STUDY OF DIFFERENT TREATMENT METHODS ON CHICKEN FEATHER BIOMASS

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ABSTRACT: Chicken feathers (CFs) make up to 10 % of total chicken dry mass and they have many potential industrial applications. CFs contain protein fibers called keratin, which is an insoluble protein. Primary sanitization phases are complex because of the presence of many blood-borne microbes, pathogens, and parasites in raw biomass. The extraction process of keratins from the unprocessed feathers is also a challenging task. Prior to the extraction, the cleaning/sanitization of feathers is a very necessary step. Thus, the present work was conducted to optimize an efficient surfactant for the cleaning process of the CFs using ionic and non-ionic surfactants. The experiment was conducted by the washing of feathers with double distilled water (ddH_2O), detergents, ether, and lastly with boiling water. The washed feathers were treated with surfactants (sodium dodecyl sulfate (SDS), cetrimonium bromide (CTAB) and polyethylene glycol (PEG)) and the effect of each surfactant was analyzed by a microbiological test that indicates the extent of the presence of different bacteria on the treated feathers. SEM, EDX, FTIR were used to study the morphology and composition of untreated and treated CFs. SEM showed no detectable fiber damage after treatment. Cetrimonium bromide (CTAB) (t3) was one of the best surfactants recommended for the treatment of CFs among all the surfactants used. The present study described the best treatment method for the CFs.

ABSTRAK: Bulu ayam (CF) terdiri daripada 10 % daripada total jisim bulu ayam kering dan terdapat pelbagai potensi aplikasi dalam industri. CF mengandungi gentian protein dinamakan sebagai keratin, iaitu merupakan protein tidak larut. Sanitasi primer adalah kompleks kerana kehadiran banyak mikrob bawaan dalam darah, patogen dan parasit dalam biojisim mentah. Proses pengekstrakan keratin daripada bulu yang masih belum diproses juga adalah tugas yang mencabar. Proses ekstrak pengeringan/sanitasi bulu juga adalah langkah yang sangat diperlukan. Oleh itu, kajian ini dijalankan untuk mengoptimumkan kadar surfaktan yang berkesan untuk proses pembersihan pada CF dengan menggunakan surfaktan ionik dan bukan ionik. Eksperimen ini dijalankan dengan membasuh bulu dengan air suling double (ddH_2O), bahan pencuci, eter dan akhir sekali dengan air mendidih. Bulu yang telah dibasuh dirawat dengan surfaktan (natrium dodecyl sulfate (SDS), cetrimonium bromide (CTAB) and polyethylene glycol (PEG)) dan kesan terhadap setiap surfaktan dianalisa dengan ujian mikrobiologi. Kehadiran bakteria pada bulu yang telah dirawat telah didedahkan melalui ujian mikrobiologi ini. SEM, EDX, FTIR telah digunakan untuk mengkaji morfologi dan komposisi CF yang tidak dirawat dan dirawat. SEM menunjukkan tiada kerosakan serat dikesan selepas rawatan. Cetrimonium bromide (CTAB) (t3) adalah salah satu surfaktan terbaik yang

disyorkan untuk rawatan CF berbanding surfaktan lain. Kajian ini menjelaskan kaedah rawatan terbaik bagi CF.

KEYWORDS: *feathers; surfactants; FTIR; sanitization*

1. INTRODUCTION

Feathers are one of the byproducts of poultry farming. Chicken feathers (CFs) are the waste biomass of the poultry industry with more than 4×10^6 tons generated per year worldwide [1]. Generally, CFs are turned into low value feedstock [2] or sent into landfills [3]. When fresh feathers are removed from the chicken, they can contain waxy, fatty substances and some blood-borne pathogens that can cause various human health hazards. It is very important to sanitize/cleanse the CFs before their use in the laboratory.

CFs are comprised of protein fibers named keratin; which is also one of the main components of wool. CFs consist of 91% keratin macromolecules with an average molecular weight of 10-20 kDa [4, 5]. The keratin is an insoluble protein commonly found in avian and mammalian species, in their beaks, claws, hooves, horns, hairs, nails, wool, and feathers [6-8]. The keratin consists of an α-helix and β-sheet conformation [9]. CFs have potential applications in biopolymers, regeneration, fabrication, bioplastics and tissue engineering [10-13]. They are abundant, biodegradable, and have guaranteed supply, which makes them an interesting choice for research. Besides this, the adequate cleaning and sanitizing of CFs before their use as laboratory material is a crucial task.

Feathers can be a habitat for some microorganisms such as *Escherichia* spp., *Salmonella,* and *Pseudomonas* spp. As the freshly plucked feathers are prone to the blood-borne pathogens, their sanitization/cleaning can be performed with different surfactants and organic solvents, as reported previously [14-16]. It is important to study the effect of the treatments on the morphology and composition of keratin proteins. Feather-borne parasites and microorganisms can be neutralized by physical and chemical means [17]. Surfactants are comprised of hydrophobic and hydrophilic chemical groups in their chemical structures and they are efficient to remove the insoluble, waxy, and fatty dirt particles [18]. Surfactants have been proven to exhibit decontamination ability but their antibacterial effects are largely undocumented with few sources [19]. Two types of hydrocarbon contaminants generally bind to feathers; one having a low boiling point and the other has a higher boiling point. The latter is difficult to remove due to its strong binding. High concentrations of the surfactant are generally used to remove large amount of contaminants [16]. These also have wide applications in removing oily particles in soft water, in contrast to hard water that adversely affects the cleaning performance.

The aim of this study is to screen for the best surfactant for the sanitization/decontamination of the CFs and effects of treatments of surfactants on the morphology and chemistry of feathers were explored. The method includes the washing of the CFs with detergent and ether after receiving them from the slaughterhouse. Different surfactant treatments were given to the CFs. Then, microbial testing, visual observation, Scanning Electron Microscope Energy Dispersive X-ray spectroscopy (SEM-EDX), Fourier Transform Infrared (FT-IR) spectroscopy, Thermo Gravimetric Analysis (TGA) were used to characterize the treated and untreated CFs.

2. MATERIALS

Fresh chicken feathers from slaughtered adult chickens with a length of 2-25 cm were supplied by a chicken processing plant at Jaya Gading, Kuantan, Malaysia. Sodium

dodecyl sulfate (SDS) and cetrimonium bromide (CTAB) were purchased from Fisher Scientific UK Ltd, Bishop, United Kingdom. Polyethylene glycol (PEG) was acquired from Merck Schuchardt OHG, Germany. All other chemicals used were provided by Sigma-Aldrich (M) Sdn. Bhd. Kuala Lumpur, Malaysia. Milli-Q water was used to make solutions and for washing. All chemicals were used without any further processing after receipt.

3. METHODS

Different treatment methods were used to decontaminate the CFs. Purchased feathers were first rinsed with water at 60 ^{o}C. After washing, these were incubated in detergent for 2 h and again rinsed at 60 ^{o}C. After that, the feathers were degreased using petroleum ether for 12 h, and then washed with distilled water and conditioned at 20 ^{o}C, 65 % RH for 24 h. After their first cleaning, the feathers were further treated with different surfactants. Each treatment step was run for 3 h. Cleaned defatted CFs were then chopped into small pieces and dried in sunlight for 48 h and stored at 4 ^{o}C for further usage. After purification, microbial testing was done in triplicate and results were compared. The methods of purification were investigated with three classes of the surfactant: SDS (an anionic surfactant), PEG (non-ionic surfactant), and CTAB (cationic surfactant). Aqueous solutions (1 g/l) of SDS, PEG, and CTAB were formed separately in beakers and 10 g of feathers having a ratio of 100:1 was added to each and named as t2, t3 and t4 respectively, whereas the name for the unwashed feathers was t0 and, the sample after first washing was named t1. The combination was mixed using a magnetic stirrer at 40 ^{o}C for 3 h under vigorous stirring at 300 rpm.

3.1 Microbiological Testing

The total aerobic microbial count for different treatment preparations was studied *via* the standard plate count method (SPC). The SPC was performed by the detection of *E coli*, *Staphylococcus aureus*, *Salmonella* spp., *Pseudomonas aeruginosa* in the central laboratory at Universiti Malaysia Pahang, Gambang, Malaysia. The samples were inoculated on the selective media for different microbes. The microbial number was calculated as colony forming units per gram (cfu/g).

4. CHARACTERIZATION

4.1 Morphological Studies

The effect of the surfactants on the CFs morphology was analyzed using digital photography, which showed a great advantage from treatment. The CFs were further investigated by SEM analysis. EDX in the SEM was used to do the elemental analysis of treated CFs. It was performed using a Hitachi TM3030Plus.

4.2 Fourier Transform Infrared Spectroscopy (FT-IR)

Chemical characterization of treated and untreated CFs was done using FT-IR spectroscopy to detect the presence of amide groups or protein [20]. Nicolet iS5 from Thermo scientific FT-IR was used for chemical characterization of treated and untreated CFs in between the 4000 cm^{-1} and 700 cm^{-1} wave number range.

4.3 Thermo Gravimetric Analysis (TGA)

Thermo gravimetric measurements of untreated and treated CFs were performed using TGA analyzer Q 500 under nitrogen atmosphere, in a temperature range between 10

^{o}C and 900 ^{o}C at a ramping time of 10 ^{o}C/min. The samples were vacuum dried at 40 ^{o}C. Samples with a mass of 3 mg were put in an aluminum crucible and the data obtained was analyzed.

5. RESULTS AND DISCUSSION

5.1 Microbiological Testing

The unwashed feathers (t0) have highest total aerobic microbial count (8.0×10^5 cfu/g) followed by washed (t1) with an aerobic microbial count of 1.8×10^5 whereas CTAB treatment (t3) displayed lowest count (3.4×10^3 cfu/g) among other treatments. This is supported by previous studies which showed that the use of CTAB as a surfactant was effective to reduce the microbial count [21]. As per the guidelines of Australian Food and drug administration for the SPC microbiological examination (December 2001), if the cfu number is $<10^4$, then the quality is satisfactory and if $<10^5$, then it is marginal and lastly if $\geq10^5$, then it is unsatisfactory and not acceptable.

Surfactants act as a decontaminants because they have two main functions such as surface activity and an intrinsic disinfecting/bactericidal function [19]. The strong detergent property of the surfactant is inversely proportional to the value of the surface tension and critical micelle concentration (CMC) [22], which means low value of surface tension and CMC will give the strong detergent properties. The values for surface tension and CMC is as follows CTAB (49.6 mN/m and 0.99×10^{-3} mol/l) < SDS (50.2 mN/m and 0.44×10^{-3} mol/l) < PEG (75.9 mN/m and 0.78×10^{-3} mol/l) [22, 23]. The reduction in the total microbial count among all surfactants was in the same trend as was observed in case of surface tension and CMC values. It was concluded that detergent mostly help in the removal of bacteria. Table 1 shows the values of the total microbial count with each surfactant. The lower count indicates the strong bactericidal competence.

Table 1: Total microbial count of treated and untreated chicken feathers

Sample No.	Surfactants /Samples	Total microbial count [cfu/g]
1	Unwashed (t0)	8.0×10^5
2	Washed (t1)	1.8×10^5
3	SDS (t2)	1.1×10^3
4	CTAB (t3)	3.4×10^2
5	PEG (t4)	1.6×10^3

There was no detection of *E. coli* after all treatments, which showed that all surfactants were efficient to abolish *E. coli* which is in agreement with the research stated by Pourjavaheri et al. regarding the presence of *E. coli*. There was a visible growth of *Pseudomonas spp.* and *Staphylococcus aureus*, thus they were detected after t0, t1, t2, t3 and t4. The treatment with surfactants were efficient in the removal of bacteria like *S. aureus* and *Pseudomonas* spp. as previously reported [21]. There was a detection of *Salmonella* spp. in t0, t1 and t2 but it was not found in t3 and t4. Among the three surfactants, two showed efficiency to destroy a gram of negative bacteria; which was unexpected as they are less efficient to destroy the *Salmonella* spp. [24]. SDS was unable to do the same for *Salmonella* spp. It may be due to insufficient concentration of the surfactant, although it was higher than suggested in the previous studies [25].

Consequently, the best purification surfactant, t3, was selected for further study in this project.

5.2 Morphological Analysis

The outcome of each treatment method on the chicken feather morphology was studied via the naked eye and using a camera. The results showed the presence of all major components of the feather and can be differentiated in treated feathers. Further, morphological analysis was done using Scanning Electron Microscopy and Energy Dispersive X-ray spectroscopy (SEM-EDX) as shown in Fig. 1.

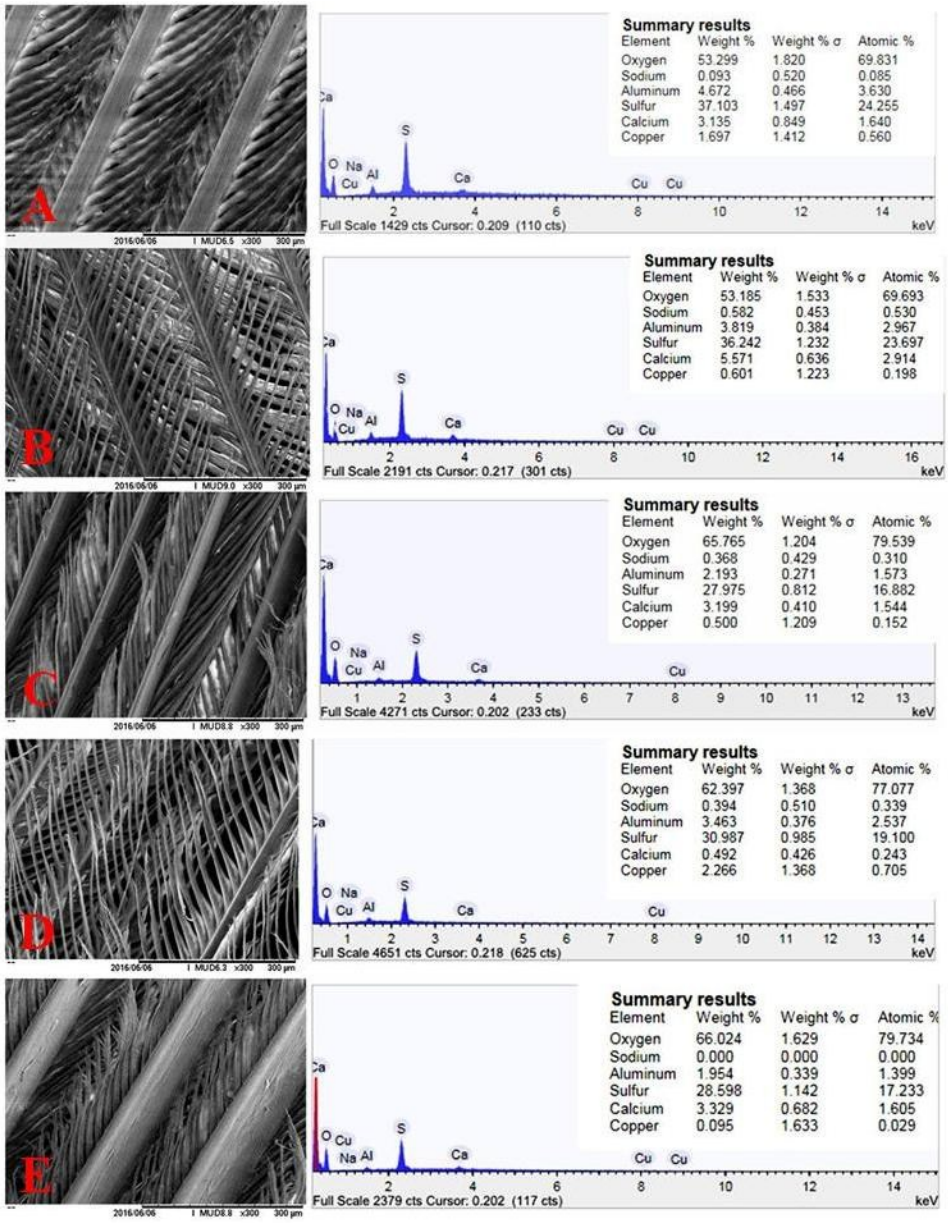

Fig. 1: SEM-EDX derived from the chicken feathers (A) unwashed (t0); (B) washed (t1); (C) treated via SDS (t2) (D) treated via CTAB (t3) (E) treated via PEG (t4).

Chicken feathers treated via t2, t3, t4 showed over erection and lack of woolly part as compared to t0 and t1 treatments along the feather structure. The major elements found in all the treatments after elimination of C were: O, Na, Al, S, Ca and Cu. The weight

proportion (% w/w) associated with S were 37.10, 36.24, 27.97, 37.18, 28.59 and Na 0.09, 0.58, 0.36, 0.29, 0.00 in the feathers with treatments t1, t2, t3, t4 respectively. There was no detectable fiber damage in the treated feathers. The results showed that there were minimum changes in the feather structure in treatment t3 followed by t4 when compared to t0 and t1. Thus, in this study, it was shown that t3 was the best surfactant among others and there was no deposition of surfactant in treatment t3 but some were seen on the t2 and t4 feathers. The deposition of surfactants on feathers can cause mild skin irritation on human skin upon contact [18].

5.3 Fourier Transform Infrared Spectroscopy (FT-IR)

In order to understand the chemical structure of treated and untreated chicken feathers, FT-IR spectra was used, (Fig. 2). After comparison of five samples, it was found that the characteristic peaks are similar with each other and are comparable with other studies [26]. On the other hand, the surfactants have little effect on the chemical structure of protein and all the treatments showed transmission bands to the peptide bonds (-CONH) and are known as Amide A, Amide I, Amide II, Amide III [10, 27, 28]. The transmission band region between 3500 cm^{-1} to 3200 cm^{-1} is attributed to stretching vibration of -O-H and -N-H (Amide A) [29]. The transmission bands appeared in the range between 3000 cm^{-1} and 2800 cm^{-1} were related to symmetrical CH_3 stretching vibration [30, 31]. The strong transmission band is attributed to C=O stretching (Amide I), which occurs in the range of 1700 cm^{-1} to1600 cm^{-1} [20, 27]. The transmission band (Amide II) in the range of 1580 cm^{-1} to 1480 cm^{-1} is for N-H bending and C-H stretching [31]. The weak band between 1300 cm^{-1} and 1220 cm^{-1} is associated with Amide III, which is derived from C-N stretching and N-H bending [32, 33, 34] and with some contribution from C=O bending and C-C stretching vibration [28, 35, 36]. The transmission band between 750 cm^{-1} and 600 cm^{-1} is related to N-H out-of-plane bending [29, 37].

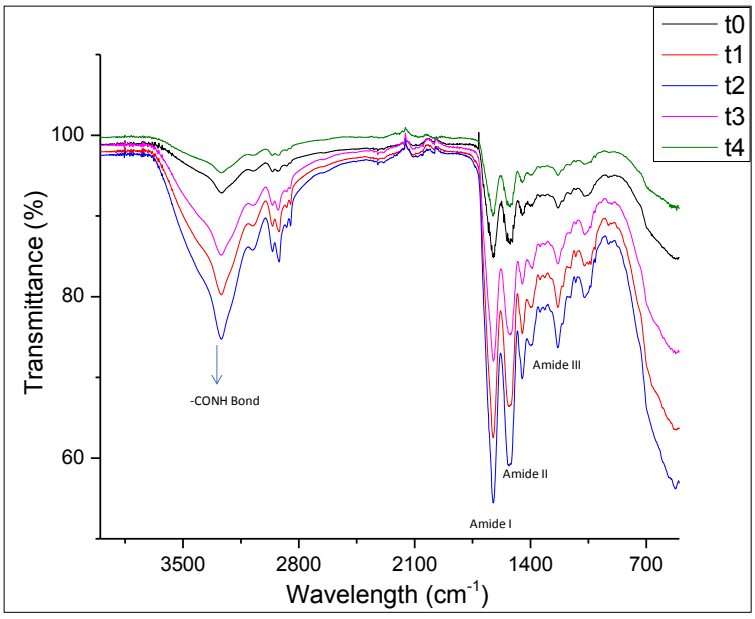

Fig. 2: FT-IR spectra of treated and untreated chicken feathers.

The transmission vibration around 1700 cm^{-1} is attributed to C=O group of fatty acid ester found in animal skins [38]. The C-O stretching vibration associated with ester linkage, attributed at 1200 cm^{-1} to 1270 cm^{-1}, was not detected. No transmission band at

1700 cm^{-1} was detected after the treatments, which confirms the action of surfactants in removing fatty materials from chicken feathers (Fig. 2). Overall, it was shown that there are no effects on the chemical composition of feathers after treatment with surfactants.

5.4 Thermo Gravimetric Analysis (TGA)

Thermal stability of the untreated and treated feather material was conducted by TGA. As shown in Fig. 3, all the untreated and treated samples have the same profiles present in a similar pattern with two stages of decomposition, which showed that the thermal stability of all samples were at par. Some percentage of weight loss occurred before 100 °C, which is due to the evaporation of water, including free water and bounded water. All samples were stable until 200°C. After that point, a steep weight loss occurred until 400 °C. This loss was allied with the helix denaturation, skeletal degradation and destruction of peptide bridge chain linkage [39-41]. With this, there was decomposition of some volatile compounds such as HCN, H_2S, CO_2 and H_2O [42]. This indicated that the treatment of feathers had no effect on their thermal stability. After pyrolysis a total weight loss of ~70 % was observed.

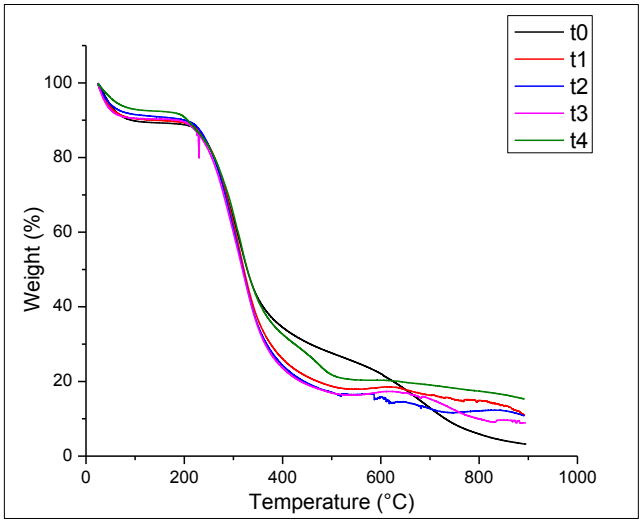

Fig. 3: TGA of untreated and treated chicken feathers.

6. CONCLUSION

Chicken feathers with treated CTAB (t3) exhibited the lowest bacterial counts as compared to other treatment preparations. This treatment removed *Salmonella* also. Treated feathers had the same morphology in optical evaluation. There was no detectable fiber damage in the treated feathers and the least changes in the feather structure in treatment t3. There was the elimination of fatty esters from the feathers confirmed with FT-IR. Therefore, t3 was selected as the best surfactant, among other tested surfactants, for the decontamination and preparation of chicken feathers for other applications.

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