Journal of Home Economics

Journal of Home Economics

Volume 25, Number (1), 2015

http://homeEcon.menofia.edu.eg ISSN 1110-2578

Effect Of Pepsin And Trypsin Enzymes Used In Textile Conservation On Natural Dyedtextiles Samples.

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Abstract: This paper discusses the pepsin and trypsin enzymes behavior towards natural fibers dyed with natural dyes. In this study four natural fibers were used: cotton, linen, wool, silk dyed with 14 of natural dyes extracted from*R.tinctoria L*(madder), *Humuluslupulus L*(common hop), *P.tormentillaneck* (blood-root), *A.cepa L*(onion skin), *Punicagranatum L*(pomegranate skin), *A.catechuwilld*(catechu tree), *Juniperuscommunis L*(common juniper tree), *R.coriaria L*(dyer's sumac), *Bongardiachyso-gonumboiss*(golden rod), *A.arabicawilld*(Egyptian *L*(dog′s fennel) and acacia),*Bixaorellana L*(annatto), *S.officinalis L*(common sage),*A.cotula Dactylopiuscoccus* (American cochineal). Alum was used as mordant to fixate dyes with fibers. The dyed samples were divided in three groups; the first group was treated with buffer solution to study the effect of buffer solution used with enzymes on the dye, the second group was treated with slight concentration of enzymes and the last group was treated with high concentration of enzymes to study the effect of enzymes concentration on natural dyes. Each treatment continues for four hours at 40C˚ with stirring the solution. The strength of color and color difference were measured for each sample to show the effect of treatment on dyes, it concluded that the pepsin and trypsin enzymes have not any side effects on dyed samples, the side effect back to the buffers solution.

Key words:conservation, enzyme, pepsin, trypsin, stain, natural fibers, natural dyes, strength of color.

1. Introduction:

Textile conservators have long recognized the benefits of enzymes in the conservation treatment of works of art. Most commonly, hydrolase-type enzymes are employed in the conservation of works on textile to assist in the breakdown of adhesive residues from previous

restorations or to facilitate the removal of some stains. The principal advantages of these enzymes are their specificity and efficiency in catalyzing hydrolytic cleavage of polymers such as proteins, polysaccharides, and lipids (Agnes et al.1998).

Protease is a class of enzymes that converts the complex proteins into simple proteins, amino acids and other non protein parts by catalyzing the hydrolysis processes. A number of such enzymes have been found in animals, plants and microorganisms. Proteases represent one of the three largest groups of industrial enzymes and find application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes. Pepsin and trypsin are two of three principal protein-degrading, or proteolytic, enzymes in the digestive system, the third one being chymotrypsin. Pepsin and trypsin enzymes consider two of the most important proteolotic enzymes which have high specificity in protein digestion, so it may use to get rid of protein stains from fibers dyed with natural dyes (Yaseenet al.2010).

Many authors have been used various enzymes"lipase, amylase, protease, diastase" in textile conservation(Whaap2007, Harby 2009, Ibrahim2010). The aim of this study was to throw a beam of light on thrt usage of pepsin and trypsin enzymes in conservation of natural dyed textiles samples.

2. Materials and Methods

2.1. Materials

Natural fibers, linen, cotton, wool and silk were used in this study,fourteen of natural dyes and two enzymes, pepsin and trypsin as well as two buffer solutions,glycine pH3 and phosphate buffer pH8.

2.1.1. Linen fiber: raw linen fiber, plain weave, the weight of one meter square in 113gm, 13 wefts and 17 warps in centimeter square. The average of tensile strength is 56.5 kg/strength, the average of elongation percent is 6.9%.42 samples of this material were cut up in sizes 10×10 cm for experiments and test them.

2.1.2. Cotton fibers: raw cotton fibers, plain weave, the weight of one meter square is 179 gm. 25 wefts and 25 warps in centimeter square, the average of tensile strength is 66.9 kg/strength ,the average of elongation percent is 19,9%. 42 samples of this material were cut up in sizes 10×10 cm for experiments and test them.

2.1.3. Wool fibers: raw wool fibers, plain weave, the weight of one meter square is 148gm, 23wefts and 23 warps in centimeter square, the average of tensile strength is 13.9kg/strength, the average of elongation percent is 12,6%.42 samples of this material were cut up in sizes 10×10 cm for experiments and test them.

2.1.4. Silk fibers:raw silk fibers, plain weave, the weight of one meter square is 143gm, 24 wefts and 25 warps in centimeter square, the average of tensile strength is 33.7 kg/strength, the average of elongation percent is 14.9%.42 samples of this material were cut up in sizes 10×10 cm for experiments and test them.

2.1.5. Natural dyes: In this study 14 natural dyes were used including the following dyes: madder dye, common hop, blood-root dye, onion skin dye, pomegranate skin dye, catechu dye, common juniper tree dye, sumac dye, Egyptian acacia, golden rod dye, annatto plant dye, common sage dye, dog's fennel dye and cochineal dye.

2.1.6. Pepsin enzyme(E.C.3.4.23.1) extracted from hog stomach(Oxford Laboratory, India), activity 3 units/mg, and temperature of activity is 40˚C, the pH of activity medium at pH 3, store at 0-4˚C.

2.1.7. Trypsin enzyme (E.C.3.4.21.4) extracted from porcine pancreas (Euromadex, France), activity 2 units /mg, and temperature of activity is 40˚C, the pH of activity medium at pH 8, store at 2-8 ˚C.

2.2. Methods

2.2.1. Dye extraction: dyes were ground in to a fine powder, soaked in cold water for 24 hours, boiled in water for 15 minutes to assure the extraction process with continuous stirring, allowed the extract to be cooled and then filtered to get a clear solution. Then the dye solution was adjusted to definite volume to give the suitable ratio 20 ml of dye solution : 1gm of fiber (Liquer ratio).

2.2.2. Dyeing procedures: thedyeingprocedures were performed in glass beakers at 40 c˚ with continuous stirring and rinsing in temperature to boiling for 15 minutes, with adding small amount of sodium chloride to cellulose(cotton,linen) dye bath and small amount of weak acid to protein fiber(wool,silk) dye bath (Landi 1987). Alum was used as a mordant with 0.1 % concentration. Unfixed dyestuff was removed with rinsing with cold water at room temperature.

2.2.3. Preparation of enzymes and buffers solution:

2.2.3.1. Glycine buffer (pH3) was used alone or buffer medium of two pepsin enzyme concentrations were used, to show the effect of increasing in enzyme concentration on fading of natural dyes.

Concentration **A**: 582 U of pepsin enzyme in 250ml of buffer solution.

Concentration **B**: 2910 U of pepsin enzyme in 250ml of buffer solution.

2.2.3.2. Phosphate buffer (pH8)was used alone or buffer medium of two trypsin enzyme concentrations were usedfor the prvious reason.
Concentration **A**: 388 U of trypsin enzyme in 250ml of buffer solution.
Concentration B: 1940 U of trypsin enzyme in 250ml of buffer solution.

Noting that:

- Pepsin enzyme was used first then followed by trypsin enzyme, according to this equation:

- Proteins $\xrightarrow{Pepsin\ enzyme}$ Proteoses + Peptone.

- Proteoses $\xrightarrow{trypsin\ enzyme}$ Amino acids. (ChristophorK.Mathews, et al.1996)

- Color strength and color difference degree of all samples were measured by using portable spectrophotometer (AmeriaCompany for Spinningand Weavinglaboratories, Ameria city, Alexandria, Egypt).
The results of thetestsasthetables(1, 2, 3, 4, 5).

Results and Discussion:

1-according to the first satage of treatment with pepsin enzyme and glycine buffer solution (pH3), observaed after pick up samples occurs bleeding of some natural dyes represent in cochineal, catechu tree, blood- root, onion skin, Dog's fennel, common hop.in the second stage of treatment with trypsin enzyme and phosphate buffer solution (pH8), observed after pick up samples occurs high bleeding of all dyes.

2- Each dye from natural dyes of study has solo behavior towards treatment solutions and this behavior differ according to the kind of dyed textile material "flax, cotton, wool, silk".

3 - Change in color strength degree and color difference degree ΔE convergent largely within the framework of one textile sample treated with first and second concentration of enzyme or buffer solution. Proving that the change in color strength degree and color difference degree ΔE is due to the buffers solution, not to pepsin and trypsin enzymes.

4- High bleeding of natural dyes used in this study is due to the alkaline pH value of buffer solution used with trypsin enzyme (pH 8) far from the isoelectric point of textile fiber. According to Agnes 1998, for conservation purposes the isoelectric region of protein materials considered to be at pH5-7,so trypsin enzyme has relative unsafety in using in natural dyed textile conservation.

5-From previous results it is concluded that due to the severe effect of the buffer′s strength on the color of natural dyes, pepsin and trypsin enzymes does not available for conservation of ancient natural dyed textiles, and conservator must use enzymes have active conditions approaching the isoelectric point of natural dyed textile fibers.

Table (1) illustrates pepsin and trypsin enzymes treatment and buffer solution results to natural dyed woven fabric samples.

Samples treated with the second concentration of enzyme "B"		Samplestreated with the first concentration of enzyme "A"	Samples treated with buffer solution pH3 followed by pH8	Fiber material	Dye source
■	70.8	85.1	77.7	Flax	Madder "dyer's madder"
▲	2.3	2.4	2.5		
■	96.9	72.2	87	Cotton	
▲	0.5	1.6	1.2		
■	101.4	101	95	Wool	
▲	2.1	3.5	3.4		
■	85	74.2	77.5	Silk	
▲	0.7	2.6	2.0		
■	64.7	69.4	72.5	Flax	Common hop
▲	6.9	6.2	8.4		
■	61.9	64.2	63.9	Cotton	
▲	6.4	7.9	8.0		
■	72.7	72	61.6	Wool	
▲	1.8	2.5	2.2		
■	50.8	51.2	42.6	Silk	
▲	10.1	9.4	9.4		
■	80.2	78.3	84.9	Flax	Blood – root
▲	4.8	4.1	4.9		
■	93.9	93.5	98.8	Cotton	
▲	3.7	3.3	4.2		
■	111.7	111.4	102.8	Wool	
▲	1.9	3.1	2.7		
■	101	109.9	108.4	Silk	
▲	4.4	5.3	5.6		

Note: Color strength degree of control sample → 100%K/S, Color difference →0

■ Color strength degree , ▲Color difference degree Δ E

Table (2) illustrates pepsin and trypsin enzymes treatment and buffer solution results to natural dyed woven fabric samples.

Samples treated with the second concentration of enzyme "B"		Samplestreated with the first concentration of enzyme "A"	Samples treated with buffer solution pH3 followed by pH8	Fiber material	Dye source
■	42.5	50.6	43.3	Flax	Onion skin
▲	6.8	6.6	8.1		
■	40.1	43.1	41.1	Cotton	
▲	6.2	6.6	7.1		
■	64.5	68	70.6	Wool	
▲	6.3	5.9	6.4		
■	84.7	95.5	82.7	Silk	
▲	8.0	6.2	7.3		
■	82.6	87.6	80.9	Flax	Pomegranate skin
▲	3.6	3.0	3.5		
■	78.1	95.9	80.1	Cotton	
▲	2.6	2.4	2.5		
■	127.6	135.4	130.5	Wool	
▲	2.2	2.1	2.0		
■	65.5	65.7	60.3	Silk	
▲	3.7	3.7	3.9		
■	74.9	75.3	76.8	Flax	Catechu tree
▲	6.7	6.3	6.9		
■	86.5	85.4	90.3	Cotton	
▲	4.5	4.1	4.8		
■	128.8	116.4	118.6	Wool	
▲	5.5	5.7	6.4		
■	120.4	120.3	129	Silk	
▲	3.4	3.4	5.1		

Note: Color strength degree of control sample → 100%K/S, Color difference →0

■ Color strength degree , ▲Color difference degree Δ E

Table (3) illustrates pepsin and trypsin enzymes treatment and buffer solution results to natural dyed woven fabric samples.

Samples treated with the second concentration of enzyme "B"		Samplestreated with the first concentration of enzyme "A"	Samples treated with buffer solution pH3 followed by pH8	Fiber material	Dye source
■	70.4	68.9	66.1	Flax	Common Juniper tree
▲	4.7	3.7	4.1		
■	87.2	77.1	77.7	Cotton	
▲	3.4	3.2	3.2		
■	103.6	95.8	104.4	Wool	
▲	1.1	0.96	1.2		
■	56.6	55.3	63.1	Silk	
▲	5.4	5.31	5.3		
■	83	81.1	85.8	Flax	Sumach
▲	2.6	2.7	2.5		
■	98.1	101.3	110.3	Cotton	
▲	1.3	1.7	1.7		
■	117.8	127.2	116.1	Wool	
▲	2.0	2.0	2.6		
■	101.1	92.2	98.1	Silk	
▲	1.6	1.5	1.9		
■	118.9	117.2	120.3	Flax	Egyptian acacia
▲	2.7	3.2	2.3		
■	83	95.9	89.7	Cotton	
▲	1.7	1.4	1.4		
■	112	102.8	112.2	Wool	
▲	1.5	0.83	1.5		
■	115.5	106.4	120	Silk	
▲	1.8	1.5	1.6		

Note: Color strength degree of control sample → 100%K/S, Color difference →0

■ Color strength degree , ▲Color difference degree Δ E

Table (4) illustrates pepsin and trypsin enzymes treatment and buffer solution results to natural dyed woven fabric samples.

Samples treated with the second concentration of enzyme "B"		Samplestreated with the first concentration of enzyme "A"	Samples treated with buffer solution pH3 followed by pH8	Fiber material	Dye source
■	58.9	56.3	60.4	Flax	Golden rod
▲	4.3	3.7	3.6		
■	62.7	57.1	64.6	Cotton	
▲	4.0	4.7	4.0		
■	103.2	97.4	106.1	Wool	
▲	0.84	0.98	0.86		
■	54.2	64.5	55	Silk	
▲	4.0	3.7	4.4		
■	119.6	125.5	121.9	Flax	Annatto dye plant
▲	1.9	2.5	2.4		
■	110.9	125.8	119.7	Cotton	
▲	3.6	4.9	3.9		
■	104.5	102.2	103.3	Wool	
▲	0.8	0.8	1.1		
■	106.6	98.9	104.6	Silk	
▲	0.5	0.7	0.7		
■	47.6	40.9	49	Flax	Common sage
▲	6.0	6.7	5.3		
■	49.8	44.8	49.2	Cotton	
▲	5.3	6.3	5.2		
■`	90.9	101.3	103	Wool	
▲	0.21	1.0	0.99		
■	55.1	62.6	58.6	Silk	
▲	4.8	4.6	3.8		

Note: Color strength degree of control sample → 100%K/S, Color difference →0

■ Color strength degree , ▲Color difference degree Δ E

Table (5) illustrates pepsin and trypsin enzymes treatment and buffer solution results to natural dyed woven fabric samples.

Samples treated with the second concentration of enzyme "B"		Samplestreated with the first concentration of enzyme "A"	Samples treated with buffer solution pH3 followed by pH8	Fiber material	Dye source
■	40.8	48.2	45.3	Flax	Dog's fennel
▲	5.6	4.9	4.6		
■	50.3	59.1	56.9	Cotton	
▲	5.0	4.7	4.4		
■	74.4	68.4	71.9	Wool	
▲	1.8	2.3	1.7		
■	50.5	53.4	50.1	Silk	
▲	4.7	5.2	4.8		
■	8.8	11.9	9.8	Flax	Cochineal
▲	20.2	21.5	19.9		
■	11.3	11.5	9.1	Cotton	
▲	22.1	22.9	14.1		
■	65.9	54.1	58.7	Wool	
▲	8.7	8.8	10.6		
■	46.1	43.8	40.1	Silk	
▲	9.3	10.0	10.2		

Note: Color strength degree of control sample → 100%K/S, Color difference →0

■ Color strength degree , ▲Color difference degree Δ E

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Journal of Home Economics, Volume 25, Number (1), 2015

تأثير إنزيمى الببسين والتربسين على العينات النسيجية المصبوغة بالصبغات الطبيعية.

إبراهيم حامد محمد ، ياسين زيدان ، نبيل صابر ، حربى أحمد

تناقش هذه الورقة البحثية سلوك إنزيمى الببسين والتربسين تجاه العينات النسيجية المصبوغة بالصبغات الطبيعية .ففى هذه الدراسة تم إستخدام أربعة من الخامات النسيجية هى الكتان والقطن والصوف والحرير ، حيث تم صباغتها بأربعة عشر من الصبغات الطبيعية متمثلة فى "الفوة ، حشيشة الدينار ، عرق إنجبار ، قشر البصل ، قشر الرمان ، كاد هندى، عرعر، سماق ، قرض السنط ، عرف الديك ، أناتو ، المرمرية ، الأقحوان ، الكوكنيل"،حيثتم ترسيخ الخامات بمرسخ الشبة أثناء الصباغة.وتم توزيع العينات المصبوغة على هيئة ثلاث مجموعات ،المجموعة الأولى تم معالجتها بالمحلول المنظم والمجموعة الثانية تم معالجتها بالتركيز الأول للإنزيم أما المجموعة الثالثة فتم معالجتها بتركيز أعلى للإنزيم ، وذلك لدراسة تأثير زيادة تركيز الإنزيم على الصبغات المتواجدة بالعينات النسيجية. وقد إستمرت كل معالجة لمدة أربع ساعات عند ٤٠مْ مع التقليب.بعد إنتهاء المعالجة تم قياس درجة قوة اللون ودرجة التغير فى إتجاه اللون لكل العينات ، والتى تم من خلالها إستنتاج أن إنزيمى الببسين والتربسين ليس لهما أية تأثيرات سلبية على الصبغات الطبيعية المتواجدة بالعينات ،وأن التأثير السلبى يرجع إلى المحاليل المنظمة المستخدمة مع الإنزيمين.