Bio-scouring of cotton fabric with pectinase combined with natural surfactant from soapnut

Tassanee KHUNKRONG[1], Arunee K. ALDRED[2] and Kanya BUTTARAJ[1,*]

[1]*Program in Chemistry, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand*
[2]*Program in Industrial Chemistry and Textile Technology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand*

([*]Corresponding author's e-mail: kanya@mju.ac.th)

Abstract

Cotton fabrics were bio-scoured by using pectinase combined with natural surfactant (soapnut extract)/commercial soap (Premasol jet) in alkaline solution. Pectinase was produced from *Colletotrichum capsici* infected chili, cultivated in different media, its activity was analyzed. Two steps were adopted for cotton fabrics scouring, firstly a solution containing soapnut extract or a commercial surfactant was used to remove the wax from cotton fabrics. In the second step to remove pectin, the cotton fabrics were further bio-scoured treated in the solution containing pectinase from a culture medium. Activity of pectinase was varied depended on type of medium and cultivation time. The soapnut extract resulted in high water absorbency fabric and the result was comparable to that of the Premasol jet treatment. The combination of pectinase and soapnut extract gave scoured fabrics with a high whiteness index. However, the breaking force of the scoured fabrics seemed to be slightly lower.

Keywords: Bio-scouring, cotton, pectinase, soapnut

Introduction

Cotton fabric is a popular and widely used textile material, due to the comfortable wearing and durability properties; moreover it can absorb and ventilate moisture well. Cotton is a cellulose fiber containing glucose units bonded together via β-1,4 linkage. Its repeat unit contains three hydroxyl groups which provide the prominent properties highlighted above regarding clothing production. However, small amounts of hydrophobic substances (such as wax and pectin) contained in cotton raw fiber, cause poor absorbability of dye stuffs and other chemicals which are applied in textile production. The scouring of cotton fiber is required to make the textile more hydrophilic, soft, and suitable for further processes. In scouring, many chemicals are used including aqueous alkaline solution and surfactant (Carr, 1995). Some of these chemicals are corrosive to equipment and cause pollution to effluents, therefore their use have been minimized. Biotechnological treatment of cellulosic textiles is an alternative to reduce environment impact. Enzymatic treatments have been developed for wet processes in textile industries covering a wide range of enzymatic operations such as cleaning, desizing, dyeing, finishing and decolourization as investigation earlier (Aly, 2004; Tanapongpipat et al. 2008; Sumathi & Manju, 2000; Maximo & Costa-Ferreira, 2004; Robinson, 2001; Ahmed & Kolisis, 2011). Scouring of cotton fabric using various enzymes, including pectinase, has been reported elsewhere (Li & Hardin, 1997; Kalliala & Talvenmaa, 2000; Chen et al. 2007; Wang et al. 2007).

Soapberry tree (*Sapindus rarak*) is a native deciduous tree, widely distributed in South, East and Southeast Asia. Extract of soapnut pericap in folk medicine as an expectorant, for cough relief and for detoxification (Shiau et al. 2009) Besides, pharmacological property as well as anti-microbial, insecticidal, molluscicidal, larvicidal and anti-oxidant activities have been investigated (Attele, 1999; Huang et al. 2003; Sparg et al. 2004; Saxena et al. 2004; Dini et al. 2009; Fauziah et al. 2014). The major active compounds of the soapnut pericap extract have been known as saponins which are surface-active compounds. Being natural surfactant with foaming and emulsifying properties, they thus provide diverse range of application, cosmetics, household and hair care products, and cleansers containing saponins have been formulated and available in the market (Shiau et al. 2009; Draelos, 1995; Kurniawan, 2011).

In the scouring process, surfactant is added to the treatment solution, which removes wax and impurities from the textile materials. However, commercial surfactants derived from petrochemical products have been exploited due to economical reason. The utilization of saponins as a surfactant in scouring textile process for ecological aspect is our attempt and is firstly reported in this paper. The scouring by a combination of soapnut extract and pectinase is applied to cotton fabric. Water absorbency, whiteness index and breaking force of the fabrics scoured with such solutions compared with unscoured fabric and fabric scoured with a commercial surfactant is described.

Materials and methods

Extraction of natural surfactant and its critical micelle concentration

Natural surfactant was extracted from 50 g of crushed soapnut pericap in 500 ml distilled water at 60 °C for 1 h. The supernatant was kept by filtration and diluted to concentrations of 0.5 to 8.0 %v/v. Absorbance of natural soapnut extract solution was measured over the range of 200-500 nm using a spectrophotometer (Hitachi, U-2900), critical micelle concentration (CMC) of the soapnut extract solution was subsequently spectrophotometrically quantified according to the method described in the previous work (Duff & Giles, 1972).

Microorganism and production media

C. capsici which was used as source of pectinase was isolated from anthracnose infected chili fruit. The infected fruit tissue was placed on Potato dextrose broth (PDB, Central Drug House), Czapek's broth and Modified V-8 for 20 days at 28 °C. The conidial suspension was prepared by adding 15 ml sterilized water to the agar plate. A sterile wire-loop was gently used to break the conidial clumps. The suspension was collected; the conidial was counted using a haemacytometer and subsequently diluted to the 50,000 spores/ml suspension. The culture was started by the addition of 10 %v/v conidial suspension and incubated at 30 °C on a rotary shaker maintaining at 110 rpm for 192 h. Growth of *C. capsici* was followed by determination of remained glucose which was determined using 3,5-dinitrosalicylate (DNS) as reagent (Miller, 1959)

Assay of pectinase

The assay of pectinase was modified from the method reported earlier (Ahlawat et al. 2008). Activity of pectinase was based on determination of viscosity of pectin solution in the presence of the enzyme using an Ostwald viscometer (Cannon). One unit of enzyme activity was defined as the amount of pectinase which 5 % decrease in relative viscosity of 0.2 %w/v citrus pectin solution at 37 °C for 1 min.

$$A = \frac{V_0 - V_t}{V_0 - V_s} \times 100 \quad (1)$$

where A is % of viscosity diminishing, V_o is flow time of 0.2 %w/v pectin solution with inactive enzyme, V_t is flow time of 0.2 %w/v pectin solution with active enzyme and V_s is flow time of solvent (0.1 M acetate buffer, pH 4.5).

Scouring of cotton fabric

First step scouring: removal of wax

Scouring solution of 100 ml containing a commercial surfactant; Premasol jet (BASF) was prepared according to supplier recommendation, soapnut extract were used separately to study the effect of natural surfactant on scouring performance. Nine recipes of scouring solution are shown in **Table 1**.

Table 1 Scouring solution containing Premasol jet and soapnut extract.

Samples	NaOH (g)	$K_2S_2O_8$ (g)	Premasol jet (g)	Soapnut extract (g)
None	0.5	0.5	-	-
Prem	0.5	0.5	0.20	-
S6	0.5	0.5	-	0.20 (6 %v/v)
S7	0.5	0.5	-	0.20 (7 %v/v)
S8	0.5	0.5	-	0.20 (8 %v/v)
S9	0.5	0.5	-	0.20 (9 %v/v)
S10	0.5	0.5	-	0.20 (10 %v/v)
S11	0.5	0.5	-	0.20 (11 %v/v)
Sap9	-	-	-	0.20 (9 %v/v)

Gray cotton fabric of 10 × 10 cm^2 piece was treated in each scouring solution at 85 °C for 1 h at a liquor ratio of 1:50, using a shaker bath at 60 rpm. The resulting fabrics were subsequently thoroughly rinsed with distilled water and air dried. The samples were labeled as None, Prem, S6, S7, S8, S9, S10, S11 and Sap9.

Second step scouring: Removal of pectin

Seven recipes of 100 ml scouring solutions in the presence of polyethylene glycol (PEG) and chelating agent in alkaline solution were prepared, various weights of Modified V-8 broth containing pectinase was added in to the solutions. The recipes of the treatment are shown in **Table 2**.

Table 2 Scouring solution containing containing pectinase from Modified V-8 broth.

Samples	PEG (g)	NaOH (g)	EDTA (g)	Modified V-8 broth (g)
0.0E	0.20	0.50	0.10	-
0.5E	0.20	0.50	0.10	0.50
1.0E	0.20	0.50	0.10	1.00
1.5E	0.20	0.50	0.10	1.50
2.0E	0.20	0.50	0.10	2.00
2.0EP	0.20	0.50	0.10	2.00
2.0ES	0.20	0.50	0.10	2.00

Cotton fabrics of 10x10 cm were immersed into 100 ml of each solution without and with various amount of pectinase in Modified V-8 broth. Scouring process of the cotton fabrics was conducted at 65 °C for 120 h using a shaker bath at 60 rpm. The samples were labeled as 0.0E, 0.5E, 1.0E, 1.5E and 2.0E. Cotton fabrics received from the first step scouring were treated in the same manner as the samples mentioned above, the samples were labeled as 2.0EP and 2.0ES for samples scoured with Premasol jet and soapnut extract, respectively. All resulting fabrics were then thoroughly rinsed with distilled water and air dried.

Testing of fabrics

Water absorbency of scoured fabrics was tested by determination of sinking time fabrics, according to the modified AATCC Test Method 17-1994. Fabric scoured with and without surfactant (samples None, Prem, S6, S7, S8, S9, S10, S11, Sap9), without and with various amount of pectinase (samples 0.0E, 0.5E, 1.0E, 1.5E, 2.0E, 2.0EP, 2.0ES) and unscoured (sample UT) were dropped into distilled water in a cylinder. Sinking time of the fabric was measured when the fabrics went into distilled water. Tri-stimulus coordinates including x, y and z values of fabrics) were measured using a colorimeter (Juki, JC801). Whiteness index of the fabrics was calculated according to CIE standard of illuminant D65, using Eq. (2);

$$W = Y + 800(x_n - x) + 1700(y_n - y) \tag{2}$$

where x_n and y_n are chromaticity co-ordinates of the light source (D65) which are 0.3137 and 0.3309, respectively, and x and y are measured chromaticity co-ordinates. Scoured cotton fabrics were cut into 2.5×10 cm^2 pieces. Breaking force of scoured fabrics (samples 0.0E-2.0E, None, Prem, S6-S11, 2.0EP and 2.0ES) and unscoured fabrics (UT) of 2.5×10 cm^2 dimension were tested according to the modified ISO 13934-1:1999 standard method using gauge length of 5 cm at an extension rate of 200 mm/min using a tensile tester (LLOYD).

Results and discussion

Critical micelle concentration of soapnut extract

In the wavelength range of 200-500 nm, soapnut extract shows maximum absorbance at 209 nm. When the concentration of soapnut extract was varied from 0.5 to 8% v/v, the curve relating the absorbance at 209 nm of soapnut extract and its concentration shows reflection point at 5.5 %v/v (see **Figure 1**) which is critical micelle concentration as suggested in previous work (Duff & Giles, 1972). Thus, concentration of soapnut extract in the range of 6-11 %v/v were further used to prepare scouring solution to study the influence of soap nut extract on scouring performance of cotton fabric.

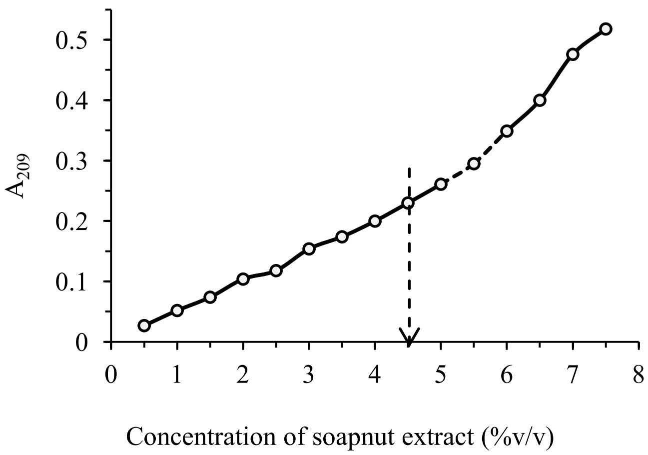

Figure 1 Relationship between absorbance of soapnut extract at 209 nm and its concentration.

Sinking time of scoured cotton fabrics

The performance to remove wax of each of the scouring solutions was evaluated by water absorbency in term of sinking times of the fabrics in distilled water; the results are shown in **Figure 2**. The sinking time of fabric scoured in solution containing NaOH and $K_2S_2O_8$ without surfactant (sample None) is 3.00 min, while the values of the cotton fabrics scoured with various concentrations of soapnut extract solution (6-11 %v/v) containing NaOH and $K_2S_2O_8$ are significantly lower and varied. The value of fabric scoured with solution containing 9 %v/v soapnut extract, NaOH and $K_2S_2O_8$ (sample S9) is lowest at 1.34 min which is closed to that scoured with the solution containing Premasol jet, NaOH and $K_2S_2O_8$ (sample Prem) (1.18 min). Significant low sinking times of the fabrics scoured with soapnut extract/Premasol jet indicate low wax remaining in cotton fabrics. Additionally, insignificant difference in sinking time of fabric scoured with soapnut extract and Premasol jet was observed. Therefore, soapnut extract possibly substitutes commercial soap; Premasol jet in scouring process for the eco-friendly treatment of cotton.

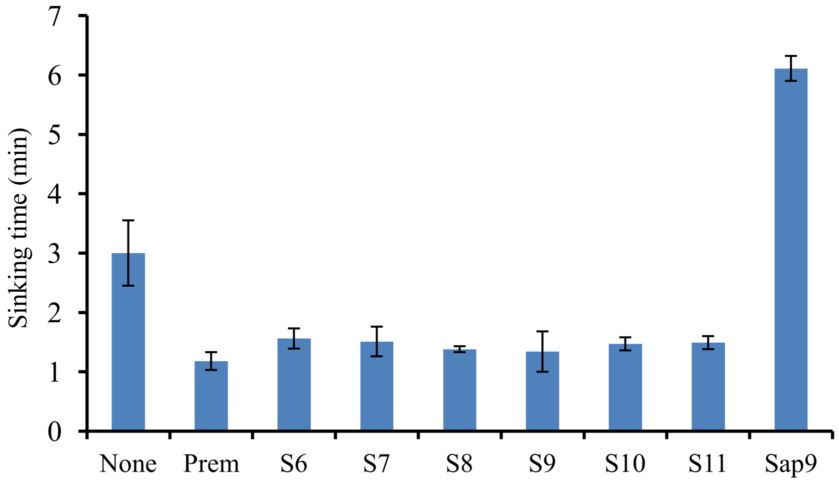

Figure 2 Sinking time of scoured cotton fabrics compared with unscoured.

Activity of pectinase in culture media

Activity of pectinase in PDB, Modified V-8 and Czapek's broth was determined based on its viscosity at 48 h interval for 20 days. The results are shown in **Figure 3**. The values are varied depending on type of medium and cultivation time. Modified V-8 shows highest activity of pectinase of 1.23 unit/ml at 144 h. Thus, growth of *C. capsici* in Modified V-8 was followed by measuring concentration of remaining sugar and pH, the results are shown in **Table 2**. Concentration of remaining sugar and pH tend to decrease with increasing cultivation time. It could be explained that *C. capsici* consumed sugar for its growth as reported earlier (Cao et al. 2008). The consumption of sugar subsequently led to acidic media from cultivation.

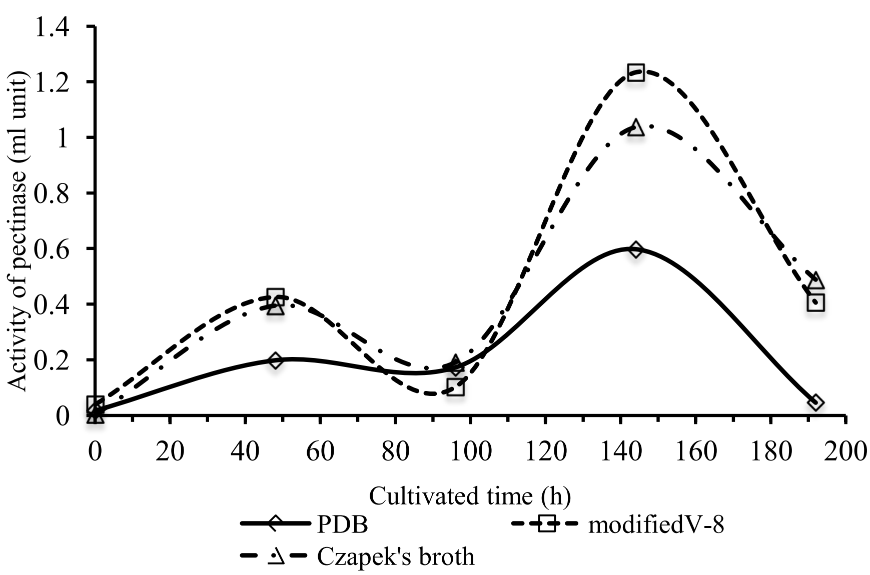

Figure 3 Relationship of pectinase activity in PDB, Modified V-8 and Czapek's broth and cultivation time.

Table 2 Relationship between cultivation time and concentration of remaining sugar, pH and activity of enzyme.

Time (h)	Concentration of remaining sugar (mg/ml)	pH	Activity (Unit/ml)
0	0.836	6.4	0.04
48	0.749	6.8	0.42
96	0.849	6.2	0.10
144	0.771	4.9	1.23
192	0.610	4.8	0.40

Modified V-8 of varied weights (0.5, 1.0, 1.5 and 2.0 g) which were cultivated for 144 h was directly added into scouring solution to remove pectin in cotton fabrics.

Properties of scoured cotton fabrics

Tri-stimulus coordinates, including x, y and z of scoured fabrics and unscoured were measured; their whiteness indices were calculated using Eq. (1) and the results are presented in **Figure 4**. Unscoured fabric (UT) shows lowest whiteness index among all samples. The cotton fabrics scoured with soapnut extract (S9) gave higher whiteness index that that of unscoured. The cotton fabrics scoured without pectinase (samples 0.0E, Prem and S9) show much lower whiteness indices than those of fabrics obtained from two-step scouring process (soaps; Premasol jet/soapnut extract and subsequently pectinase (samples Prem and S9)). The results attributed to the removal of wax from cotton fabrics in the first scouring step, penetration of pectinase into the cotton fibers for pectin removal was easier, thereby increasing the whiteness index of the samples.

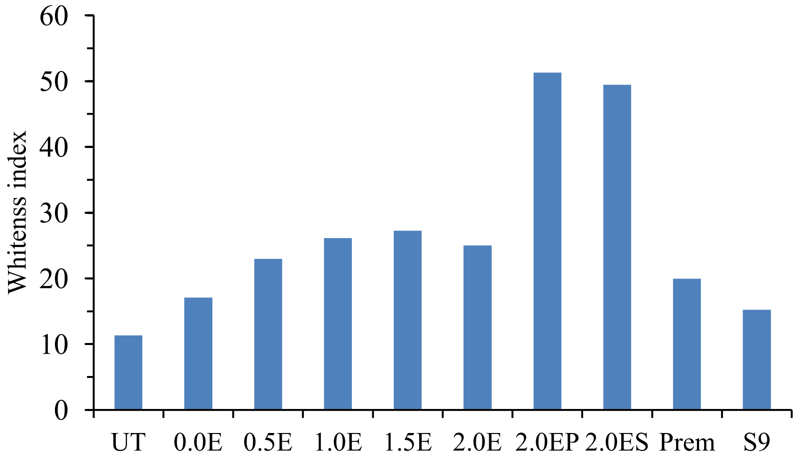

Figure 4 Whiteness indices of scoured cotton fabrics comparing with that of unscoured.

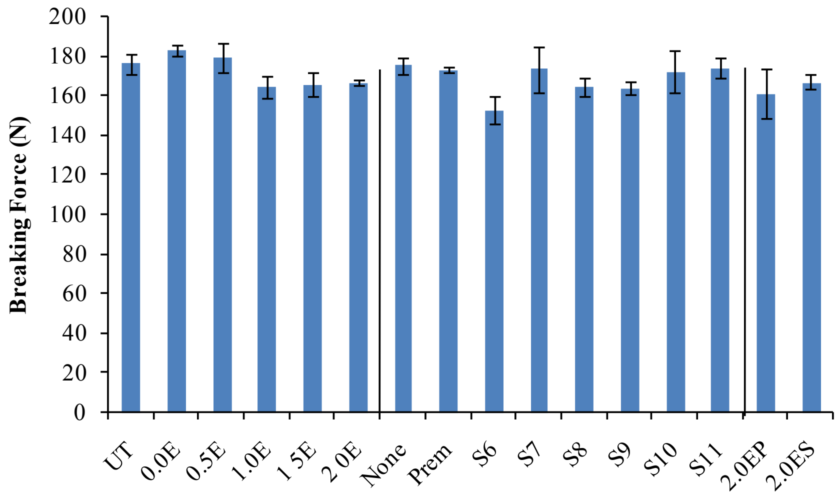

Figure 5 Breaking forces of scoured cotton fabrics comparing with that of unscoured.

In set of fabrics scoured with pectinase, the breaking forces of cotton fabrics scoured in solution containing high amount of pectinase (samples 1.0E, 1.5E and 2.0E) are slightly lower as compared with those scoured without and with small amount of pectinase (sample 0.0E and 0.5E, respectively) and unscoured as shown in **Figure 5**. In sets of fabrics scoured with surfactants, 6 %v/v soapnut extract (sample S6) gives lowest breaking force whereas the values of the fabrics scoured with higher concentration of soapnut extract (samples S10 and S11) is comparable to those scoured with Premasol jet (sample Prem) and without soap (sample None). Slightly, low breaking forces of fabrics scoured with pectinase and both soaps (sample 2.0EP and 2.0ES) are observed. This indicates damage of cotton fabrics upon scouring with an enzyme and alkaline solution.

Conclusions

C.capsici isolated from anthracnose infected chili fruit was successfully cultivated in PDB, Modified V-8 and Czapek's broths. Being cultivated up to 192 h, Modified V-8 gave highest activity, low sugar remaining and pH of pectinase at 144 h. In scouring process, the use of soapnut extract of 9.0 %v/v gave the highest performance of scouring in the first step. Combination of soapnut extract and pectinase in alkaline solution improved water absorbency and whiteness indices of cotton fabrics when compared with the set without soapnut and pectinase. However, breaking force of cotton fabrics scoured with soapnut extract/Premasol jet combined with pectinase were slightly lower which was affected by alkali used in scouring process. Performance of scouring process using soapnut extract is comparable to that of the commercial Premasol jet, thus soapnut extract may replace commercial surfactant for ecological purposes.

References

Ahlawat, S., Dhiman, S., Battan, B., Mandhan, R.P., & Sharma, J. (2009). Pectinase production by Bacillus subtilis and its potential application in biopreparation of cotton and micropolyfabrics. *Process Biochemistry 44*, 521-526.

Ahmed, H.E., & Kolisis, F.N. (2011). An investigation into the removal of starch paste adhesives from historical textiles by using the enzyme-amylase. *Journal of Cultural Heritage 12*(2), 169-179.

Aly, A.S., Moustafa, A.B., & Hebeishref, A. (2004). Bio-technological treatment of cellulosic textile. *Journal of Cleaner Production 12*(7), 697-705.

Attele A.S., Wu, J.A., & Yuan, C.S. (1999). Ginseng pharmacology. Multiple constituents and multiple actions. *Biochemical Pharmacology 58*(11), 1685-1693.

Cao, S., Zheng, Y., Tang, S., & Wang, K. (2008). Improved control of anthracnose rot in loquat fruit by a combination treatment of Pichia membranifaciens with $CaCl_2$. *International Journal of Food Microbiology 126*, 216-220.

Carr, C.M. (1995). *Chemistry of the textiles industry*. Blackie Academic & Professional, Glassgow, pp. 258-259.

Chen, J., Wang, Q., Hua, Z., & Du, G. (2007). Research and application of biotechnology in textile industries in China. *Enzyme and Microbial Technology 40*(7), 1651-1655.

Dini, I., Carlo, G., & Dini. A. (2009). Saponin in Ipomoea batatas tubers: Isolation, characterization, quantification and antioxidant properties. *Food Chemistry 113*(2), 411-419.

Draelos, Z.K. (1995). *Cosmetics: An overview*. *Current Problems in Dermatology 7*(2), 45-64.

Duff G.D., & Giles, H.C. (1972). Spectrophotometric determination of the critical micelle concentration of soaps. *Journal of Colloid and Interface Science 41*, 407-414.

Huang H.C., Liao, S.C., Chang, F.R., Kuo, Y.H., & Wu, Y.C. (2003). Molluscicidal Saponins from Sapindus mukorossi, Inhibitory Agents of Golden Apple Snails, Pomacea canaliculata. *Journal of Agricultural and Food Chemistry 51*(17), 4916-4919.

Kalliala, E. & Talvenmaa, P. (2000). Environmental profile of textile wet processing in Finland. *Journal of Cleaner Production 8*(2), 143-154.

Kurniawan, A., Sutiono, H., Ju, Y.H., Soetaredjo, F.E., Ayucitra, A., Yudha, A., & Ismadji, S. (2011). Utilization of rarasaponin natural surfactant for organo-bentonite preparation: Application for methylene blue removal from aqueous effluent. *Microporous and Mesoporous Materials 142*(1), 184-193.

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

Maximo, C., & Costa-Ferreira, M. (2004). Decolourisation of reactive textile dyes by Irpex lacteus and lignin modifying enzymes. *Process Biochemistry 39*, 1475-1479.

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

Robinson, T., Chandran, B., & Nigam, P. (2001). Studies on the production of enzymes by white-rot fungi for the decolourisation of textile dyes. *Enzyme and Microbial Technology 29*(8-9), 575-579.

Safarina, R., Sudarsono, F., & Mulyaningsih, B. (2014). Larvicidal activity of the mixture of cashew nut shell liquid (CNSL) and aqueous extract of Sapindus rarak DC against larvae of Culex quinquefasciatus. *Biology, Medicine, & Natural Product Chemistry 3*(1) 23-26.

Saxena, D., Pal, R., Dwivedi, A. K., & Singh, S. (2004). Characterisation of sapindosides in Sapindus mukorosii saponin (reetha saponin) and quantitative determination of sapindoside B. *Journal of Scientific and Industrial Research 63*(2), 181-186.

Shiau, I.L., Shih, T.L., Chen, H.T., Lan, H.F., Lin, H.C., Yang, B.Y., Ko, C.H., & Murase, Y. (2009). Quantification for saponin from a soapberry (Sapindus mukorossi Gaertn) in cleaning products by a chromatography and two colorimetric assays. *Journal Faculty of Agriculture Kyushu University 54*(1), 215-221.

Sparg S.G., Light M.E., & Staden J.V. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology 94*(2-3), 219-243.

Sumathi, S., & Manju, B.S. (2000). Uptake of reactive textile dyes by Aspergillus foetidu. *Enzyme and Microbial Technology 27*(6), 347-355.

Tanapongpipat, A., Khamman, C., Pruksathorm, K., & Hunsom, M. (2008). Process modification in the scouring process of textile industry. *Journal of Cleaner Production 16*(1), 152-158.

Wang, Q., Fan, X., Hua, Z., Gao, W., & Chen, G. (2007). Degradation kinetics of pectins by an alkaline pectinase in bioscouring of cotton fabrics. *Carbohydrate Polymers 67*(4), 572-575.

Wina, E., Muetzel, S., Hoffmann, E., Makkar, H.P.S., & Becker, K. (2005). Saponins containing methanol extract of *Sapindus rarak* affect microbial fermentation, microbial activity and microbial community structure *in vitro*. *Animal Feed Science and Technology 121*(1-2), 159-174.