(REFEREED RESEARCH)

INVESTIGATION OF BIOSURFACTANT USAGE IN RAW WOOL SCOURING BY RESPONSE SURFACE METHODOLOGY

YÜNÜN YIKANMASINDA BİYOYÜZEY AKTİF MADDE KULLANIMININ TEPKİ YÜZEY YÖNTEMİYLE İNCELENMESİ

Ebru BOZACI

Ege University, Faculty of Engineering, Department of Textile Engineering, Bornova 35100, Izmir, Turkey

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ABSTRACT

In recent years, there is a growing demand for eco-friendly chemicals for sustainable textiles. This paper examines an eco-friendly chemical, plant-derived biosurfactant, for scouring of raw wool. Box–Behnken response surface experimental design was chosen to study and optimize the influence of process variables; on the whiteness, weight loss and grease content of wool fibers. The regression model provided a good explanation of the relationship among the independent variables and the response. The morphological and chemical changes of wool fibers were investigated by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR), respectively. The chemical oxygen demand (COD) of the effluents was measured in order to determine wastewater quality. The achieved results indicated that the plant-derived biosurfactant *Quillaja saponaria* saponin could be an environmental friendly alternative for scouring of raw wool.

Keywords: Sustainability; Eco-friendly; Wool; Biosurfactant; Saponin; Box-Behnken Experimental Design

ÖZET

Son yıllarda, sürdürülebilir tekstil ürünleri için çevre dostu kimyasallara yönelik artan bir talep vardır. Bu çalışmada, yünün yıkanması için çevre dostu kimyasal olarak bitkisel kaynaklı biyoyüzey aktif madde kullanımı incelenmiştir. İşlem değişkenlerinin yün liflerinin beyazlığına, ağırlık kaybına ve yağ içeriğine etkisini incelemek ve optimize etmek için Box-Behnken tepki yüzey deneysel tasarımı kullanılmıştır. Regresyon modeli, bağımsız değişkenler ve yanıt arasındaki ilişki hakkında iyi bir açıklama sağlamıştır. Yün liflerinin morfolojik ve kimyasal değişimleri taramalı elektron mikroskobu (SEM) ve Fourier dönüşüm infrared spektroskopisi (FTIR) kullanılarak araştırılmıştır. Atık suyun kalitesini belirlemek için atık suların kimyasal oksijen ihtiyacı (COD) ölçülmüştür. Elde edilen sonuçlar, bitkisel kaynaklı biyoyüzey aktif madde *Quillaja saponaria* saponin'in yünün yıkanması için çevre dostu bir alternatif olabileceğini göstermiştir.

Anahtar Kelimeler: Sürdürülebilirlik; Çevre dostu; Yün; Biyoyüzey aktif madde; Saponin; Box-Behnken Deneysel Tasarım

Corresponding Author: Ebru Bozacı, ebrubozaci@gmail.com; ebru.bozaci@ege.edu.tr.

1. INTRODUCTION

The world is faced with major environmental problems like pollution of water, air, and soil (1). The textile processing industry is one of the main environmental polluters with high amounts of chemical, water and energy consumption (2). In recent decades, the interest in sustainable production methods and chemicals in textile industry has increased in accordance with health and environmental concerns (3).

Wool fibers are one of the most important natural fibers for textile industry due to their unique properties like warmth retention, resiliency, and handle. Raw wool generally contains less than 50% of clean fiber, being heavily contaminated by sand, dirt, wool wax, suint, and vegetable matter. In order to achieve satisfying dyeings, it is necessary to remove these impurities efficiently by scouring. The waste water load of a wool scouring mill can be equivalent to the normal discharge level of a small town (4-8). Scouring is the

first stage of wool processing and several methods (emulsion, suint, solvent extraction and refrigeration, ultrasound etc.) can be used to remove dirt, suint and wool grease from the surface of the fiber (4). Conventionally, scouring of wool is carried out by using detergent and alkali. This treatment emulsifies the waxes and breaks down peptide bonds into water-soluble or water-emulsifiable products. This process effectively removes all the dirtiness that present in wool fibers but the effluent has high biochemical and chemical oxygen demand, and alkalinity, so its ecologically unwanted. Wool scouring effluents cannot be discharged without further chemical or biological treatments. In addition, some of the detergents and auxiliaries used in wool scouring have adverse effects, so these drawbacks led to a consideration of ecofriendly wool scouring alternatives (9). Hereof various ecofriendly methods like ultrasound, plasma and enzyme usage were studied by several researches (4, 7, 9-18). The use of ecofriendly chemicals can be another way to limit the environmental impact of scouring process. There are only a few researches in the literature about usage of environmentally friendly washing agents (19, 20).

Surfactants can be derived from chemical (synthetic surfactants) or biological (biosurfactants) sources (21, 22). Although chemically synthesized surfactants have been utilized in various industrial applications, some of them have important drawbacks like polluting the environment, disrupting endocrine hormones, slow degradation process, persistence in nature, toxicity and leading secondary pollution etc. (22-27). Biosurfactants are eco-friendly materials which are produced by fungi, bacteria, and yeasts (28, 29). Biosurfactants have several advantages compared to the synthetic surfactants including non-toxicity, effectiveness under high pH and temperature, biodegradability and biocompatibility (29). Besides they do not cause secondary pollution even if they are leaked and discharged to the ecosystem (30).

Saponins are a structurally diverse class of compounds broadly spread in the plants. They can be isolated from different parts of plant like roots, pericarps, stems, bark, leaves, seeds, fruits and flowers. Saponins are surface active substances because of their hydrophilic and hydrophobic components (31). These natural surface active compounds are able to reduce surface and interfacial tension between different fluid phases owing to their water and fat-soluble components (3). There are numbers of papers concerning saponin usage in many different industries (3, 14, 30-34). On contrary to synthetic surfactants, plant derived saponin is cost-effective, environmentally safe, readily biodegradable, renewable as well as ecologically adaptable and is more effective than the synthetic surfactants (22-25, 35). One of the most important sources of saponin is *Quillaja saponaria* tree. The bark of this tree is used for the production of triterpenoid saponin owing to its high content of saponin and ease of transportation (30-31).

In this study, plant-derived *Quillaja saponaria* saponin usage for ecofriendly wool scouring process is optimized by Box-Behnken experimental design and correlated with process variables. In order to evaluate effects of wool scouring, the whiteness, weight loss, grease content and chemical oxygen demand were tested. FTIR, SEM, the energy dispersive X-ray (EDX) analysis of samples were also evaluated. According to our knowledge this is the first report describing the usage of *Quillaja saponaria* saponin for scouring of raw wool. The results showed that owing to its unique properties, *Quillaja saponaria* is a promising surfactant for wool scouring when compared to commercial washing agents.

2. MATERIAL AND METHODS

2.1. Materials

The raw greasy wool fiber of Turkish origin with a mean diameter of 25.5 μm was used for the scouring experiments. Biosurfactant used for scouring was plant-derived *Quillaja saponara* saponin (Sigma-Aldrich) and contained β-D-glucuronic acid with carboxyl group of sugar moiety in hydrophilic fraction Molecular structure of Quillaja saponin is given in Figure 1 (4, 31). A biosurfactant and commercial nonionic washing agent (fatty alcohol ethoxylates based) was used as detergent, with sodium carbonate as the builder. The chemicals were used as received.

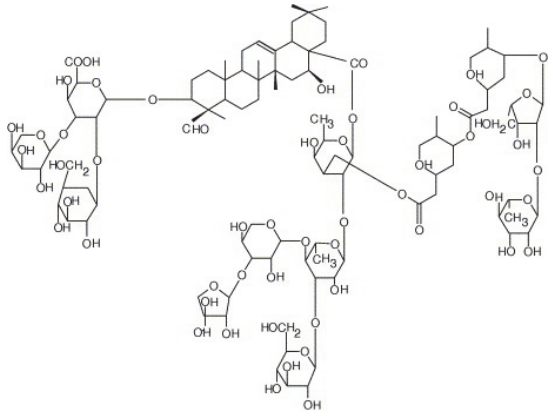

Fig.1. Chemical structure of saponin (31)

2.2. Scouring Procedure

The scouring process was performed with a liquor ratio of 1:50, for 20 minutes at 50 °C temperature. According to the experimental design this process was repeated for one to three times. After the scouring process, all samples were rinsed at 95 °C for 10 minutes, 65 °C for 10 minutes and rinsed under tab water for 10 minutes. The samples were air dried.

2.3. Experimental design

The Box–Behnken design (BBD) is an independent, rotatable quadratic design with no embedded factorial or fractional factorial points, where the variable combinations are at the midpoints of the edges of the variable space and at the center (36-37). The Box–Behnken design has been used for many industrial applications. It not only helps in determining the accurate optimum values of experimental parameters but also provides the possibility to evaluate the interaction between variables with a reduced number of experiments (38-41).

The Box-Behnken response surface experimental design basically involves three major steps; performing the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response and checking the adequacy of the model (39, 41). In this study, three factors with three levels of Box–Behnken response surface experimental design (BBD) was chosen to study and optimize the influence of surfactant type (saponin, saponin and alkali, washing agent and alkali), surfactant concentration (1–3 g/L) and washing steps (1-3 times), on the whiteness, weight loss and grease content of raw wool. The low, middle, and high levels of each variable were designated as −1, 0, and +1, and given in Table 1. The actual design of experiments is shown in Table 2.

Table 1. The levels of variables chosen for the trials

	−1	0	1
Surfactant type, X_1	Saponin (S)	Saponin+Alkali (S+A)	Washing agent+ Alkali (N+A)
Surfactant concentration , X_2, g/L	1	2	3
Washing steps, X_3	1	2	3

Table 2. The Box-Behnken design for the three independent variables

Trial No	X_1	X_2	X_3
1	-1	-1	0
2	0	1	1
3	0	0	0
4	0	0	0
5	1	-1	0
6	0	1	-1
7	1	0	1
8	0	-1	-1
9	1	0	-1
10	0	0	0
11	1	1	0
12	-1	1	0
13	-1	0	-1
14	0	-1	1
15	-1	0	1

Experimental data were fitted to a second-order polynomial mathematical equation in order to express the relationship between independent variables and responses. The generalized form of second-order polynomial equation was given as follows:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} x_i x_j + e_i \quad (1)$$

where Y is the response; x_i and x_j are the variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are the interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters ($k = 3$ in this study); and e_i is the error (39, 41).

2.4. Evaluation

The weight loss (WL) of the samples after washing was determined as follows:

$$\text{Weight loss, \%} = \frac{W1 - W2}{W1} \times 100 \quad (2)$$

Where, W_1 is the constant weight of unwashed sample and W_2 is the constant weight of the washed sample, respectively. The whiteness (WI) degrees of the samples were measured with Hunter Lab ColorQuest II spectrophotometer according to CIELab formula. Ten measurements were carried and the average values were calculated. The grease content (GC, %) of all the samples were analyzed according to the procedure described elsewhere (42). The Fourier transform infrared spectra of the samples were recorded on Perkin Elmer (Spectrum 100, USA) in the attenuated total reflectance (ATR) mode using a diamond/zinc selenide crystal in 4000-650 cm^{-1}. The surface morphology of the fibers was investigated by a scanning electron microscope (SEM, Carl Zeiss 300VP, 1000x). Furthermore, the energy dispersive X-ray (EDX) analysis was also performed to obtain the elemental composition of each fiber surface. The chemical oxygen demand (COD) was tested photometrically with a Merck SQ 300 Water and Wastewater Spectrometer. Also, the results were compared using ANOVA.

3. RESULTS AND DISCUSSION

The mathematical relationship between the experimental variables (X_1, X_2 and X_3) and weight loss (WL, %), whiteness index (WI) and grease content (GC, %) of wool samples in coded values was determined as:

The statistical significance of the above equations was checked by the F test and the analysis of variance (ANOVA) second order of regression model is presented in Table 3. The F value and P value represent the significance level of generated model and coefficients of regression, respectively (40-41, 43-44).

$$WL, \% = 37.3 + 1.31\ X_1 + 1.29\ X_2 - 0.58\ X_3 - 0.023\ X_1{\times}X_1 + 0.070\ X_1{\times}X_2 - 0.023\ X_1{\times}X_3 - 0.012\ X_2{\times}X_3 - 0.254\ X_2{\times}X_2 + 0.268\ X_3{\times}X_3 \quad (3)$$

$$WI = -40.3 + 3.86\ X_1 + 4.74\ X_2 + 2.62\ X_3 - 0.560\ X_1{\times}X_1 - 0.445\ X_1{\times}X_2 + 0.450\ X_1{\times}X_3 + 0.203\ X_2{\times}X_3 - 0.857\ X_2{\times}X_2 - 0.298\ X_3{\times}X_3 \quad (4)$$

$$GC, \% = 1.53 - 0.548\ X_1 + 0.055\ X_2 - 0.249\ X_3 + 0.102\ X_1{\times}X_1 - 0.0008\ X_1{\times}X_2 - 0.0060\ X_1{\times}X_3 + 0.0119\ X_2{\times}X_3 - 0.0289\ X_2{\times}X_2 + 0.0313\ X_3{\times}X_3 \quad (5)$$

Table 3. Regression analyses for the WL, WI, GC

	WL, %						WI						GC, %					
Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*	DF	Seq SS	Adj SS	Adj MS	*F*	*P*	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Regression	9	16.886	16.886	1.8762	6.64	0.025	9	92.1	92.1	10.2333	4.46	0.057	9	0.346101	0.3465	0.0385	10.3	0.01
Linear	3	16.317	16.317	5.4389	19.3	0.004	3	86.566	86.566	28.8555	12.57	0.009	3	0.29926	0.2995	0.099828	26.7	0.002
Square	3	0.5471	0.5471	0.1824	0.65	0.618	3	3.764	3.7639	1.2546	0.55	0.672	3	0.046133	0.0463	0.015429	4.12	0.081
Interaction	3	0.0221	0.0221	0.0074	0.03	0.994	3	1.769	1.7691	0.5897	0.26	0.854	3	0.000709	0.0007	0.000242	0.06	0.977
R-Sq = 92.3% R-Sq(adj) = 78.4%							R-Sq = 88.9% R-Sq(adj) = 69.0%						R-Sq = 94.9% R-Sq(adj) = 85.7%					

It can be seen from Table 3, that the *F*-statistics values for the regressions are high. This result shows that most of the variation in the response can be explained by the regression model equation. The lower *P*-value indicates that the model is considered to be statistically significant (36-37). The adequacy of the model was further verified with analysis of variance (ANOVA). The correlation coefficient (R^2) between the observed and the calculated values were obtained from the above equations. For weight loss, whiteness index and grease content correlation coefficients are recorded as 0.923, 0.889 and 0.949 respectively (Table 3). According to literature data, R^2 should be bigger than 80%. All the R^2 values were calculated >80%, indicating that the regression models could explain 92.3, 88.9 and 94.9% of the variability in the response. This displays that the models can explain the responses successfully (36, 45-46).

The significance of the regression coefficients on responses was determined using *P*-value and the results are given in Table 4. The *P*-values were used to check the consequences of interactions among the variables. In general, the smaller the *P*-value indicates the greater significance of the corresponding coefficient term. After ANOVA analyses, all the model terms having *P*-values greater than 0.05 were eliminated, as they were not statistically significant at the 95% confidence level. It can be seen from the Table 4 that surfactant type (*P*=0.001) for WL, %; surfactant type (*P*=0.029) and washing steps (*P*=0.004) for WI; surfactant type (*P*=0.001) and washing steps (*P*=0.003) for GC, % were the most significant factors on the model (40-41, 45-47).

Table 4. ANOVA and estimated regression coefficients

	WL, %				WI				GC, %			
Term	Coef	StDev	T	*P*	Coef	StDev	T	*P*	Coef	StDev	T	*P*
Constant	41.423	0.3069	134.987	0.000	-23.9	0.8749	-27.313	0.000	0.487	0.03527	13.808	0.000
X_1	1.3074	0.1879	6.957	0.001	1.63	0.5358	3.04	0.029	-0.1524	0.0216	-7.055	0.001
X_2	0.3873	0.1879	2.061	0.094	0.83	0.5358	1.551	0.182	-0.0389	0.0216	-1.803	0.131
X_3	0.4247	0.1879	2.26	0.073	2.73	0.5358	5.104	0.004	-0.1126	0.0216	-5.212	0.003
$X_1 \times X_1$	-0.0234	0.2766	-0.085	0.936	-0.56	0.7886	-0.71	0.509	0.1022	0.03179	3.215	0.024
$X_2 \times X_2$	-0.2539	0.2766	-0.918	0.401	-0.86	0.7886	-1.087	0.327	-0.0289	0.03179	-0.911	0.404
$X_3 \times X_3$	0.2679	0.2766	0.969	0.377	-0.3	0.7886	-0.378	0.721	0.0313	0.03179	0.985	0.37
$X_1 \times X_2$	0.0696	0.2658	0.262	0.804	-0.45	0.7577	-0.588	0.582	-0.0008	0.03054	-0.025	0.981
$X_1 \times X_3$	-0.0234	0.2658	-0.088	0.933	0.45	0.7577	0.594	0.578	-0.006	0.03054	-0.196	0.852
$X_2 \times X_3$	-0.0119	0.2658	-0.045	0.966	0.2	0.7577	0.268	0.799	0.0119	0.03054	0.388	0.714

3.1. Weight Loss

Figure 2 shows the results of the weight loss values (WL, %) for wool fibers which were subjected to three different treatment solutions.

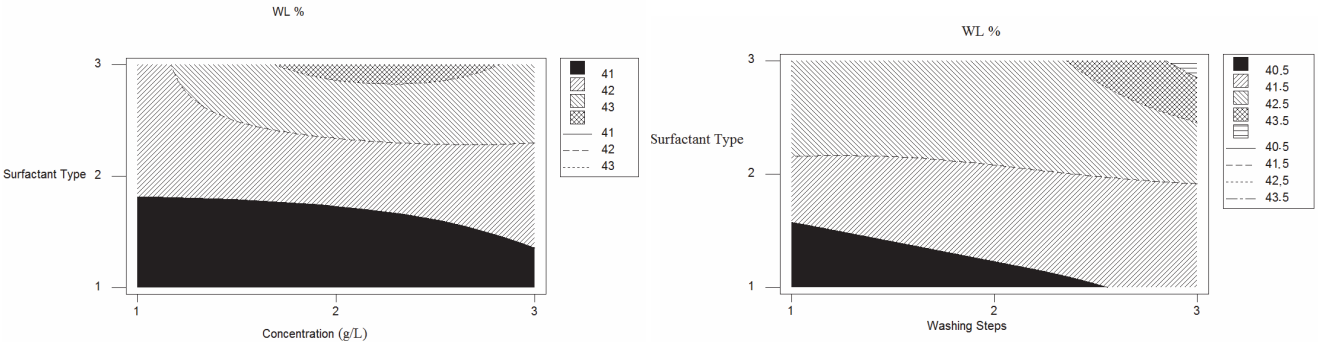

Fig. 2 Contour plots representing the effects of surfactant type, concentration and washing steps on weight loss % (WL, %)

The weight loss occurred in scouring was due to the removal of sand, dirt, wool wax, suint, and vegetable matter (4, 5). As seen from the Figure 2 surfactant type affects the weight loss of the raw wool fibers after scouring process. The order of weight loss is found to be only saponin< saponin+alkali<washing agent+alkali. Although the minimum weight loss was obtained after scouring with only saponin, the achieved results were not too low when compared to alkali containing solutions. Unlike commercial washing agent, the increase in concentration did not have any effect on the weight loss for saponin containing solutions. The weight losses after consecutive washings were increased for all washing solutions, but the increases were insignificant. These results showed that in the first step of the washing, the main extent of the contamination was removed. Therefore, it is thought that high weight loss after first step was caused by the removal of the dirt and sand, which are heavy in weight (7). From the weight loss results, it can be concluded that saponin has comparable cleaning effect in mild acidic and alkali solution when checked against commercial product.

3.2. Whiteness Determination

Wool has a natural creamy color, but when wool is sheared from the sheep it is not possible to see this color. The reason for this is raw wool fibers are contaminated by the sheep's environment, and some of these unwanted impurities give wool a soiled color. Chemicals used during scouring process are especially focused on the removal of these contaminations. Removal of these impurities by scouring process also causes whiteness improvement. In order to evaluate the effect of the different scouring solutions on color of the wool fibers, whiteness index values have been collected according to the CIELab formula (7, 48). The results of the whiteness measurement values of wool fibers are given in Figure 3.

The results showed that the whiteness values obtained were the same for only saponin and saponin+alkali solutions at 1 g/L (Figure 3). Above this concentration, the increase in whiteness values was not in the same rate, but the maximum difference was only 2% under the experimental conditions. The concentration of washing agent+alkali containing solution, should be between 1.4 g/L and 2.7 g/L to reach the maximum whiteness value. However, no further increase was observed at concentrations higher than 2.7 g/L. The increase in whiteness index for all scouring solutions was ~60% after first washing step and reached up to ~65-70% after third step. The whiteness index (WI) of the raw wool fiber was measured as -62.86 according to CIELab formula, which indicates that the fibers used in the study had a very dirty appearance. After scouring, the light brownish appearance of the raw wool samples became creamy on all of the treated samples. When the samples were observed with the naked eye, there was no obvious difference between the color of the samples scoured by saponin containing solutions by the color of samples scoured by washing agent.

3.3. Grease content

Raw wool is difficult to spin because of the natural grease, wax, suint, vegetable materials, dirt and etc. Finer varieties of wool may contain fats and suint as much as 50% of the weight of raw wool. Therefore unlike natural cellulose fibers, wool fibers are scoured in fleece form. During scouring, approximately 50-60% of the wool grease is saponified while the rest wool grease is emulsified into the detergent solution. Meanwhile the suint dissolves, and the mineral particles, many of them less than 1μm in diameter become suspended. Wool grease is insoluble in water but soluble in many organic solvents. Wool grease is a mixture of high molecular alcohol, and fatty acids in free state and wax-like esters. Natural soap, which is formed by saponified fatty acids during scouring also promotes washing and low consumption of detergent (49). The optimal residual wax content should be of the order of 0.5 to 0.75%.

If these amounts are exceeded, this causes problems in carding, doffing and subsequent dyeing process. On the contrary, very low wax content damages the handling of wool, as well as its spinning properties (8). In this study, saponin and a washing agent were examined to achieve satisfactory residual grease content on wool.

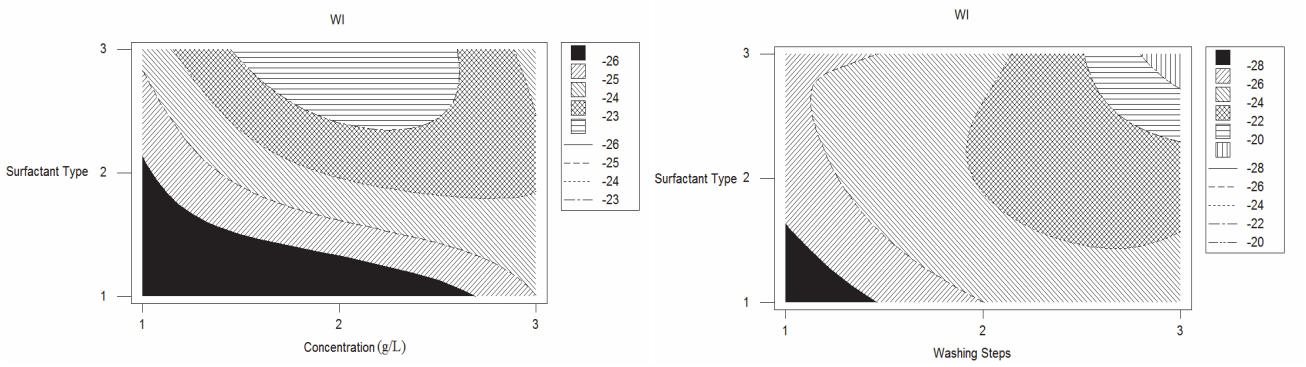

Fig. 3 Contour plots representing the effects of surfactant type, concentration and washing steps on whiteness index (WI)

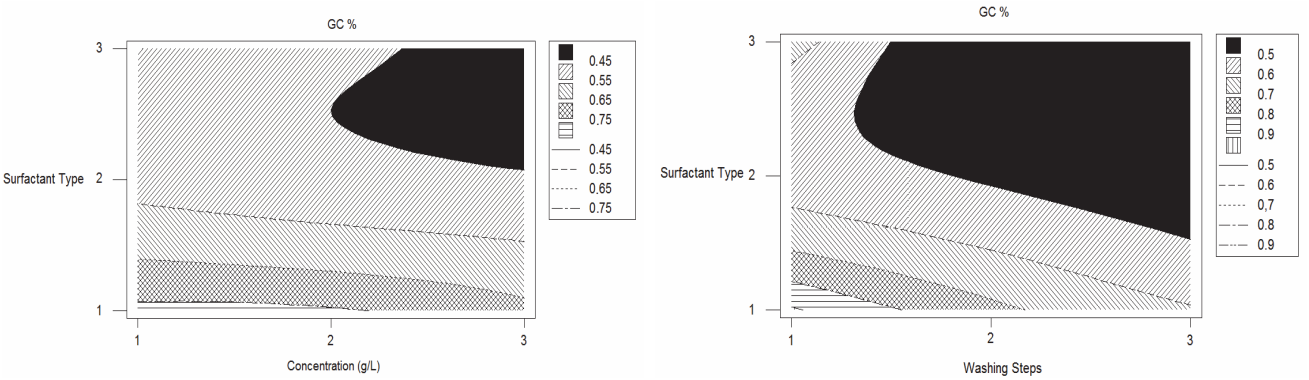

Fig. 4 Contour plots representing the effects of surfactant type, concentration and washing steps on grease content (GC, %)

It was found that the increase in concentration of the saponin and washing agent is resulted with lower residual grease content. But the decrease was not significant according to the *P*-test (Figure 4). The values have decreased from higher than 0.75% to 0.65% and 0.55% to 0.45%, respectively. Unlike other detergents, the increase in concentration of surfactant in saponin+alkali containing solution did not have any effect on the grease content (0.55%). When the washing steps were increased from 1 to 3, the residual grease content decreased for saponin scouring from 0.8% to 0.7%, for saponin+alkali scouring from 0.6% to 0.5%, and for washing agent+alkali scouring from 0.7% to 0.5%, respectively. The lowest residual grease contents were achieved by washing agent+alkali treatment on all treatment parameters. The sizes of Quillaja saponin micelles have little dependence on pH and saponin solutions are useful in exploring their ability to extract solutes of biological significance (51). The findings of this study is consistent with the literature information that Quillaja saponins were effective in different pH's and gave sufficient scouring in terms of residual grease content (51). It is well known that wool is sensitive to higher pH values and usage of saponin without alkali may have several advantages. The usage of 1 g/L saponin and 3 washing steps; 1 g/L saponin+alkali and 1 washing step; 1 g/L washing agent+alkali and 1 washing step could be sufficient to achieve an acceptable residual grease content after scouring the wool (8, 51).

3.4. FTIR Analyses

The FTIR analysis was performed to find out the effect of scouring on the chemical functional groups of wool samples (13). The FTIR spectra of raw and scoured wool samples are shown in Figure 5.

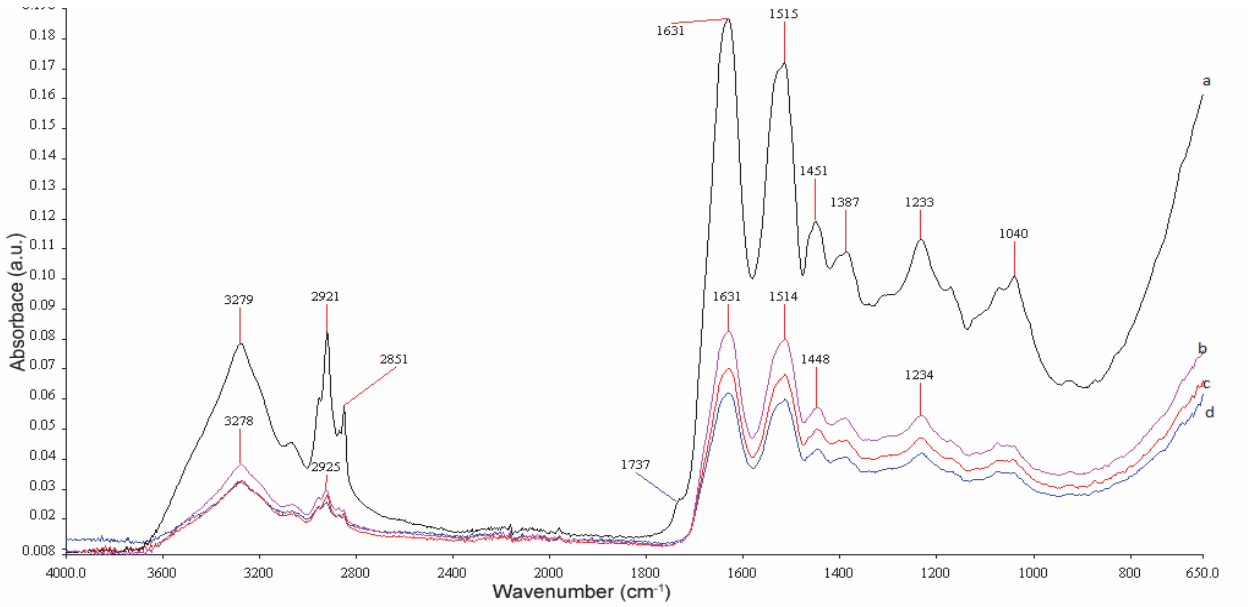

Fig.5 ATR-FTIR spectra of a) raw, b) saponin, c) saponin+alkali, and d)washing agent+alkali treated wool samples

In general, lower absorption values with different intensity levels were observed in the case of scoured wool fibers compared to raw wool, and no new chemical bonds or free residues were occurred in wool fiber (9, 13-14). The peak at 3278 cm^{-1} is associated with hydrogen bond stretch of OH groups. Probably the hydroxyl group containing amino acid residues in the wool protein, was originally attached with the fatty acids of greasy substances. The intensity of the peak was lowered after different scouring treatments (13). The alkane group is one of the major components that form lipids and the wave number range of 3100-2800 cm^{-1} can be used to examine lipid chain packing (52). Therefore, it can be concluded that lower absorbance at this region for scoured wool samples is due to the removal of some waxy lipids from the fiber. The decrement of waxy lipids was confirmed by the results of grease content measurements for scoured and raw wool fibers (14). The two sharp peaks in the range of 2921-2851 cm^{-1} for raw wool fiber samples corresponds to C-H asymmetric/ symmetric stretch and similarly at 1485-1445 cm^{-1} for C-H bend (9). The intensity of these peaks has reduced after scouring treatment with saponin and washing agent containing solutions (C-H stretch and bend). This decrease suggests a disturbance of alkane groups in wool and removal of 18-MEA acid covalently bonded to epicuticle fibers (9, 14). Saponin, saponin+alkali and washing agent+alkali treated samples gave a lower absorbance in the region between 2200-1800 cm^{-1} associated with the ester linkage, apparently removal of lipid substances (13). The absorbency peak around 1737 cm^{-1} for raw wool is assigned to C = O stretching of carboxylic acid functional group which is covalently bonded to epicuticle through sulfoester (9, 52). It can be seen from the FTIR spectras of S, S+A and N+A treated wool fibers that peak at 1737 cm^{-1} disappeared, meaning that this bond has been broken down (9). Wool is a very complicated fiber and infrared absorption spectra of wool fiber keratin indicate characteristic absorption bands assigned mainly to the peptide bond (–CONH–) which represents the primary structural unit of the polypeptide chain (53). In the FTIR spectroscopy, the main characteristic peaks of wool appeared between 1000 and 1700 cm^{-1}, which are related to amide I (1600^{-1},700 cm^{-1}), amide II (1500–1600 cm^{-1}), and sulfoxide (1000–1150 cm^{-1}) bands (52, 53). The spectra of the raw and scoured wool samples showed similar structures between 1000 and 1700 cm^{-1}, which arise from the comparable absorbance values of absorption bands correlated with the peptide group being characteristic of keratin, and only the intensity of some functional groups was changed (52). All these changes in peak intensities point out the removal of greasy substances after scouring of raw wool fibers in the order of saponin<saponin+alkali<nonionic washing agent+alkali with small differences, which is confirmed by the grease residual test.

3.5. SEM-EDX Analyses

Scanning electron microscope (SEM) images were taken at 1000× and 5000× magnifications to examine the surface morphology of the wool fibers (Fig.6).

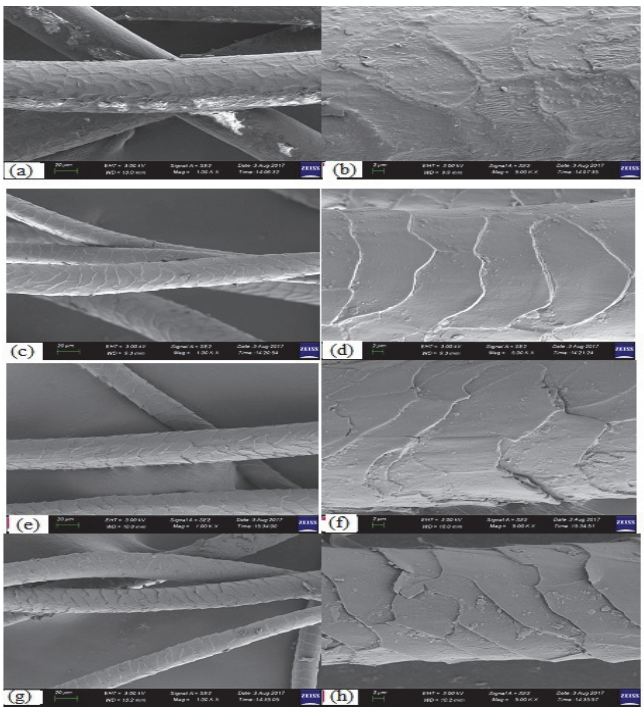

Fig. 6. SEM micrographs of a) and b) raw wool, 1000×- 5000×; c) and d) saponin scoured wool, 1000×- 5000×; e) and f) saponin+alkali scoured wool, 1000×- 5000×; g) and h) washing agent+alkali scoured wool, 1000×- 5000× samples.

The layers of greasy substances and the impurities which are covering the surface scales of the raw wool fibers can be seen clearly in Figure 6 (a) and (b). It can be seen from Figure 6 (c) to (h) that the scoured surfaces were clean, the continuous lipid layer on all samples was removed and the scales of wool became apparent. The peeling and damage of the cuticle structure occurred after scouring with washing agent, and also fractures and small scale particles were found on the wool fibers (Figure 6-h). The washing agent scoured wool fiber showed sharper cuticle cell edges compared to the saponin scoured fibers. These sharp edges are responsible for creating the distinctive friction and also fabric shrinkage during laundering. Considering SEM images, it can be said that saponin scouring is milder and caused less damage to wool fibers than the washing agent (10, 13, 54-55).

To evaluate the chemical composition of the fibers after scouring process, EDX analysis was carried out at magnification 1000 and the result are given Figure 7.

As seen from Figure 7, the ratio of chemical elements has changed on the surface of the scoured fibers. After three scouring processes, in contrary to increase in weight and atomic percentage of oxygen and nitrogen, carbon has decreased. The main reason for the decrease of carbon content is the removal of lipids etc. from the surface of the fibers. After scouring, the thin layer of lipids was cleaned and the protein structure became more apparent. This was also confirmed by SEM images. Consequently, the percentage of nitrogen atom increased after treatments with different scouring solutions. The sample treated with saponin in alkali conditions had the highest amount of nitrogen, 18.21% (weight, %) and saponin and washing

agent treated samples had nearly the same percentage, ~14%. The O/C ratio of saponin, saponin+alkali and washing agent+alkali scroured samples were 0.36, 0.52 and 0.41, respectively. In general, the increase in oxygen/carbon ratio demonstrates better wettability properties of the fibers (56). The Si and Al peaks of raw wool fiber disappeared after the scouring process. Therefore, it can be concluded that the 3 scouring solutions were effective to remove sand and soiled impurities.

3.6. COD

In order to determine the environmental impact of scouring, the chemical oxygen demand (COD) values for scouring wastewaters with different treatment solutions were measured and shown in the Figure 8.

Wool scouring wastewater is a highly concentrated and major pollutant source in the textile industry related to its very high oxygen demand. It contains detergent, suint, sheep dung, short wool and organic materials, such as lanonin, which was removed from the fiber during scouring and caused the increase in COD value. Figure 8 shows that, as the number of washing steps were increased, the COD values decreased for all the scouring processes. The reason for this is most probably the removal of impurities and contaminants during the first washing step. These results are consistent with the weight loss analysis (57). The total COD values of alkali containing solutions were higher than only saponin containing solutions. The pH value of effluents plays an important role in decreasing environmental impact. According to reported papers, the increase in pH value causes the increase in chemical oxygen demand. The findings of this paper were in accordance with the literature data (10, 58). Consequently, the use of only saponin and saponin+alkali has reduced the COD of scouring effluents 28% and 15%, respectively when compared to commercial washing agent.

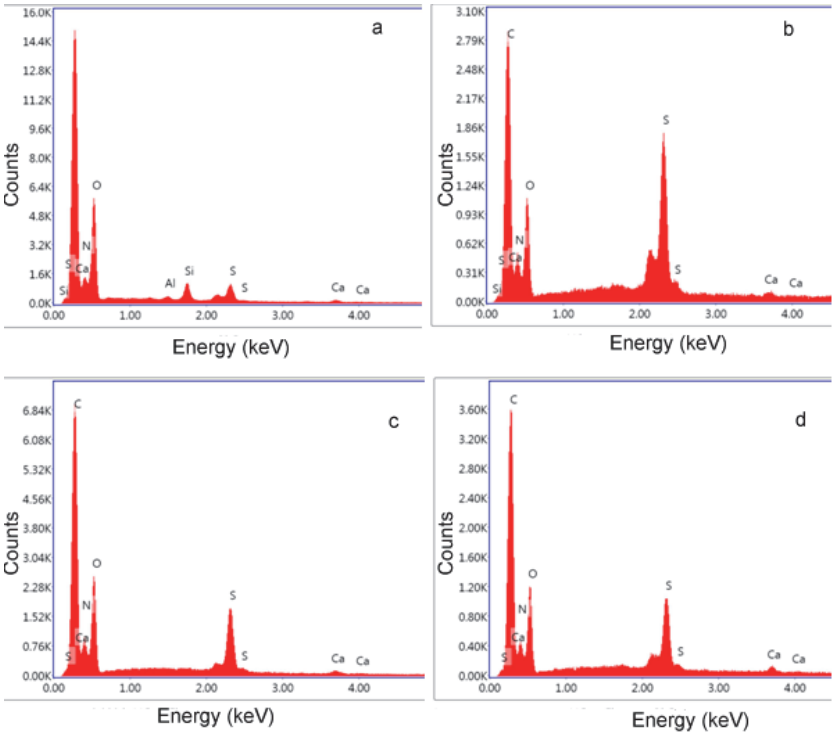

Fig. 7 EDX spectra of a) raw wool; b) saponin, c) saponin+alkali and d) washing agent + alkali scoured wool

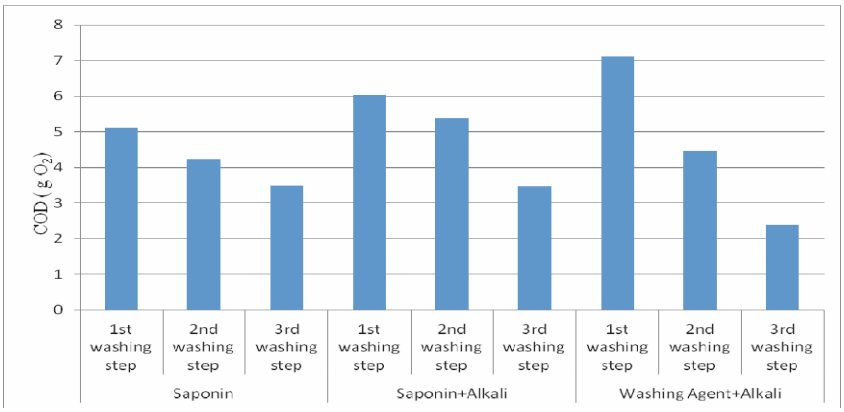

Fig. 8 Chemical oxygen demand of scouring effluents

4. CONCLUSION

This paper has focused on the usage of plant derived biosurfactant as an alternative bioscouring agent for wool scouring. The impact of plant derived Quillaja saponin on weight loss, whiteness degree, residual grease content, quality of wastewater, chemical structure, and surface morphology of wool was investigated and discussed. The findings were also checked against the results of commercial washing agent. The biosurfactant treated samples exhibited nearly similar, whiteness degree, and residual grease content with the washing agent. The reduction in COD of effluents was achieved with saponin usage both in mild acidic and alkali pH values. The COD of scouring effluents decreased up to 28% when compared to commercial washing agent. The FTIR spectras of the S; S+A and N+A scoured wool samples showed same peaks with different intensities. From SEM images, it can be concluded that saponin scouring is milder and caused less damage to wool fibers than the washing agent. The ATR-FTIR spectra and SEM-EDX images of raw and scoured wool samples confirmed the removal of greasy substances, sand, and soiled impurities after scouring. The bioscouring process of wool fiber was optimized with three-level three-factor Box-Behnken response surface experimental design. Regression equations were developed for weight loss, whiteness degree, and residual grease content using experimental data. It has been observed that model predictions are consistent with experimental results. As a result of Box-Behnken experimental design, the optimum conditions to achieve maximum WL, WI, and GC after scouring were 2.7 g/L saponin and 3 washing steps, 2 g/L saponin+alkali and 2 washing steps, 2.2 g/L washing agent and 2 washing steps, respectively. *Quillaja saponaria* saponin was used for raw wool scouring and the optimized conditions identified in this study may provide reference values for future studies. In conclusion, the results presented in this study showed that owing to its unique properties, *Quillaja saponaria* saponin could be an ecofriendly, promising alternative for scouring of raw wool.

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