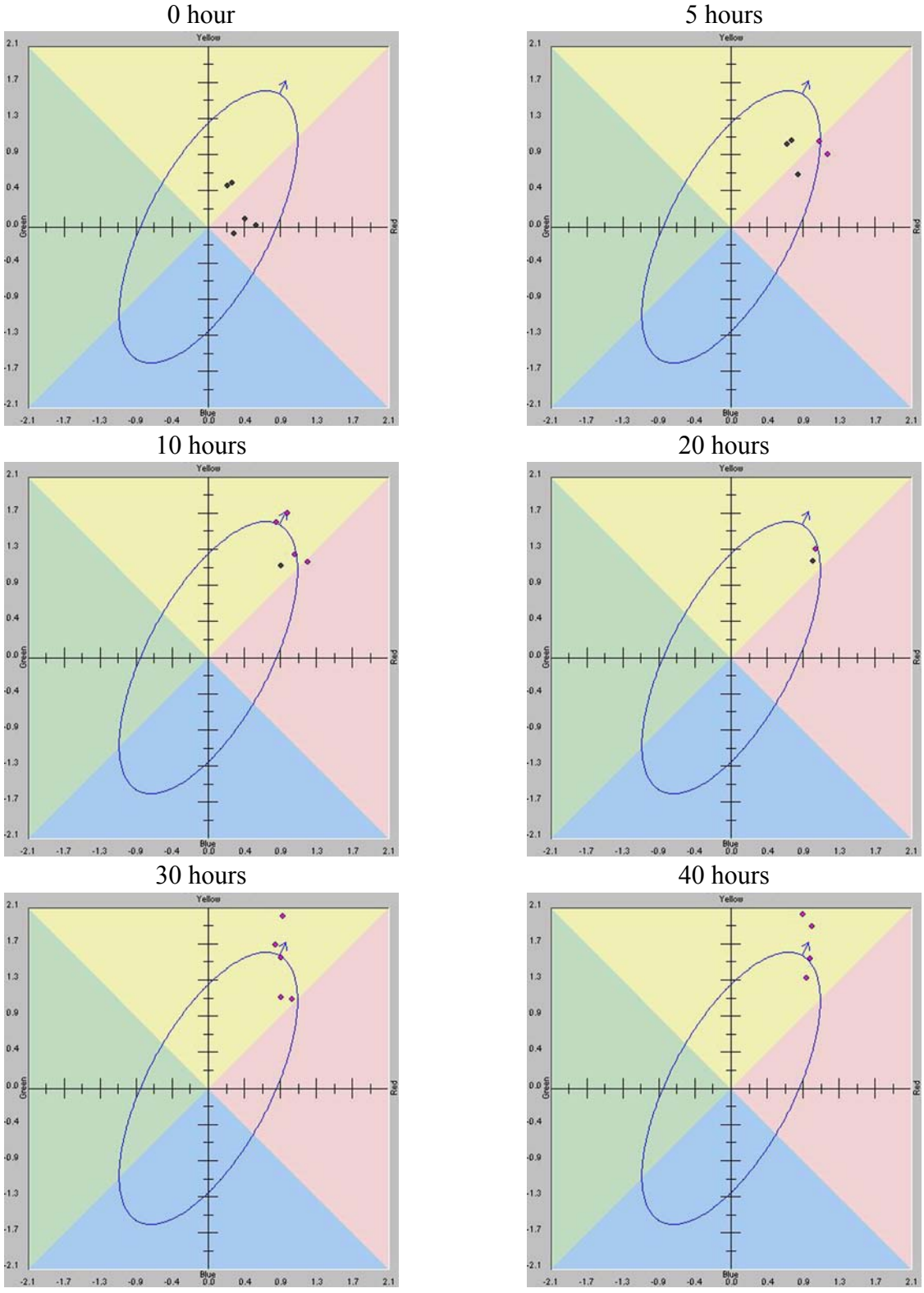


Figure B-23. Effect of UV exposure hours on hue differences of coyote brown colored cotton (cellulase + lipase treated)

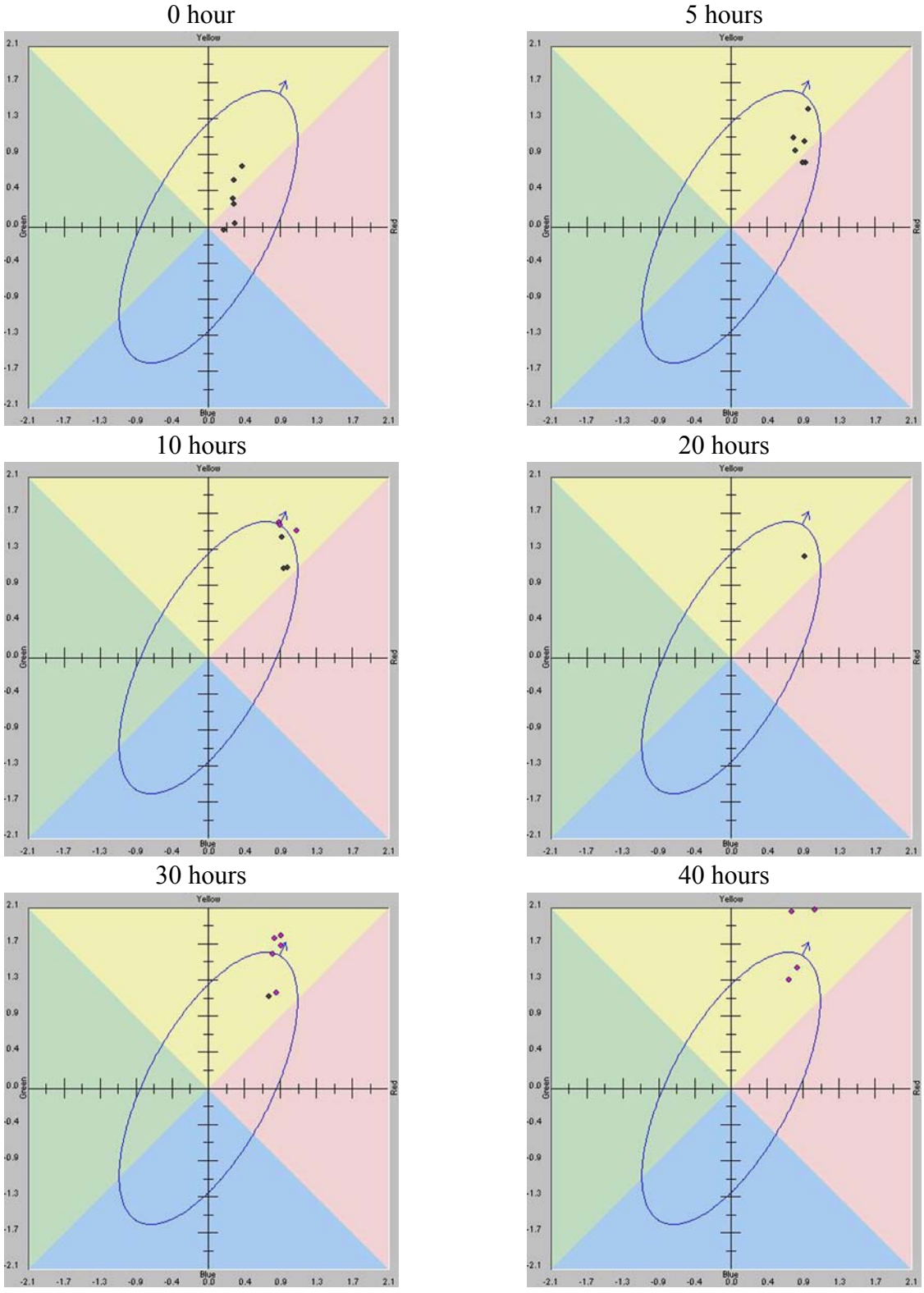


Figure B-24. Effect of UV exposure hours on hue differences of coyote brown colored cotton (cellulase + protease treated)

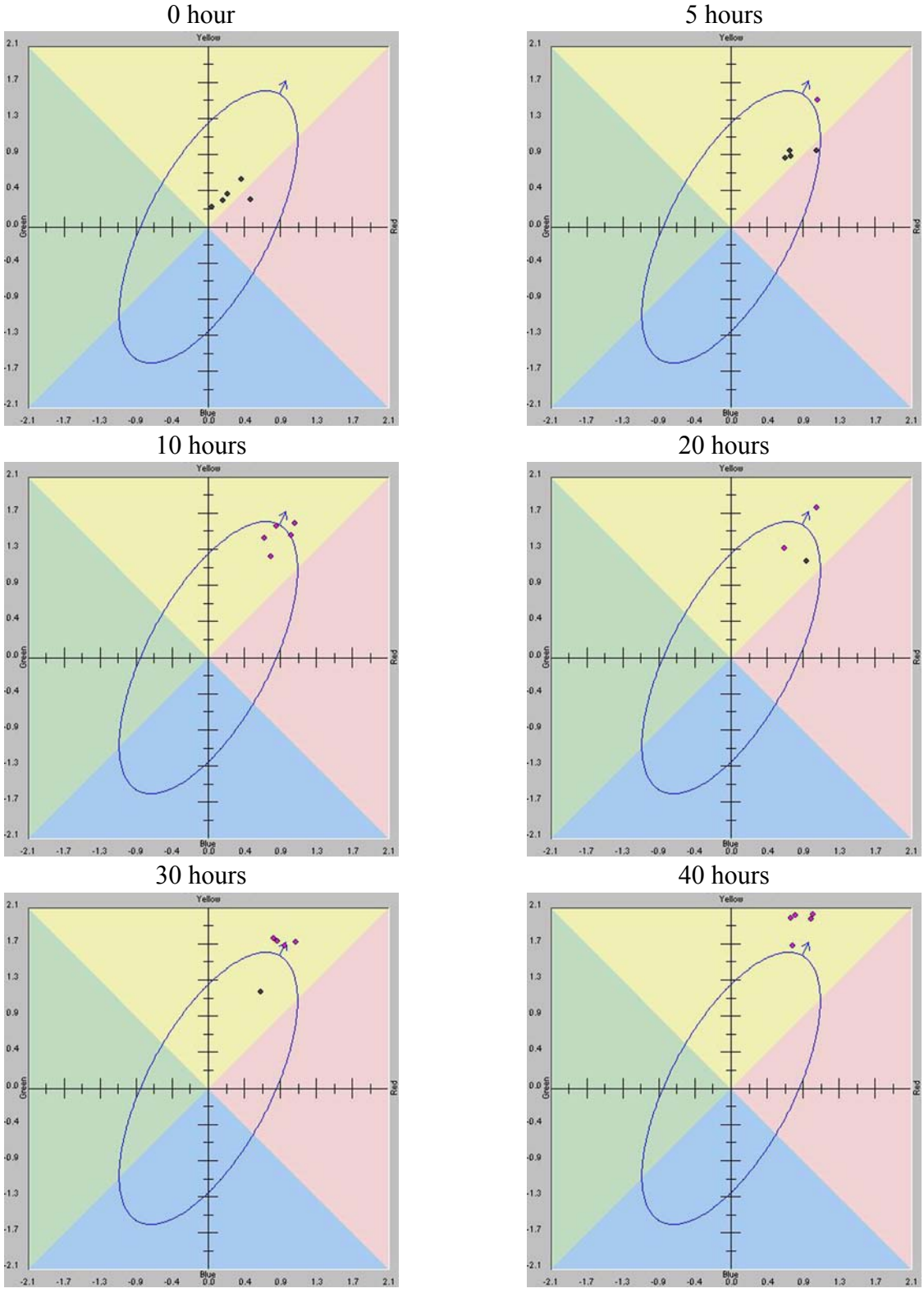


Figure B-25. Effect of UV exposure hours on hue differences of coyote brown colored cotton (lipase + protease treated)

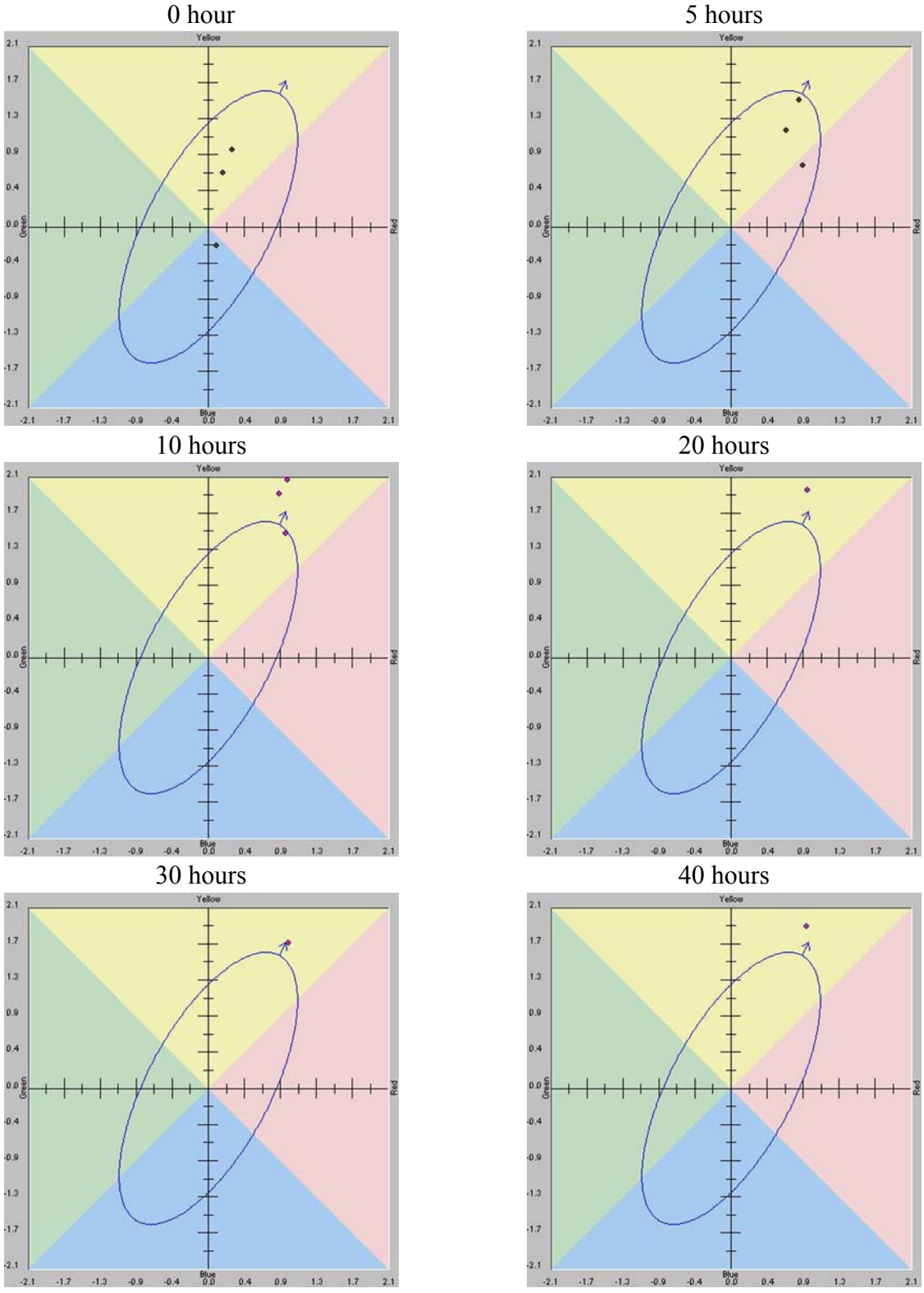


Figure B-26. Effect of UV exposure hours on hue differences of coyote brown colored cotton (amylase + cellulase + lipase + protease treated)

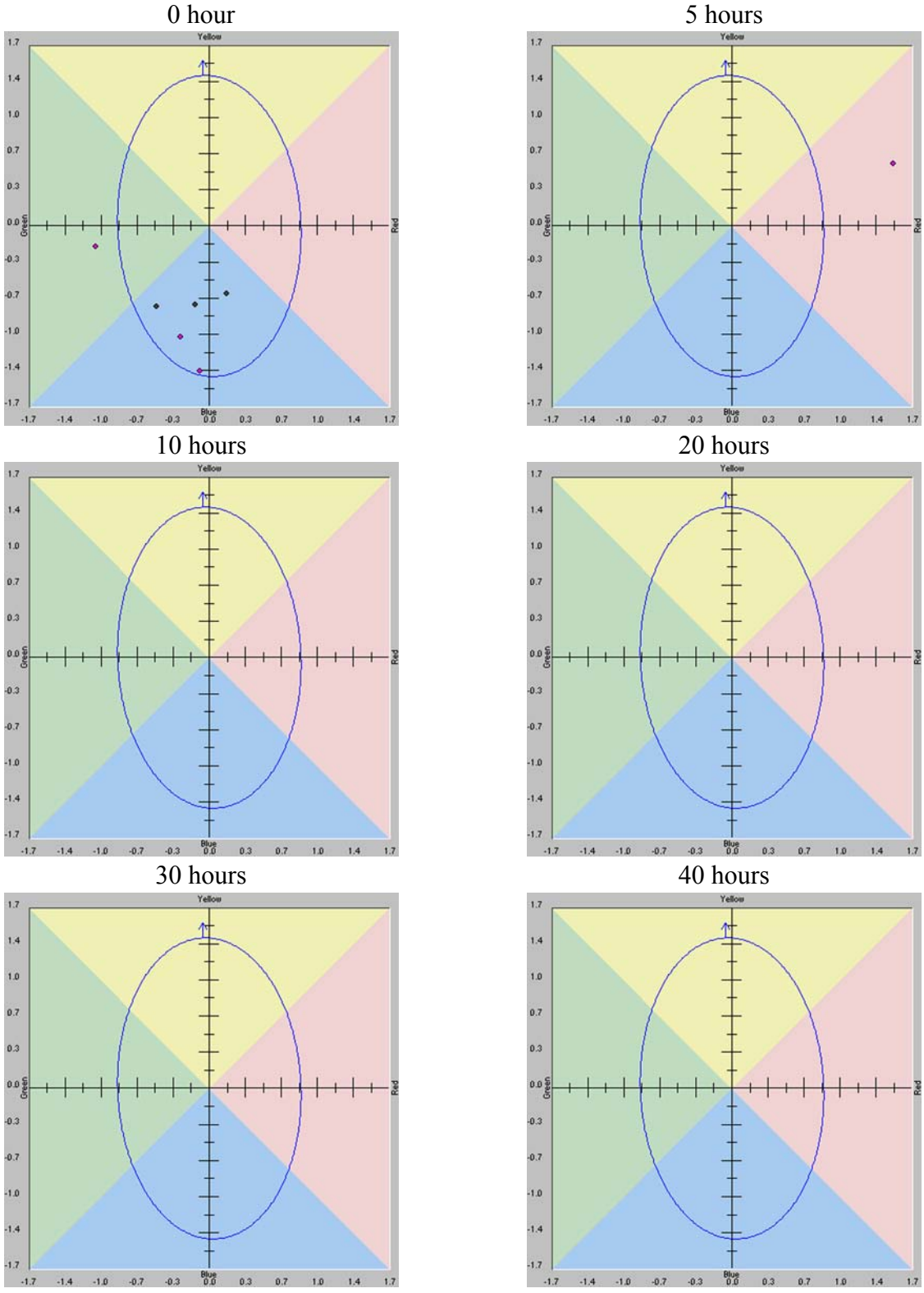


Figure B-27. Effect of UV exposure hours on hue differences of green colored cotton (amylase + cellulase treated)

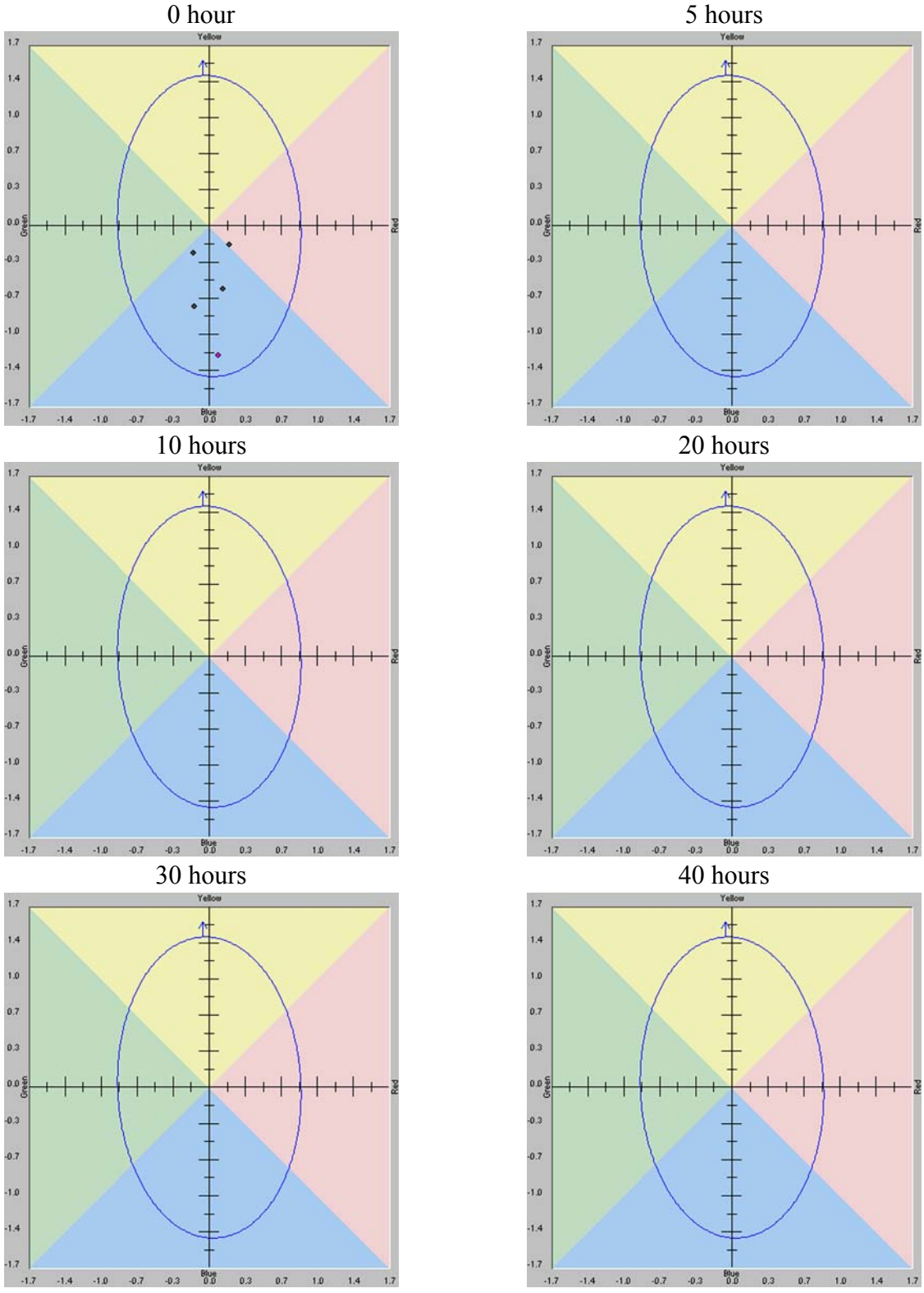


Figure B-28. Effect of UV exposure hours on hue differences of green colored cotton (amylase + lipase treated)

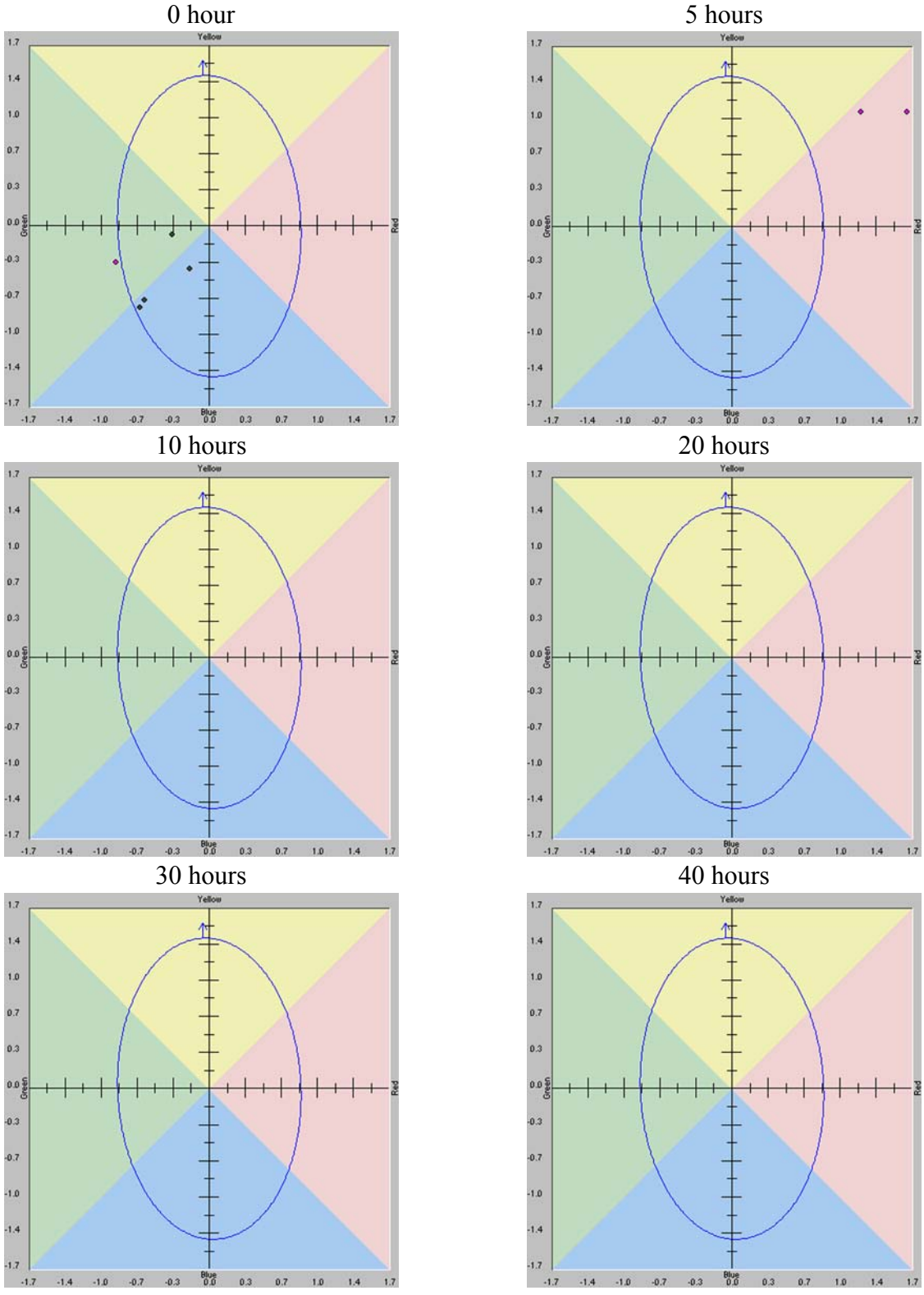


Figure B-29. Effect of UV exposure hours on hue differences of green colored cotton (amylase + protease treated)

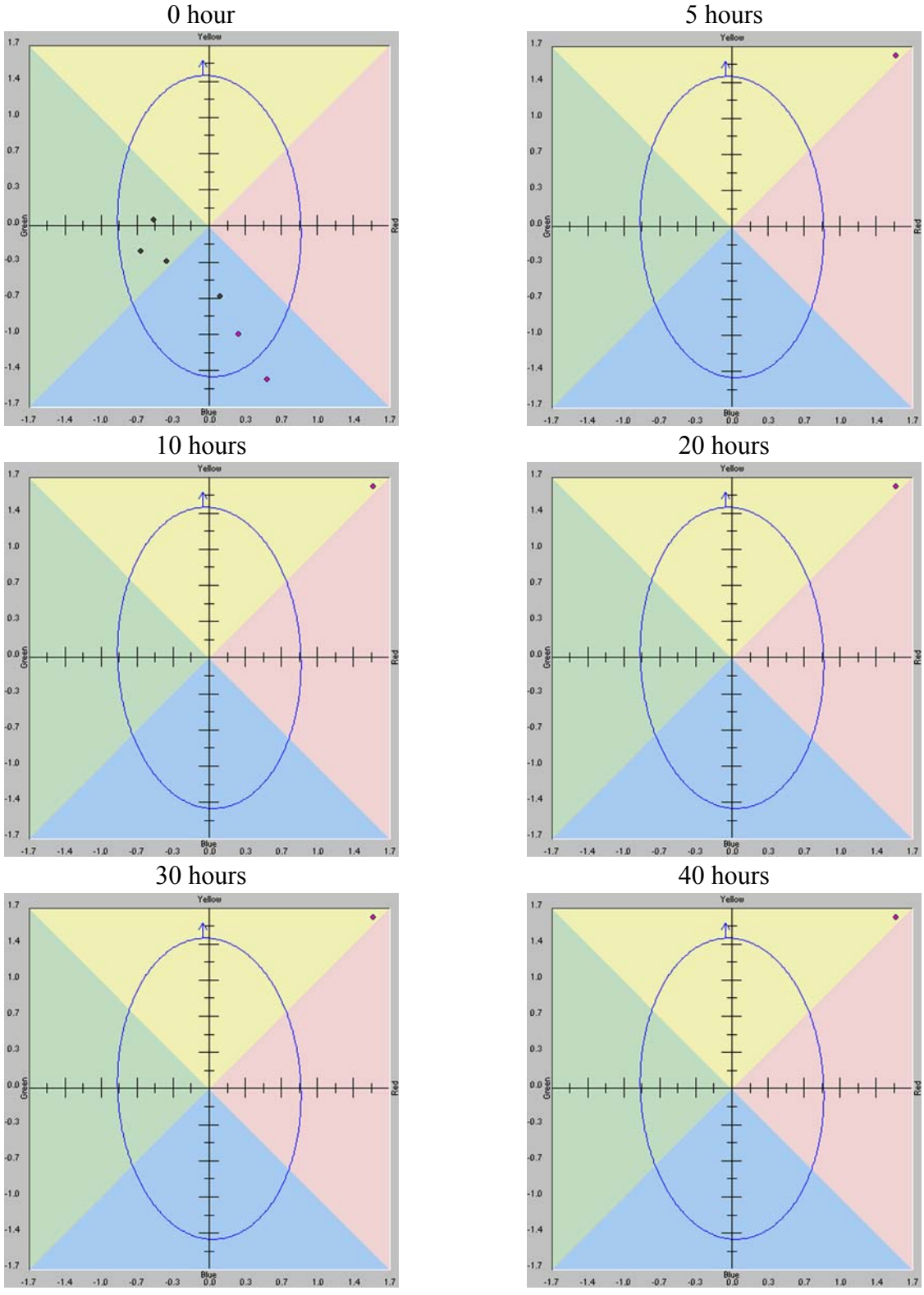


Figure B-30. Effect of UV exposure hours on hue differences of green colored cotton (cellulase + lipase treated)

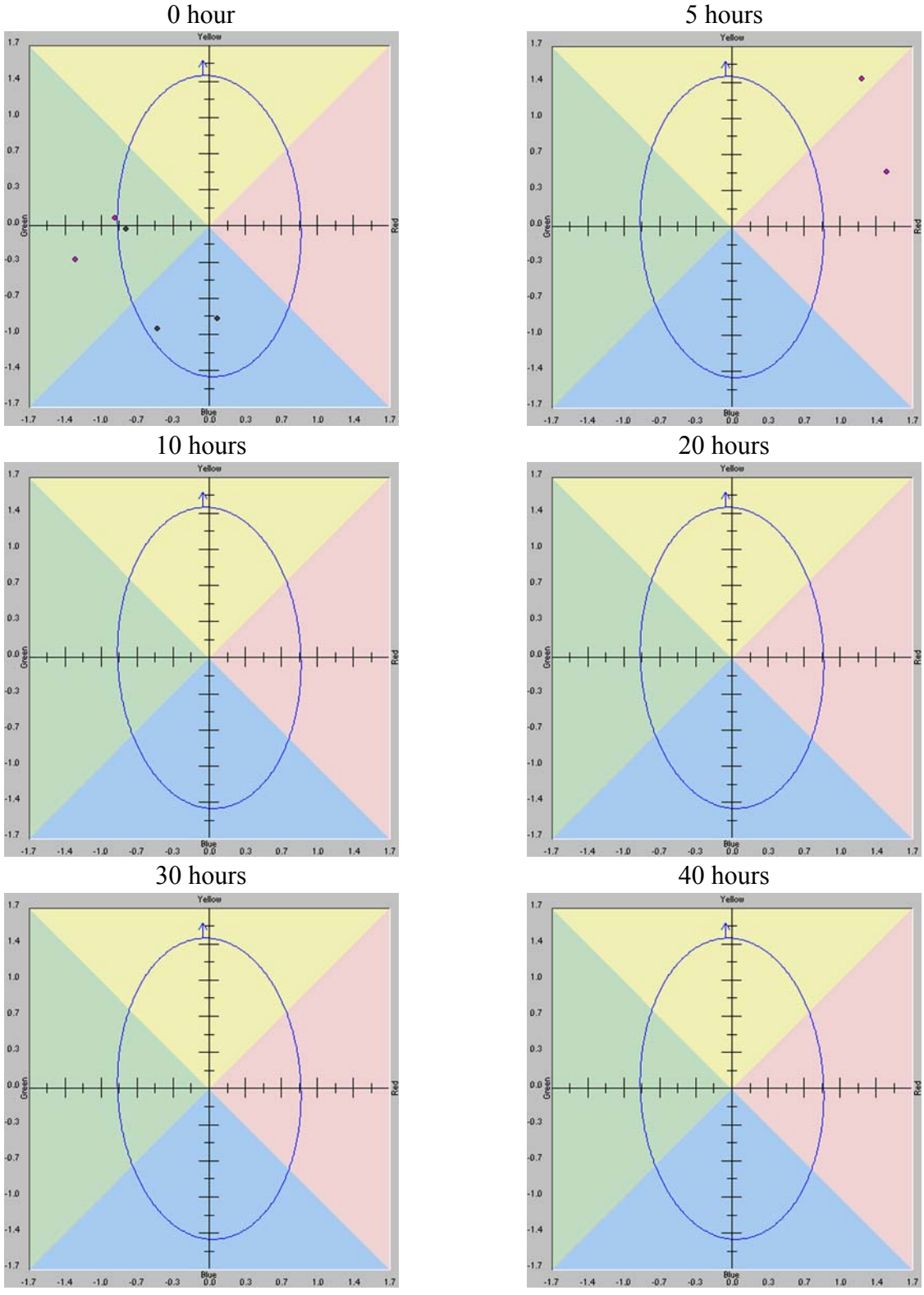


Figure B-31. Effect of UV exposure hours on hue differences of green colored cotton (cellulase + protease treated)

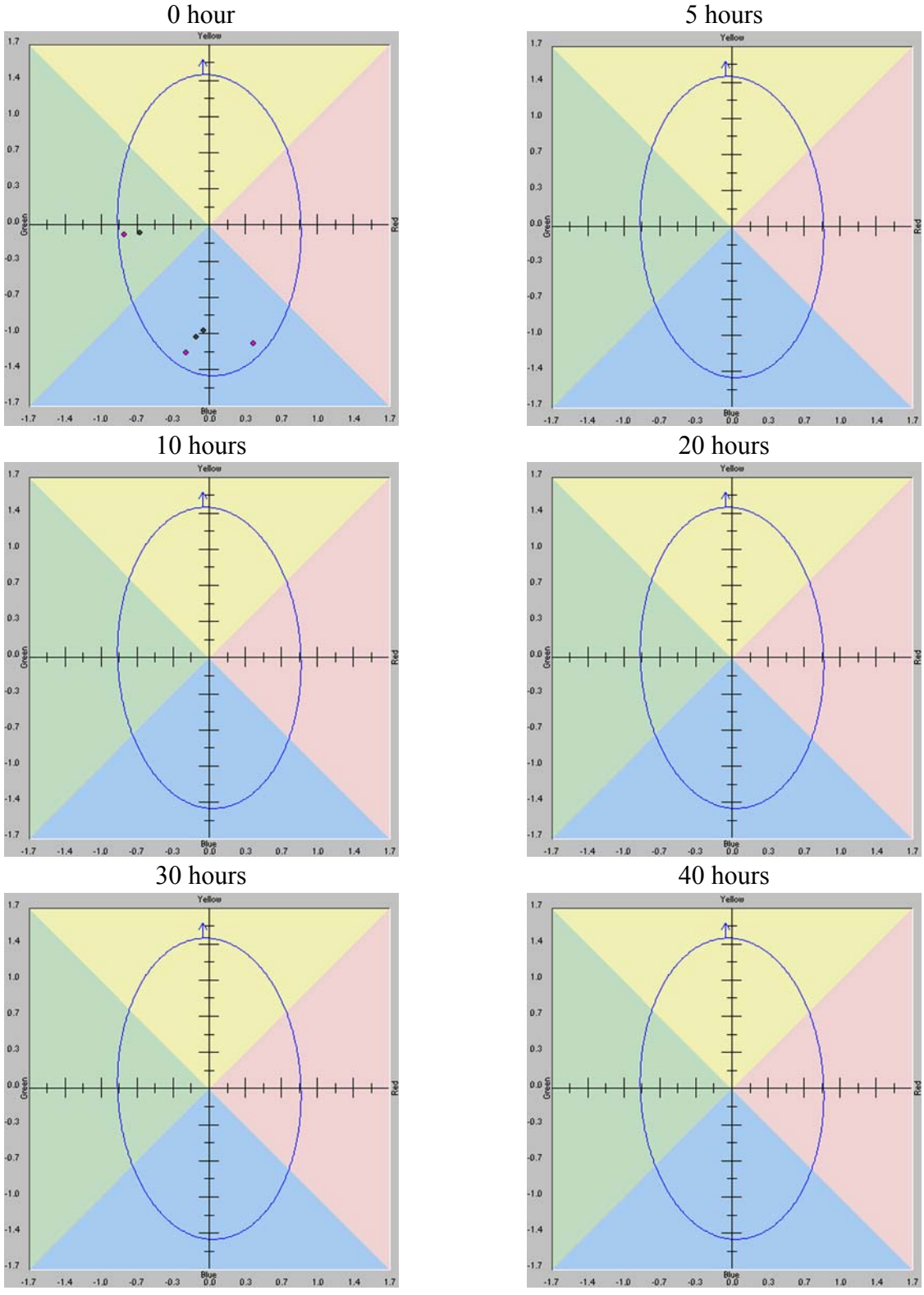


Figure B-32. Effect of UV exposure hours on hue differences of green colored cotton (lipase + protease treated)

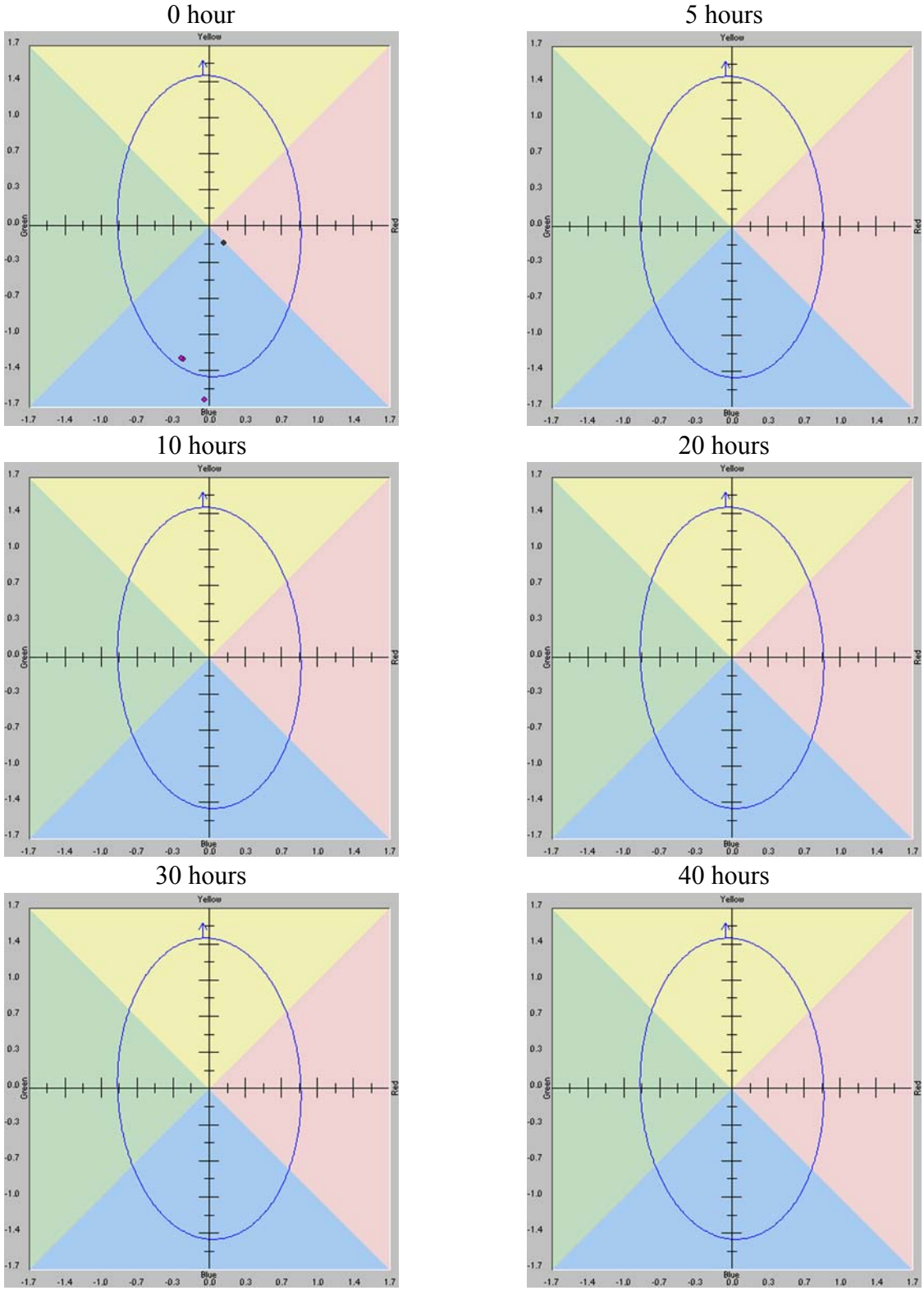


Figure B-33. Effect of UV exposure hours on hue differences of green colored cotton (amylase + cellulase + lipase + protease treated)

Appendix C

Statistical Analysis of Moisture regain (%) of Naturally Colored Cotton after Enzyme Treatment

Moisture regain (%) changes of naturally colored cotton after scouring and enzyme treatment

Table 5. Moisture Regain of Buffalo Brown Colored Cotton (%)

	Enzyme (%)	Moisture Regain (%)
Raw Cotton	-	6.991
Scoured Cotton	-	7.491
Amylase	0.2	8.325
	0.5	8.361
	1.0	8.300
	1.5	8.445
Cellulase	0.2	8.886
	0.5	8.729
	1.0	8.648
	1.5	8.575
Lipase	0.2	8.569
	0.5	8.547
	1.0	8.374
	1.5	8.238
Protease	0.2	8.830
	0.5	8.633
	1.0	8.412
	1.5	8.429

Amylase + Cellulase	0.5	9.753
	1.0	10.027
Amylase + Lipase	0.5	10.277
	1.0	10.136
Amylase + Protease	0.5	10.307
	1.0	9.923
Cellulase + Lipase	0.5	10.432
	1.0	9.192
Cellulase + Protease	0.5	10.218
	1.0	9.466
Lipase + Protease	0.5	9.851
	1.0	9.835
Amylase + Cellulase + Lipase + Protease	0.5	8.561
	1.0	7.285

Table 6. Moisture Regain of Coyote Brown Colored Cotton (%)

	Enzyme (%)	Moisture Regain (%)
Raw Cotton	-	6.603
Scoured Cotton	-	7.342
Amylase	0.2	8.313
	0.5	8.176
	1.0	8.120
	1.5	7.981
Cellulase	0.2	7.704
	0.5	7.787
	1.0	8.833
	1.5	8.723
Lipase	0.2	8.529
	0.5	8.618
	1.0	6.606
	1.5	6.743

Protease	0.2	8.353
	0.5	8.440
	1.0	8.484
	1.5	8.326
Amylase + Cellulase	0.5	9.954
	1.0	9.508
Amylase + Lipase	0.5	10.171
	1.0	10.048
Amylase + Protease	0.5	10.002
	1.0	9.649
Cellulase + Lipase	0.5	9.694
	1.0	9.864
Cellulase + Protease	0.5	9.686
	1.0	9.658
Lipase + Protease	0.5	8.207
	1.0	9.557
Amylase + Cellulase + Lipase + Protease	0.5	8.242
	1.0	8.075

Table 7. Moisture Regain of Green Colored Cotton (%)

	Enzyme (%)	Moisture Regain (%)
Raw Cotton	-	5.825
Scoured Cotton	-	6.211
Amylase	0.2	6.818
	0.5	6.829
	1.0	7.397
	1.5	6.975
Cellulase	0.2	7.130

	0.5	7.170
	1.0	6.830
	1.5	6.675
Lipase	0.2	7.413
	0.5	8.258
	1.0	7.361
	1.5	8.641
Protease	0.2	6.695
	0.5	6.495
	1.0	6.918
	1.5	7.669
Amylase + Cellulase	0.5	9.096
	1.0	7.668
Amylase + Lipase	0.5	8.367
	1.0	7.981
Amylase + Protease	0.5	7.961
	1.0	7.815
Cellulase + Lipase	0.5	8.156
	1.0	9.326
Cellulase + Protease	0.5	7.935
	1.0	7.933
Lipase + Protease	0.5	7.917
	1.0	8.084
Amylase + Cellulase + Lipase + Protease	0.5	7.317
	1.0	7.067

SAS code for single enzyme treatment

```
proc import datafile="D:\cc2\moisture\sas\ccs.xls" out=ccs dbms=excel;
getnames=yes;
run;
proc print data=ccs;
run;
proc glm data=ccs;
```

```
class enzyme fiber;
model mr = enzyme fiber enzyme*fiber /solution;
lsmeans enzyme*fiber /pdiff cl adjust=tukey;
run;
data ccsnew;
set ccs;
if fiber="BB" and enzyme="s" then cond="bbs";
if fiber="BB" and enzyme="a" then cond="bba";
if fiber="BB" and enzyme="c" then cond="bbc";
if fiber="BB" and enzyme="p" then cond="bbp";
if fiber="BB" and enzyme="l" then cond="bbl";
if fiber="CB" and enzyme="s" then cond="cbs";
if fiber="CB" and enzyme="a" then cond="cba";
if fiber="CB" and enzyme="c" then cond="cbc";
if fiber="CB" and enzyme="p" then cond="cbp";
if fiber="CB" and enzyme="l" then cond="cbl";
if fiber="SG" and enzyme="s" then cond="sgs";
if fiber="SG" and enzyme="a" then cond="sga";
if fiber="SG" and enzyme="c" then cond="sgc";
if fiber="SG" and enzyme="l" then cond="sgl";
if fiber="SG" and enzyme="p" then cond="sgp";
run;
proc print data=ccsnew;
run;
proc anova data=ccsnew;
class cond;
model mr = cond;
means cond/tukey;
means cond/duncan;
run;
```

SAS output

Obs	enzyme	fiber	conc	rep	mr
1	s	BB	0.0	1	7.49403
2	s	BB	0.0	2	7.53726
3	s	BB	0.0	3	7.44150
4	a	BB	0.2	1	8.35609
5	a	BB	0.2	2	8.33829
6	a	BB	0.2	3	8.21368
7	a	BB	0.5	1	8.54695
8	a	BB	0.5	2	8.36379
9	a	BB	0.5	3	8.42008
10	a	BB	1.0	1	8.81267
11	a	BB	1.0	2	8.97719

12	a	BB	1.0	3	8.88030
13	a	BB	1.5	1	8.81605
14	a	BB	1.5	2	8.64744
15	a	BB	1.5	3	8.72301
16	c	BB	0.2	1	8.58081
17	c	BB	0.2	2	8.65821
18	c	BB	0.2	3	8.71134
19	c	BB	0.5	1	8.60809
20	c	BB	0.5	2	8.56598
21	c	BB	0.5	3	8.55121
22	c	BB	1.0	1	8.53426
23	c	BB	1.0	2	8.72044
24	c	BB	1.0	3	9.21102
25	c	BB	1.5	1	8.69023
26	c	BB	1.5	2	8.54373
27	c	BB	1.5	3	8.64841
28	p	BB	0.2	1	8.34538
29	p	BB	0.2	2	8.40817
30	p	BB	0.2	3	8.48682
31	p	BB	0.5	1	8.38556
32	p	BB	0.5	2	8.48035
33	p	BB	0.5	3	8.42657
34	p	BB	1.0	1	8.43185
35	p	BB	1.0	2	8.60850
36	p	BB	1.0	3	8.61696
37	p	BB	1.5	1	8.51295
38	p	BB	1.5	2	8.63511
39	p	BB	1.5	3	8.48898
40	l	BB	0.2	1	8.30783
41	l	BB	0.2	2	8.40050
42	l	BB	0.2	3	8.41982
43	l	BB	0.5	1	8.17587
44	l	BB	0.5	2	8.23329
45	l	BB	0.5	3	8.30286
46	l	BB	1.0	1	8.33508
47	l	BB	1.0	2	8.19839
48	l	BB	1.0	3	8.21292
49	l	BB	1.5	1	8.06205
50	l	BB	1.5	2	8.15707
51	l	BB	1.5	3	8.19270
52	s	CB	0.0	1	7.34200
53	s	CB	0.0	2	7.27400
54	s	CB	0.0	3	7.42957
55	a	CB	0.2	1	8.26129
56	a	CB	0.2	2	8.12023
57	a	CB	0.2	3	8.55806

58	a	CB	0.5	1	8.15468
59	a	CB	0.5	2	8.17531
60	a	CB	0.5	3	8.19861
61	a	CB	1.0	1	8.16611
62	a	CB	1.0	2	8.04992
63	a	CB	1.0	3	8.11313
64	a	CB	1.5	1	8.04377
65	a	CB	1.5	2	8.00441
66	a	CB	1.5	3	7.86456
67	c	CB	0.2	1	7.61163
68	c	CB	0.2	2	7.72794
69	c	CB	0.2	3	7.77387
70	c	CB	0.5	1	7.81358
71	c	CB	0.5	2	7.74304
72	c	CB	0.5	3	7.80485
73	c	CB	1.0	1	8.94183
74	c	CB	1.0	2	9.02799
75	c	CB	1.0	3	8.53040
76	c	CB	1.5	1	8.71153
77	c	CB	1.5	2	8.59512
78	c	CB	1.5	3	8.86099
79	p	CB	0.2	1	8.31323
80	p	CB	0.2	2	8.37525
81	p	CB	0.2	3	8.36999
82	p	CB	0.5	1	8.52626
83	p	CB	0.5	2	8.27268
84	p	CB	0.5	3	8.52228
85	p	CB	1.0	1	8.46015
86	p	CB	1.0	2	8.52268
87	p	CB	1.0	3	8.47050
88	p	CB	1.5	1	8.29558
89	p	CB	1.5	2	8.33500
90	p	CB	1.5	3	8.34620
91	l	CB	0.2	1	8.45575
92	l	CB	0.2	2	8.46005
93	l	CB	0.2	3	8.67060
94	l	CB	0.5	1	8.57147
95	l	CB	0.5	2	8.60433
96	l	CB	0.5	3	8.67826
97	l	CB	1.0	1	6.64799
98	l	CB	1.0	2	6.52977
99	l	CB	1.0	3	6.63928
100	l	CB	1.5	1	6.76506
101	l	CB	1.5	2	6.71395
102	l	CB	1.5	3	6.75005
103	s	SG	0.0	1	6.21129

104	s	SG	0.0	2	6.68499
105	s	SG	0.0	3	5.31667
106	a	SG	0.2	1	6.70433
107	a	SG	0.2	2	6.81876
108	a	SG	0.2	3	6.93220
109	a	SG	0.5	1	6.88621
110	a	SG	0.5	2	6.73776
111	a	SG	0.5	3	6.86222
112	a	SG	1.0	1	7.46545
113	a	SG	1.0	2	7.45234
114	a	SG	1.0	3	7.27470
115	a	SG	1.5	1	7.11065
116	a	SG	1.5	2	7.01010
117	a	SG	1.5	3	6.80355
118	c	SG	0.2	1	7.27936
119	c	SG	0.2	2	7.24161
120	c	SG	0.2	3	6.86954
121	c	SG	0.5	1	7.19605
122	c	SG	0.5	2	7.12318
123	c	SG	0.5	3	7.18981
124	c	SG	1.0	1	6.64376
125	c	SG	1.0	2	6.84082
126	c	SG	1.0	3	7.00577
127	c	SG	1.5	1	6.88868
128	c	SG	1.5	2	6.43425
129	c	SG	1.5	3	6.70261
130	p	SG	0.2	1	6.71662
131	p	SG	0.2	2	6.66097
132	p	SG	0.2	3	6.70733
133	p	SG	0.5	1	6.54289
134	p	SG	0.5	2	6.50065
135	p	SG	0.5	3	6.44286
136	p	SG	1.0	1	7.58023
137	p	SG	1.0	2	7.58024
138	p	SG	1.0	3	5.59284
139	p	SG	1.5	1	7.68404
140	p	SG	1.5	2	7.67100
141	p	SG	1.5	3	7.65300
142	l	SG	0.2	1	7.32266
143	l	SG	0.2	2	7.41600
144	l	SG	0.2	3	7.50080
145	l	SG	0.5	1	8.32126
146	l	SG	0.5	2	8.27800
147	l	SG	0.5	3	8.17532
148	l	SG	1.0	1	7.02756
149	l	SG	1.0	2	7.12756

150	l	SG	1.0	3	7.92756
151	l	SG	1.5	1	8.64097
152	l	SG	1.5	2	8.65098
153	l	SG	1.5	3	8.63098

The GLM Procedure

Class Level Information

Class	Levels	Values
enzyme	5	a c l p s
fiber	3	BB CB SG

Number of observations 153

Dependent Variable: mr mr

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	67.37842216	4.81274444	23.98	<.0001
Error	138	27.69446286	0.20068451		
Corrected Total	152	95.07288501			

R-Square	Coeff Var	Root MSE	mr Mean
0.708703	5.684950	0.447978	7.880074

Source	DF	Type I SS	Mean Square	F Value	Pr > F
enzyme	4	7.96146638	1.99036660	9.92	<.0001
fiber	2	45.74716294	22.87358147	113.98	<.0001
enzyme*fiber	8	13.66979284	1.70872410	8.51	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
enzyme	4	7.96146638	1.99036660	9.92	<.0001
fiber	2	35.16217201	17.58108601	87.61	<.0001
enzyme*fiber	8	13.66979284	1.70872410	8.51	<.0001

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		6.070983826 B	0.25864036	23.47	<.0001
enzyme	a	0.933871326 B	0.28916872	3.23	0.0015
enzyme	c	0.880303449 B	0.28916872	3.04	0.0028
enzyme	l	1.847318172 B	0.28916872	6.39	<.0001

enzyme	p	0.873405672 B	0.28916872	3.02	0.0030
enzyme	s	0.000000000 B	.	.	.
fiber	BB	1.419945820 B	0.36577271	3.88	0.0002
fiber	CB	1.277540507 B	0.36577271	3.49	0.0006
fiber	SG	0.000000000 B	.	.	.
enzyme*fiber a BB		0.166493804 B	0.40894632	0.41	0.6845
enzyme*fiber a CB		-0.139889923 B	0.40894632	-0.34	0.7328
enzyme*fiber a SG		0.000000000 B	.	.	.
enzyme*fiber c BB		0.297411773 B	0.40894632	0.73	0.4683
enzyme*fiber c CB		0.033070015 B	0.40894632	0.08	0.9357
enzyme*fiber c SG		0.000000000 B	.	.	.
enzyme*fiber l BB		-1.088384251 B	0.40894632	-2.66	0.0087
enzyme*fiber l CB		-1.571962288 B	0.40894632	-3.84	0.0002
enzyme*fiber l SG		0.000000000 B	.	.	.
enzyme*fiber p BB		0.121264219 B	0.40894632	0.30	0.7673
enzyme*fiber p CB		0.178886576 B	0.40894632	0.44	0.6625
enzyme*fiber p SG		0.000000000 B	.	.	.
enzyme*fiber s BB		0.000000000 B	.	.	.
enzyme*fiber s CB		0.000000000 B	.	.	.
enzyme*fiber s SG		0.000000000 B	.	.	.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey-Kramer

enzyme	fiber	LSMEAN mr LSMEAN	LSMEAN Number
a	BB	8.59129478	1
a	CB	8.14250574	2
a	SG	7.00485515	3
c	BB	8.66864487	4
c	CB	8.26189780	5
c	SG	6.95128728	6
l	BB	8.24986357	7
l	CB	7.62388022	8
l	SG	7.91830200	9
p	BB	8.48559954	10
p	CB	8.40081658	11
p	SG	6.94438950	12
s	BB	7.49092965	13
s	CB	7.34852433	14
s	SG	6.07098383	15

Least Squares Means for effect enzyme*fiber

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: mr

i/j	1	2	3	4	5	6	7	8
1		0.4802	<.0001	1.0000	0.8938	<.0001	0.8651	<.0001
2	0.4802		<.0001	0.2206	1.0000	<.0001	1.0000	0.2409
3	<.0001	<.0001		<.0001	<.0001	1.0000	<.0001	0.0611
4	1.0000	0.2206	<.0001		0.6472	<.0001	0.5997	<.0001
5	0.8938	1.0000	<.0001	0.6472		<.0001	1.0000	0.0451
6	<.0001	<.0001	1.0000	<.0001	<.0001		<.0001	0.0252
7	0.8651	1.0000	<.0001	0.5997	1.0000	<.0001		0.0548
8	<.0001	0.2409	0.0611	<.0001	0.0451	0.0252	0.0548	
9	0.0250	0.9962	0.0002	0.0059	0.8595	<.0001	0.8889	0.9538
10	1.0000	0.8608	<.0001	0.9996	0.9962	<.0001	0.9937	0.0006
11	0.9993	0.9849	<.0001	0.9791	1.0000	<.0001	1.0000	0.0035
12	<.0001	<.0001	1.0000	<.0001	<.0001	1.0000	<.0001	0.0223
13	0.0166	0.6262	0.9354	0.0066	0.3369	0.8654	0.3633	1.0000
14	0.0029	0.2895	0.9972	0.0010	0.1130	0.9883	0.1256	0.9998
15	<.0001	<.0001	0.0938	<.0001	<.0001	0.1503	<.0001	<.0001

i/j	9	10	11	12	13	14	15
1	0.0250	1.0000	0.9993	<.0001	0.0166	0.0029	<.0001
2	0.9962	0.8608	0.9849	<.0001	0.6262	0.2895	<.0001
3	0.0002	<.0001	<.0001	1.0000	0.9354	0.9972	0.0938
4	0.0059	0.9996	0.9791	<.0001	0.0066	0.0010	<.0001
5	0.8595	0.9962	1.0000	<.0001	0.3369	0.1130	<.0001
6	<.0001	<.0001	<.0001	1.0000	0.8654	0.9883	0.1503
7	0.8889	0.9937	1.0000	<.0001	0.3633	0.1256	<.0001
8	0.9538	0.0006	0.0035	0.0223	1.0000	0.9998	<.0001
9		0.1304	0.3544	<.0001	0.9774	0.8118	<.0001
10	0.1304		1.0000	<.0001	0.0521	0.0108	<.0001
11	0.3544	1.0000		<.0001	0.1165	0.0284	<.0001
12	<.0001	<.0001	<.0001		0.8540	0.9863	0.1591
13	0.9774	0.0521	0.1165	0.8540		1.0000	0.0128
14	0.8118	0.0108	0.0284	0.9863	1.0000		0.0446
15	<.0001	<.0001	<.0001	0.1591	0.0128	0.0446	

The ANOVA Procedure

Class Level Information

Class	Levels	Values
cond	15	bba bbc bbl bbp bbs cba cbc cbl cbp cbs sga sgc sgl sgp sgs

Number of observations 153

Dependent Variable: mr mr

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	67.37842216	4.81274444	23.98	<.0001
Error	138	27.69446286	0.20068451		
Corrected Total	152	95.07288501			

R-Square	Coeff Var	Root MSE	mr Mean
0.708703	5.684950	0.447978	7.880074

Source	DF	Anova SS	Mean Square	F Value	Pr > F
cond	14	67.37842216	4.81274444	23.98	<.0001

Tukey's Studentized Range (HSD) Test for mr

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	138
Error Mean Square	0.200685
Critical Value of Studentized Range	4.88454

Duncan's Multiple Range Test for mr

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	138
Error Mean Square	0.200685
Harmonic Mean of Cell Sizes	7.5

NOTE: Cell sizes are not equal.

Number of Means	2	3	4	5	6	7	8
Critical Range	.4574	.4814	.4974	.5092	.5183	.5257	.5319
Number of Means	9	10	11	12	13	14	15
Critical Range	.5371	.5416	.5455	.5490	.5521	.5549	.5574

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	cond
A	8.6686	12	bbc
A			
B A	8.5913	12	bba
B A			
B A	8.4856	12	bbp
B A			

B	A	C	8.4008	12	cbp
B	A	C			
B	A	C	8.2619	12	cbc
B	A	C			
B	A	C	8.2499	12	bbl
B		C			
B		C	8.1425	12	cba
		C			
	D	C	7.9183	12	sgl
	D				
E	D		7.6239	12	cbl
E	D				
E	D		7.4909	3	bbs
E					
E	F		7.3485	3	cbs
	F				
	F		7.0049	12	sga
	F				
	F		6.9513	12	sgc
	F				
	F		6.9444	12	sgp
	G		6.0710	3	sgs

SAS code for multiple enzyme treatment

```
proc import datafile="D:\cc2\moisture\sas\ccm.xls" out=ccm dbms=excel;
getnames=yes;

run;

data ccm2;
set ccm;
if fiber="BB" and enzyme="s" then condi="sb";
if fiber="BB" and enzyme="ac" then condi="1b";
if fiber="BB" and enzyme="al" then condi="2b";
if fiber="BB" and enzyme="ap" then condi="3b";
if fiber="BB" and enzyme="cl" then condi="4b";
if fiber="BB" and enzyme="cp" then condi="5b";
if fiber="BB" and enzyme="lp" then condi="6b";
if fiber="BB" and enzyme="aclp" then condi="7b";
if fiber="CB" and enzyme="s" then condi="sc";
if fiber="CB" and enzyme="ac" then condi="1c";
if fiber="CB" and enzyme="al" then condi="2c";
if fiber="CB" and enzyme="ap" then condi="3c";
if fiber="CB" and enzyme="cl" then condi="4c";
```

```
if fiber="CB" and enzyme="cp" then condi="5c";
if fiber="CB" and enzyme="lp" then condi="6c";
if fiber="CB" and enzyme="aclp" then condi="7c";
if fiber="SG" and enzyme="s" then condi="sg";
if fiber="SG" and enzyme="ac" then condi="1g";
if fiber="SG" and enzyme="al" then condi="2g";
if fiber="SG" and enzyme="ap" then condi="3g";
if fiber="SG" and enzyme="cl" then condi="4g";
if fiber="SG" and enzyme="cp" then condi="5g";
if fiber="SG" and enzyme="lp" then condi="6g";
if fiber="SG" and enzyme="aclp" then condi="7g";
run;
proc print data=ccm2;
run;
proc anova data=ccm2;
class condi;
model mr = condi;
means condi/duncan;
run;
```

SAS output

Obs	enzyme	fiber	conc	rep	mr	condi
1	s	BB	0.0	1	7.3171	sb
2	s	BB	0.0	2	7.2800	sb
3	s	BB	0.0	3	6.9943	sb
4	ac	BB	0.5	1	9.7866	1b
5	ac	BB	0.5	2	9.7200	1b
6	ac	BB	0.5	3	9.7160	1b
7	ac	BB	1.0	1	9.9770	1b
8	ac	BB	1.0	2	10.0910	1b
9	ac	BB	1.0	3	10.0143	1b
10	al	BB	0.5	1	10.2236	2b
11	al	BB	0.5	2	10.1799	2b
12	al	BB	0.5	3	10.4286	2b
13	al	BB	1.0	1	10.0000	2b
14	al	BB	1.0	2	10.1742	2b
15	al	BB	1.0	3	10.2330	2b
16	ap	BB	0.5	1	10.4112	3b
17	ap	BB	0.5	2	10.3432	3b
18	ap	BB	0.5	3	10.1671	3b
19	ap	BB	1.0	1	9.9173	3b
20	ap	BB	1.0	2	9.9289	3b
21	ap	BB	1.0	3	9.8736	3b
22	cl	BB	0.5	1	10.3670	4b

23	cl	BB	0.5	2	10.4000	4b
24	cl	BB	0.5	3	8.7969	4b
25	cl	BB	1.0	1	9.8359	4b
26	cl	BB	1.0	2	9.7946	4b
27	cl	BB	1.0	3	9.6710	4b
28	cp	BB	0.5	1	10.2215	5b
29	cp	BB	0.5	2	10.1719	5b
30	cp	BB	0.5	3	10.2611	5b
31	cp	BB	1.0	1	9.4274	5b
32	cp	BB	1.0	2	9.1241	5b
33	cp	BB	1.0	3	9.8463	5b
34	lp	BB	0.5	1	9.5484	6b
35	lp	BB	0.5	2	10.0967	6b
36	lp	BB	0.5	3	9.9080	6b
37	lp	BB	1.0	1	9.7454	6b
38	lp	BB	1.0	2	9.9207	6b
39	lp	BB	1.0	3	9.8375	6b
40	aclp	BB	0.5	1	8.8434	7b
41	aclp	BB	0.5	2	8.2790	7b
42	aclp	BB	0.5	3	8.2329	7b
43	aclp	BB	1.0	1	8.9726	7b
44	aclp	BB	1.0	2	7.9512	7b
45	aclp	BB	1.0	3	8.4306	7b
46	s	CB	0.0	1	6.5810	sc
47	s	CB	0.0	2	7.0241	sc
48	s	CB	0.0	3	7.0823	sc
49	ac	CB	0.5	1	9.9685	1c
50	ac	CB	0.5	2	9.9457	1c
51	ac	CB	0.5	3	9.9472	1c
52	ac	CB	1.0	1	9.5143	1c
53	ac	CB	1.0	2	9.6661	1c
54	ac	CB	1.0	3	9.3436	1c
55	al	CB	0.5	1	10.2031	2c
56	al	CB	0.5	2	10.1298	2c
57	al	CB	0.5	3	10.1822	2c
58	al	CB	1.0	1	9.6572	2c
59	al	CB	1.0	2	9.4491	2c
60	al	CB	1.0	3	9.6588	2c
61	ap	CB	0.5	1	9.7745	3c
62	ap	CB	0.5	2	10.2897	3c
63	ap	CB	0.5	3	9.9417	3c
64	ap	CB	1.0	1	9.5190	3c
65	ap	CB	1.0	2	9.7766	3c
66	ap	CB	1.0	3	9.6079	3c
67	cl	CB	0.5	1	9.8984	4c
68	cl	CB	0.5	2	9.4259	4c

69	cl	CB	0.5	3	9.7012	4c
70	cl	CB	1.0	1	9.9352	4c
71	cl	CB	1.0	2	9.9602	4c
72	cl	CB	1.0	3	9.6974	4c
73	cp	CB	0.5	1	9.6638	5c
74	cp	CB	0.5	2	9.8789	5c
75	cp	CB	0.5	3	9.5154	5c
76	cp	CB	1.0	1	9.3496	5c
77	cp	CB	1.0	2	9.9831	5c
78	cp	CB	1.0	3	9.6409	5c
79	lp	CB	0.5	1	9.6646	6c
80	lp	CB	0.5	2	10.1100	6c
81	lp	CB	0.5	3	9.8199	6c
82	lp	CB	1.0	1	9.3962	6c
83	lp	CB	1.0	2	9.6867	6c
84	lp	CB	1.0	3	9.5891	6c
85	aclp	CB	0.5	1	8.3167	7c
86	aclp	CB	0.5	2	8.2661	7c
87	aclp	CB	0.5	3	8.1421	7c
88	aclp	CB	1.0	1	7.6018	7c
89	aclp	CB	1.0	2	8.5488	7c
90	aclp	CB	1.0	3	8.2740	7c
91	s	SG	0.0	1	6.7643	sg
92	s	SG	0.0	2	6.3240	sg
93	s	SG	0.0	3	6.2337	sg
94	ac	SG	0.5	1	8.1249	1g
95	ac	SG	0.5	2	8.1705	1g
96	ac	SG	0.5	3	7.9931	1g
97	ac	SG	1.0	1	7.6256	1g
98	ac	SG	1.0	2	7.8303	1g
99	ac	SG	1.0	3	7.54796	1g
100	al	SG	0.5	1	8.50843	2g
101	al	SG	0.5	2	8.37769	2g
102	al	SG	0.5	3	8.22739	2g
103	al	SG	1.0	1	8.22583	2g
104	al	SG	1.0	2	7.95427	2g
105	al	SG	1.0	3	7.76448	2g
106	ap	SG	0.5	1	7.95087	3g
107	ap	SG	0.5	2	7.90247	3g
108	ap	SG	0.5	3	8.03102	3g
109	ap	SG	1.0	1	7.78670	3g
110	ap	SG	1.0	2	7.96719	3g
111	ap	SG	1.0	3	7.69123	3g
112	cl	SG	0.5	1	8.45554	4g
113	cl	SG	0.5	2	7.98376	4g
114	cl	SG	0.5	3	8.02871	4g

115	cl	SG	1.0	1	8.00888	4g
116	cl	SG	1.0	2	8.17017	4g
117	cl	SG	1.0	3	8.72969	4g
118	cp	SG	0.5	1	8.06245	5g
119	cp	SG	0.5	2	7.80795	5g
120	cp	SG	0.5	3	7.93374	5g
121	cp	SG	1.0	1	7.94191	5g
122	cp	SG	1.0	2	7.92356	5g
123	cp	SG	1.0	3	7.93282	5g
124	lp	SG	0.5	1	7.80578	6g
125	lp	SG	0.5	2	7.92409	6g
126	lp	SG	0.5	3	8.02004	6g
127	lp	SG	1.0	1	8.26326	6g
128	lp	SG	1.0	2	7.90494	6g
129	lp	SG	1.0	3	8.07093	6g
130	aclp	SG	0.5	1	7.39223	7g
131	aclp	SG	0.5	2	7.24200	7g
132	aclp	SG	0.5	3	7.30864	7g
133	aclp	SG	1.0	1	7.24293	7g
134	aclp	SG	1.0	2	6.89217	7g
135	aclp	SG	1.0	3	7.09649	7g

The ANOVA Procedure

Class Level Information

Class	Levels	Values
condi	24	1b 1c 1g 2b 2c 2g 3b 3c 3g 4b 4c 4g 5b 5c 5g 6b 6c 6g 7b 7c 7g sb sc sg

Number of observations 135

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	156.6585002	6.8112391	86.69	<.0001
Error	111	8.7209096	0.0785668		
Corrected Total	134	165.3794098			

R-Square	Coeff Var	Root MSE	mr Mean
0.947267	3.145657	0.280298	8.910624

Source	DF	Anova SS	Mean Square	F Value	Pr > F
condi	23	156.6585002	6.8112391	86.69	<.0001

Duncan's Multiple Range Test for mr

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	111

Error Mean Square 0.078567

Harmonic Mean of Cell Sizes 5.333333

NOTE: Cell sizes are not equal.

Number of Means	2	3	4	5	6	7	8	9	10	11	12	13
Critical Range	.3401	.3580	.3698	.3785	.3852	.3907	.3952	.3991	.4024	.4052	.4078	.4100

Number of Means	14	15	16	17	18	19	20	21	22	23	24
Critical Range	.4120	.4138	.4155	.4170	.4183	.4196	.4207	.4218	.4228	.4237	.4245

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	condi
	A		10.2066	6	2b
	A				
B	A		10.1069	6	3b
B	A				
B	A	C	9.8841	6	1b
B	A	C			
B	A	C	9.8800	6	2c
B	A	C			
B	A	C	9.8428	6	6b
B	A	C			
B	A	C	9.8421	6	5b
B	A	C			
B	A	C	9.8182	6	3c
B		C			
B		C	9.8109	6	4b
B		C			
B		C	9.7697	6	4c
B		C			
B		C	9.7309	6	1c
B		C			
B		C	9.7111	6	6c
		C			
		C	9.6719	6	5c
	D		8.4516	6	7b
	D				
E	D		8.2295	6	4g
E	D				
E	D		8.1916	6	7c
E	D				
E	D		8.1763	6	2g
E					

```
E              7.9982     6    6g
E
E              7.9337     6    5g
E
E              7.8882     6    3g
E
E              7.8821     6    1g

    F          7.1972     3    sb
    F
    F          7.1957     6    7g
    F
    F          6.8958     3    sc

     G          6.4407     3    sg
```