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Purification of avian biological material to refined keratin fibres

Firoozeh Pourjavaheri[a], Farzad Mohaddes[a], Prue Bramwell[a], Frank Sherkat[a], Robert A. Shanks*[a]

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Keratin derived from chicken feather fibres (CFFs) has many potential applications that are constrained by the quality and pathogen content after purification treatment. The pathogen activity after purification has not been evaluated elsewhere. Plucked chicken feathers are prone to impose biological hazards due to accommodating blood-borne pathogens; therefore, establishing an efficient purification process is crucial. Bactericidal performance of surfactants (anionic, non-ionic, and cationic), bleach (ozone and chlorine dioxide), ethanol extraction, and a combination method comprising surfactant-bleach-ethanol extraction on chicken feathers was investigated via A) Standard Plate Count and enumeration of *Escherichia coli*, *Pseudomonas* species, coagulase positive staphylococcus, aerobic and anaerobic spore-formers and B) *Salmonella* and *Campylobacter* detection tests. Among purification methods, only ethanol extraction and combination methods eliminated *Salmonella* from the untreated feathers. Although ethanol extraction exhibited superior bactericidal impact compared to the combination method, the feathers treated through the latter method demonstrated superior morphological and mechanical properties. Scanning electron microscopy–energy dispersive spectroscopy was employed to determine the remaining content of selected purifiers on treated CFFs. Fourier-transform infrared spectroscopy confirmed the successful removal of fatty esters from CFFs using nominated purifiers. Ethanol extraction was found to be the most efficacious single treatment, while combination of surfactant and oxidative sterilizer with ethanol was superior.

1. Introduction

Poultry industry generates millions of tonnes of feathers as a by-product per year worldwide [1]. Utilization of chicken feather fibres (CFFs) will not only be beneficial for poultry industry, but will also reduce health hazards, & benefit the environment, by reducing solid wastes being sent to landfills [2]. Although CFFs are abundant, inconvenient and troublesome waste product, they contain over 90 % of keratin protein [3, 4]. According to Rouse and Van Dyke [5], keratins extracted from bio-fibres such as CFFs and wool are capable of forming self-assembled structures that regulate cellular recognition and behaviour; these qualities have led to the development of keratin biomaterials with applications in wound healing, drug delivery, tissue engineering, trauma and medical devices. Given the fibrous structure of CFFs, their application in bio-degradable and green composites has been studied [6].

Due to contamination with intestinal contents, blood, fatty acids, offal fat, preen oil, and debris, fresh plucked feathers can be a suitable habitat for many microorganisms such as *Campylobacter*, *Salmonella* and *Escherichia* species (spp.), which are known to cause gastroenteritis [7]. The presence of pathogens in plucked feathers can impose potentially fatal biological hazards for humans; however, many microorganisms existing in feathers can be killed via either physical or chemical means [8-10]. Efficient and non-degradative methods are required for purification and separation of CFF keratin to render it safe, clarified and an accessible abundant resource for a variety of uses.

Disinfectants are nonsporicidal agents that destroy pathogenic microorganisms [11]. Rutala et al. [12] reported the disinfecting capacity of ethanol at various concentrations against a variety of microorganisms; *Pseudomonas aeruginosa* was killed in 10 s by ethanol at concentrations between 30 %·v/v and 100 %·v/v. *Escherichia coli* (*E. coli*) and *Salmonella typhosa* were killed in 10 s by any ethanol concentrations between 40 %·v/v and 100 %·v/v. *Staphylococcus aureus* (*S. aureus*) was slightly more resistant, requiring higher concentrations of ethanol, between 60 %·v/v and 95 %·v/v for the same period.

Sanitizers are defined as chemical agents capable of killing 99.999 % of specific bacterial populations within 30 s, yet they may or may not destroy pathogenic or harmful bacteria [12, 13]. Ozone (O_3) is a well-known sanitizer capable of killing various pathogens and bacteria including spores [14, 15]. The bactericidal effect of O_3 is associated with its high oxidation potential and its ability to diffuse through biological cell membranes [14]. Naidu [16] reported that 0.35 mg/L of O_3 reduces *E. coli*, *Salmonella* Typhi and *S. aureus* by at least 5 $\log_{10}$, and reduces the spores of *Bacillus* and *Clostridium* spp. by almost 3 $\log_{10}$. Chlorine dioxide (ClO_2) is an oxidizing agent acting as an antimicrobial sterilizing sanitizer, which is commonly used in hospitals for the removal of dirt, and disinfection [17, 18]. The oxidizing

[a] School of Applied Sciences, RMIT University, VIC 3001 Australia. Fax: +61 3 9925 3747; Tel: +61 3 9925 2122; E-mail: robert.shanks@rmit.edu.au

effect of ClO_2 can be used for whitening of CFFs [14, 19]. According to Trakhtman and Manual [20, 21], ClO_2 is effective against *E. Coli* and *Bacillus anthracoides* at dosages in the range of 1 to 5 mg/L and against *Salmonella* Paratyphi B., *Pseudomonas aeruginosa* and *Staphylococcus aureus* (*S. aureus*) at concentrations lower than 1 mg/L.

Surfactants are a class of chemicals comprising both hydrophobic and a hydrophilic groups in their chemical structure; thus being able to dispersing fatty dirt particles that are normally insoluble in water [22]. Anionic surfactants are widely used for removing oily dirt and stains in the presence of soft water; however, the minerals available in hard water adversely affect their cleaning performance. Although the general decontamination ability associated with different type of surfactants is proven, the information regards the antibacterial effect of them is limited [23].

The aim of this research was to compare microbiological and mechanical properties of CFFs purified by surfactants, disinfectants, sanitizers and their combinations. The effectiveness of different purification methods on microbiological and mechanical properties of CFFs are evaluated, and the most suitable candidates for keratin extraction and development of bio-composite application are represented.

2. Experimental

2.1. Material

White chicken feathers (varying between 3 cm and 20 cm in length) of freshly slaughtered adult chickens were supplied by Baiada Poultry Pty Ltd, Melbourne, Australia. Sodium lauryl sulphate (SLS) 99.0 % was acquired from The British Drug Houses Ltd., Poole, England. Poly(ethylene glycol) (PEG) 99 % with number average molecular weight (Mn) of 400 g/mol, sodium chlorite ($NaClO_2$) 99 %, hydrochloric acid (HCl) 99 %, Peptone diluent solution, and Tween 80 (TW80) emulsifier were purchased from Sigma-Aldrich, Sydney, Australia. Cetyltrimethylammonium chloride (CTAC) 25 %·w/w aqueous solution was provided by Aldrich Chemical Company, Milwaukee, USA. Nutrient Broth CM0001, Nutrient Agar (NA) CM0003, Plate Count Agar (PCA) CM0325, Urinary Tract Infection (UTI) Brilliance agar CM0949, Baird-Parker Agar (BPA) CM0275, Tryptone Soy Agar (TSA) CM0131, Wilkins-Chalgren Anaerobic Agar (WCA) CM0619, Xylose Lysine Desoxycholate Agar (XLD) CM0469, Bismuth Sulphite Agar (BSA) CM0201, Campylobacter Selective Agar (CM0689), Laked Horse Blood SR0048, Campylobacter Growth Supplement (FBP supplement) SR0232, and Staphlytect Plus X240E were obtained from Oxoid, Altrincham, England. Columbia blood agar base was obtained from Acumedia, a division of Neogen, USA. Biochemical detection strips API 20 E were purchased from Biomerieux, Baulkham Hills, NSW, Australia. All the chemicals were used upon receipt without further purification.

2.2. Purification Methods

The untreated CFFs were purified via different methods: Soxhlet extraction with ethanol (SEEt) (T1), Ozonation (T2), purification by ClO_2 (T3), purification with a non-ionic surfactant (poly(ethylene glycol)) (T4) , purification with an anionic surfactant (sodium lauryl sulphate) (T5), purification with a cationic surfactant (cetyltrimethylammonium chloride) (T6), and purification via a combination method (SLS-ClO_2-SEEt) (T7) .

Except for the CFFs treated by T1 and T7, the feathers purified by other methods were rinsed in distilled water for 10 min. All treated CFFs were dried in an incubator at 34 °C ± 1 °C for 3 d and conditioned at 20 °C ± 2 °C and 60 % ± 2 % RH for 72 h. Due to working with unknown type and count of bacteria present in the untreated CFFs, each purification method was timed for total length of 5 h. The microbiological tests were conducted in triplicate after each purification method, and the results were compared.

2.2.1. Purification by Surfactants

The purification effect associated with three classes of surfactants (anionic sodium lauryl sulphate (SLS), non-ionic poly(ethylene glycol) (PEG), and cationic cetyltrimethylammonium chloride (CTAC)) was investigated on untreated CFFs.

1.0 g/L aqueous solutions of SLS, PEG, and CTAC were prepared in separate containers and 10.0 g of untreated CFFs, liquid to solid ration of 100:1, was added to each. The mixtures were agitated using magnetic stirrers (400 rpm) over hot plates at 20 °C. Then the temperature was gradually increased to 35 °C, and stirring continued for 5 h.

2.2.2. Purification via Soxhlet Extraction with Ethanol

Given the higher antimicrobial effect of alcohol at higher concentrations, continuous Soxhlet extraction with ethanol (SEEt) was carried out for 5 h on CFFs. The extraction time chosen was longer than suggested in the literature [12] due to the unknown type and load of bacteria in the untreated CFFs.

2.2.3. Purification by Ozone

Ozonation of untreated CFFs was carried out using an Enaly Trade Co., Ltd Ozone Generator, Model OZX-300U, Canada (ozone output 200 mg/h). One gram of untreated CFFs was ozonated in an air-sealed flask containing 100 mL of distilled water (liquid to solid ratio of 100:1) at 20 °C for 5 h. Upon contact with water, O_3 reacts to create an oxidizing solution of hydrogen peroxide (H_2O_2), as shown in Equation 1 [24], which is expected to kill bacteria, fungus and spores [25].

$$O_3 + H_2O \rightarrow H_2O_2 + O_2 \qquad \text{Equation 1}$$

2.2.4. Purification by Chlorine Dioxide

Chlorine dioxide can be produced from the reaction between sodium chlorite and hydrochloric acid, Equation 2 [26]. In order to make 100 mg/L ClO_2 aqueous solution, 1.85×10^{-3} mol of $NaClO_2$ and 1.48×10^{-3} mol of HCl were dissolved in distilled water to result in 1 L of purification solution, in which 10 g of untreated CFFs was stirred (liquid to solid ration of 100:1) at 400 rpm and 20 °C for 5 h.

$$5\ NaClO_2 + 4\ HCl \rightarrow 4\ ClO_2 + 5\ NaCl + 2\ H_2O \qquad \text{Equation 2}$$

2.2.5. Combined Purification Treatment

The effect of combining SLS, ClO_2, and SEEt methods was studied on the untreated CFFs. 10 g of CFFs was added to 1 g/L

SLS aqueous solution (liquid to solid ratio of 100:1); meanwhile, 1.85×10^{-3} mol of $NaClO_2$ and 1.48×10^{-3} mol of HCl were added to the same container in order to generate 100 mg/L ClO_2 in the system. CFFs were stirred at 400 rpm at 20 °C for 3 h, rinsed with distilled water, then Soxhlet extracted with ethanol for the remaining 2 h of the 5 h purification cycle as in previous treatments.

2.3. Microbiological Tests on CFFs

The bactericidal efficacy of different purification treatments on CFFs was investigated via a standard plate count (SPC), followed by detection of hazardous bacteria such as *Escherichia coli*, *Pseudomonas* spp., coagulase positive staphylococcus (CPS), aerobic and anaerobic spore-formers, *Salmonella* spp. and *Campylobacter* spp., in a PC2 microbiology laboratory. As shown in Figure 1, serial dilutions were performed according to Australian Standard AS 5013.11.1-2004 under a sterile Class II cabinet using aseptic technique. Tween 80 (TW80) emulsifier was used for the initial dilution to separate the possible fat globules from CFFs as per the Oxoid Manual [27]. To formulate the initial dilution, 10^{-2}, 0.25 g ± 0.02 g of CFFs was added to 25 mL 0.1 % peptone water (PW) ± 0.1 mL of 0.1 % TW80 followed by serial dilutions with 9 mL ± 0.1 mL of 0.1 % PW. Prepared dilutions ranged from 10^{-2} to 10^{-6} unpurified and 10^{-2} to 10^{-4} for purified CFFs. Using the spread plate method, 0.5 mL of each sample dilution was plated on selective media in triplicate. The specific growth condition for each microorganism is shown in Table 1. Plates chosen for counting were dilutions having colony counts in the range of 10-150 typical colonies per plate according to the spread plate method unless the initial dilution had less than 10 colonies in which case all typical colonies on that plate were counted. The corresponding microbial numbers are reported as colony forming units per gram (cfu/g) according to AS 5013.14.1-2006.

2.3.1. Bacterial enumeration

Standard plate count (SPC) was performed according to AS 5013.14.3-2012. Enumeration of *S. aureus* individual colonies was performed as per AS 5013.12.1-2004 and was confirmed using Staphlytect Plus X240E (Oxoid). *Pseudomonas fluorescence*, *E. coli.*, *S. aureus*, *Bacillus subtilis* and *Clostridium sporogenes* were used as positive controls and non-inoculated BPA, TSA and WCA plates were used as negative controls.

To determine the aerobic and anaerobic spore count of each of *Bacillus* and *Clostridium* spp. each dilution of the sample was heated in a water bath at 80 °C for 10 min before plating on TSA and WCA agars respectively to harvest the spores since spores need heat treatment before they can germinate. The aerobic growth on TSA plate was considered as *Bacillus* spp. after confirmation as Gram-positive/catalase positive rods, and the anaerobic growth on WCA plates was considered as *Clostridium* spp. after confirmation as Gram-positive/oxidase negative rods [28].

2.3.2. Detection of Salmonella spp. and Campylobacter spp.

Salmonella and *Campylobacter* spp. were detected according to AS 5013.10-2009 and AS 5013.6-2004, respectively. *Salmonella* Typhimurium and *Campylobacter jejuni* were used as positive controls. After inoculation of the sample into standard pre-enrichment and selective enrichment broth, typical *Salmonella* colonies on XLD and BSA were inoculated onto NA plates, and the oxidase negative colonies were further tested using API 20 E for *Salmonella* spp. confirmation. In order to detect *Campylobacter,* samples were inoculated into Preston broth, in microaerophilic conditions for 24 h at 42 °C to select for *Campylobacter* spp., after which a loopful of this enrichment was plated on Campylobacter Selective Agar and incubated in the same conditions as mentioned. Typical *Campylobacter* colonies were then confirmed by a Gram stain.

2.4. Characterization

2.4.1. Morphological Analysis and Scanning Electron Microscopy-Energy Dispersive Spectroscopy

The overall impact of each purification method on morphology of treated feathers was investigated using macro digital photography. The CFFs that showed superior bactericidal efficacy from purification methods were further analyzed using scanning electron microscope (SEM) analysis. Elemental analysis of the treated CFFs was carried out using energy dispersive spectroscopy (EDS) in the SEM. SEM imaging was performed using an FEI Quanta200 (tungsten filament) with an attached Oxford Instruments $XMax^N20$ spectrometer. The selected CFF samples were carbon coated using a SPI-coduleTM Sputter Coater (IMBROS Pty Ltd) prior to analysis to remove charging.

2.4.2. Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was employed for chemical characterization of treated and untreated CFFs. Infrared spectroscopy can detect specific alterations in the chemical composition of peptides [29]. A PerkinElmer Spectrum 100/Universal diamond attenuated reflectance (ATR), FTIR was used for chemical characterization of the superior bactericidal efficacy purified and untreated CFFs barbs in a wavenumber range between 4000 cm^{-1} and 650 cm^{-1}.

2.4.3. Mechanical properties of purified CFFs

Tensile and viscoelastic properties of the CFFs barbs purified with methods of superior bactericidal effect were evaluated via tensile mechanical analysis (stress–strain analysis) and modulated force thermomechanometry (MF-TM or dynamic mechanical analysis (DMA)), respectively. Sampling for a single fibre tensile testing was carried out according to ASTM D3822, using a paper template to mount the fibre and grip in the tensile clamps. Prior to testing, the diameter of the CFFs barbs (Figure 3 a) were measured by a Dino-Lite digital microscope (Dino-Lite AM4013T-M40 from AnMon Electronics Co., using DinoCapture 2.0 operating software).

Tensile testing was performed using a TA Instruments DMA Q800 (at 30 ℃; ramped force from 0.001 to 1 N at 0.01 N/min), to measure stress-strain properties of the CFF barbs. MF-TM was carried out using a PerkinElmer Diamond DMA to determine storage modulus (E') as an indication of elasticity, loss modulus (E"), representing the amount of energy absorbed, and tanδ, showing damping associated with CFFs. Test conditions included specimen

gauge length of 10 mm, deformation of 20 µm, frequency at 0.5 Hz, and temperature at 18 °C.

3. Results and Discussion

3.1. Standard Aerobic Plate Count

The unpurified CFFs (T0) showed the highest count (1.2×10^7 cfu/g) of feathers whereas SEEt treatment (T1) showed the lowest count (3.5×10^2 cfu/g) among all purification treatments, with a mean reduction of *ca.* 5 $\log_{10}$ (Figure 2). This is in agreement with literature as ethanol is capable of eliminating a broad spectrum of bacteria [12]. The SLS-ClO_2-SEEt combined method (T7) exhibited the second favourable results with average count of 4.2×10^3 cfu/g, which may be due to the bactericidal effects of ethanol. Although the time required for ethanol to be effective against different bacteria was suggested to be 30 s [12], the large gap in bacteria count after 2 h (T7) and 5 h (T1) of ethanol Soxhilation suggested the continuation of Soxhilation for several hours. Prolonged treatment time can be due to dealing with unknown types and/or loads of bacteria on unpurified CFFs.

Surfactants are promising purifiers due to benefiting from their dual functionality namely surface activity and intrinsic disinfecting/bactericidal performance [23]. The surface activity of a surfactant is reliant on different factors such as pH, temperature, and concentration. Low values of surface tension and critical micelle concentration (CMC) translates as strong detergent properties associated with a surfactant [30]. The values of surface tension and CMC of the used surfactants followed below order:
PEG (74.5 mN/m and 0.78 mol/L) > SLS (47.5 mN/m and 0.44 mol/L) > CTAC (37.0 mN/m and 0.0015 mol/L) [30, 31].

As shown in Figure 2, the highest to lowest reduction in all counts including SPC, aerobic- and anaerobic sporeformers belonged to CTAC (T6), SLS (T5), and PGE (T4), respectively, which was the same trend observed for corresponding CMC and surface tension values. It can be concluded that detergents may more engage in removal of bacteria mechanically than destroying them.

As shown in Figure 2, the counts resulted from O_3 (T2) (5.9×10^3 cfu/g) and ClO_2 (T3) (2.9×10^4 cfu/g) treatments were substantially lower than those obtained from detergents. The lower counts signify the superior bactericidal efficiency associated with the used bleaches compared to the selected surfactants.

The SPC is an incapable method for distinguishing pathogens from non-pathogens; therefore, further evaluation of the selected purification treatments requires targeting individual pathogens and indicator organisms.

3.2. Aerobic and anaerobic spore-formers, coagulase positive staphylococcus, Escherichia coli, Pseudomonas spp. Salmonella and Campylobacter spp.

The viable count of aerobic and anaerobic spore-formers resulted from T0 to T7 treatments are shown in Figure 2. The T1 treatment was found to be the most effective in removing both aerobic (average of 1.1×10^4 cfu/g) and anaerobic spore-formers (average of 1.5×10^2 cfu/g). Even though ethanol is not effective in destroying spores [12], the viable spore count was lower than T0 (Figure 2). The lower count could be owing to the spores being washed away in the purification process. The T2 treatment was effective in reducing spore counts as ozone has been found to have sporicidal properties [14]. The T3 treatment was effective in reducing both aerobic and anaerobic spore counts[15]. Surfactants are not known to have sporicidal properties, hence the most likely reason for reduced of spore counts in T4, T5 and T6 purification treatments is the spores were washed away in solution within the surfactant micelles.

The T4 treatment presented relatively higher count of spore-formers (Figure 2) than other purification treatments, which is in agreement with a study reported by Vardaxis et al. [32] regarding PEG that has been supported the growth of spores.

E. coli was detected on T0 (4×10^2 cfu/g), whereas, it was not observed on T1 to T7 treatments, suggesting that all purification methods used were effective at eliminating *E. coli*. The absence of visible growth of presumptive *Pseudomonas* spp., coagulase positive *S. aureus* (CPSA), and *Campylobacter* spp. on T0 (< 1×10^2 cfu/g), does not necessarily imply that the purification treatments T1 to T7 were effective in eliminating the above species in the purified samples. The *S. aureus* was possibly unable to compete with the other microflora on the CFFs, due to a combination of inadequate time and temperature to allow *S. aureus* to flourish. The positive control (*Pseudomonas fluorescence* 283/2) was confirmed by Gram morphology, and the isolates appeared Gram-negative and at the same time, oxidase negative. Colonies that appeared similar to the *E. coli* positive control on the interpretation guide were considered as *E. coli*. All positive controls were effective in growing CPSA and *Campylobacter*.

Salmonella was detected in T0, T2, T3, T4, T5 and T6 but was not found in T1 and T7, as ethanol is known to destroy *Salmonella* [12]. Surfactants are known to be less efficient against Gram-negatives [33], therefore *Salmonella* spp. were detected. Detection of *Salmonella* in T2 and T3 was unexpected since O_3 and ClO_2 are known to destroy *Salmonella* [34]. Furthermore, the treatment period of over 5 h was assumed to be sufficient. It could be argued that the concentrations may have not been adequate for a 10 g sample loading, even though the concentration used were higher than those suggested in the literature [16, 21]. The T7 result demonstrated that 2 h ethanol treatment is not reducing the microbial loads as effectively as 5 h treatment, however it was sufficient for disinfecting pathogens such as *Salmonella*, when combined with surfactant and bleach.

The ineffective treatments in detecting the *Salmonella* should not be employed as they do not eliminate human pathogens carried by chickens. The microbiological findings over pathogenic bacteria in T0 and T7 will be utilized in this project and in industries to limit the exposure risk of human pathogens.

3.4. Morphological Analysis

The impact of each purification method on the morphology of CFFs was investigated via visual observation. Except for T1, which exhibited a significant shrinkage and crippling of the treated CFFs, the major components of the feather were distinguishable in other purified CFFs (Figure 3 b).

3.5. Scanning Electron Microscopy and Energy Dispersive Spectroscopy

Figure 4 shows the SEM electron images, SEM-EDS maps, and elemental analysis associated with the total map spectrum obtained from CFF treated via T1 and T7 treatments. Comparing the CFFs resulted from both treatments, CFFs treated via T1 exhibited over-erection along the feather structure as well as lacking in the woolly part of the CFFs shown in Figure 4. These effects can be attributed to the over-drying nature of ethanol on CFFs. None of CFFs purified via T1 and T7 showed any signs of detectable fibre damages. Eliminating N and C from elemental analysis, other major elements in T1 and T7 samples were found to be O, S, Na, Cu, and Ca. The relative weight proportions associated with S (22.9 %·w/w) and Na (2.8 %·w/w) in the CFFs treated by T7 were 9.9 %·w/w and 2.3 %·w/w higher than those of the CFFs treated by T1, respectively. This confirms the partial deposition of SLS on the purified CFFs, which from safety point of view, can cause mild to moderate skin irritation on human skin upon contact [22].

3.6. FTIR Spectroscopy

In order to examine the effects of purification processes on CFFs, FTIR spectra of the untreated feather (T0) and CFFs purified via T1 and T7 were obtained. The broad absorption band region from 3500 cm^{-1} to 3200 cm^{-1} is attributed to the stretching vibration of N-H and O-H bonds [35]. Bands that fall in the 3000 cm^{-1} to 2800 cm^{-1} range are related to C-H stretching modes [36]. The amide I band is attributed to C=O stretching vibration, which occurs in the range of 1700 cm^{-1} to 1600 cm^{-1} [29, 37]. N-H bending stretching vibration associated with amide II occur between 1580 cm^{-1} and 1480 cm^{-1} [36]. The amide III band occurs in the range of 1300 cm^{-1} to 1220 cm^{-1}, which can be due to the phase combination of C-N stretching and N-H in-plane bending [38, 39]. N-H out-of-plane bending associated with the amide group occurs in a range between 750 cm^{-1} and 600 cm^{-1} [35].

In the FTIR spectrum of unpurified CFFs, [39]the stretching vibration at around 1710 cm^{-1} can be associated with carbonyl groups (C=O) of a fatty acid ester namely adipic acid ester usueally found on animal skins [40]. As the amide peaks cover the range between 1700 cm^{-1} and 1220 cm^{-1} [29, 37], the C-O stretching vibration associated with the ester-linkage occurring at 1267 cm^{-1} [40] was undetectable. Elimination of the stretching vibration at 1710 cm^{-1} associated with C=O of ester in T1 and T7 spectra confirms the capability of both purification methods in removing fatty materials from the untreated CFFs (Figure 5).

3.7. Mechanical Properties

As many bacteria such as aerobic, anaerobic and enteric bacteria could adversely affect the mechanical properties of the untreated CFFs [41], the mechanical properties of the CFFs was evaluated after implementing T1 and T7 treatments.

The stress-strain properties of purified CFFs barbs were evaluated (Figure 6). The maximal strength values resulted from CFFs purified by T7 and T1 were 104.9 MPa and 14.1 MPa, and the corresponding strain values were 9.3 % and 6.5 %, respectively. The elastic modulus (E), which is the initial slope of the stress–strain curve, was higher for T7 (2.0 GPa) than T1 (0.3 GPa). As the area below the stress-strain curve associated with T7 was considerably larger than that of T1, it was concluded that the CFFs barbs treated via T7 were significantly tougher than those treated via T1, which confirm the visual difference in feather structure in Figure 3 b.

Table 2 shows the average measures of E', E", tanδ, standard deviation (SD), and standards error values associated with 20 similar CFFs barbs purified via T7 and T1 determined within 90 % of confidence. The each DMA test performed at a constant temperature at 18 ℃, T7 demonstrated lower E' but higher values of E" and tanδ than T1. The stress–strain test showed T7 to have greater modulus and strength than T1, in contrast to the DMA test where T1 had somewhat higher elastic modulus is interpreted as due to the higher rate of strain in the DMA test at 0.5 Hz. The T7 had a slightly greater loss modulus showing greater energy dissipation or viscoelasticity than T1 consistent with the increased ultimate strain in the stress–strain result. The barbs from CFF are potential applicable to reinforcement in natural fibre composites (bio-composites) in lieu of cellulose fibres.

4. Conclusions

The chicken feathers resulting from ethanol-extraction purification (T1) were confirmed to have fatty esters and *Salmonella* removed and they exhibited minimal bacterial counts (3.5×10^2 cfu/g) compared to other practised methods. Combined surfactant-oxidant-ethanol purification (T7) was found to be the second efficient purification technique in reducing bacterial counts (4.2×10^3 cfu/g) and destroying *Salmonella.*

The elimination of fatty esters from the CFFs purified via T1 and T7 was confirmed by FTIR. T7 resulted superior morphological and mechanical properties compared to T1. Optical evaluation of the treated CFFs suggested the similar morphology for the CFFs purified via ozonation and chlorine to the CFFs purified by anionic, non-ionic and cationic surfactants.

SEM-EDS results confirmed the presence of SLS remnants in CFFs treated via T7; therefore, T1 was chosen as the safest single purification treatment among other practices. However, as far as benefiting from superior mechanical properties in bio-composites- or similar technologies is concerned, the combined reagent treatment, T7 was found more promising due to offering fibres of superior tensile strength (104.9 MPa) than T1 (14.1 MPa)."

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Notes and references

1. 1.C. Williams, C. Richter, J. Mackenzie and J. C. Shih, *Applied and Environmental Microbiology*, 1990, **56**, 1509-1515.

2. B. Shi, T. G. Shannon and E. Pelky, *BioResources*, 2010, **5**, 1425-1435.
3. M. A. Khosa, J. Wu and A. Ullah, *RSC Advances*, 2013, **3**, 20800-20810.
4. Y. Dou, X. Huang, B. Zhang, M. He, G. Yin and Y. Cui, *RSC Advances*, 2015, **5**, 27168-27174.
5. J. G. Rouse and M. E. Van Dyke, *Materials*, 2010, **3**, 999-1014.
6. H.-y. Cheung, M.-p. Ho, K.-t. Lau, F. Cardona and D. Hui, *Composites Part B: Engineering*, 2009, **40**, 655-663.
7. J. Mills, ed., *In Meat industry Microbiological methods*, Agresearch, 2011.
8. F. Pourjavaheri, F. Mohaddes, R. A. Shanks, M. Czajka and A. Gupta, *Advanced Materials Research*, 2014, **941-944**, 1184-1187.
9. G. Sudalaiyandi, Master of Engineering, Thesis, University of Waikato, 2012.
10. S. K. Rai, R. Konwarh and A. K. Mukherjee, *Biochemical Engineering Journal*, 2009, **45**, 218-225.
11. FDA, Guidance for Industry and FDA Reviewers: Content and Format of Premarket Notification - Submissions for Liquid Chemical Sterilants/High Level Disinfectants, 2000.
12. W. A. Rutala, D. J. Weber and C. f. D. Control, Guideline for disinfection and sterilization in healthcare facilities, Centers for Disease Control (US), 2008.
13. W. Joklik, *Sterilization and disinfection. Zinsser Microbiology*, 1992, 188-200.
14. N. Singh, R. Singh, A. Bhunia and R. Stroshine, *LWT-Food Science and Technology*, 2002, **35**, 720-729.
15. P. Foegeding, *Food Microbiology*, 1985, **2**, 123-134.
16. A. Naidu, *Natural food antimicrobial systems*, CRC press, 2000.
17. M. Shemesh, R. Kolter and R. Losick, *Journal of bacteriology*, 2010, **192**, 6352-6356.
18. E. M. Aieta and J. D. Berg, *Journal (American Water Works Association)*, 1986, 62-72.
19. T. A. Augurt and J. van Asten, *Kirk-Othmer Encyclopedia of Chemical Technology*, 2000.
20. N. Trakhtman, Chlorine dioxide in water disinfection. Chemical Abstracts, 1949, pp. 1508.
21. E. G. Manual, *Disinfectant Use in Water Treatment, Washington, DC*, 1999.
22. I. Effendy and H. I. Maibach, *Contact dermatitis*, 1995, **33**, 217-225.
23. J. G. Davis, *Journal of Applied Bacteriology*, 1960, **23**, 318-344.
24. B. Meunier and A. Sorokin, *Accounts of chemical research*, 1997, **30**, 470-476.
25. S. E. Newman, 20th Annual Conference on Pest Management on Ornamentals, 2004, pp. 20-22.
26. D. Mattei, S. Cataudella, L. Mancini, L. Tancioni and L. Migliore, *Water, air, and soil pollution*, 2006, **177**, 441-455.
27. E. Bridson, *The oxoid manual*, Unipath limited, 1990.
28. Z. Durmic, D. Pethick, J. Pluske and D. Hampson, *Journal of applied microbiology*, 1998, **85**, 574-582.
29. A. K. Mohanty, M. Misra and L. T. Drzal, *Natural Fibers, Biopolymers, and Biocomposites*, CRC Press, 2005.
30. R. MANDAVI, S. K. SAR[1] and N. RATHORE, *Oriental Journal of Chemistry*, 2008, **24**, 559-564.
31. Q. Xu, L. Wang and F. Xing, *Journal of surfactants and detergents*, 2011, **14**, 85-90.
32. N. Vardaxis, M. Hoogeveen, M. Boon and C. Hair, *Journal of clinical pathology*, 1997, **50**, 429-433.
33. L. Pérez, M. T. Garcia, I. Ribosa, M. P. Vinardell, A. Manresa and M. R. Infante, *Environmental toxicology and chemistry*, 2002, **21**, 1279-1285.
34. K. Muthukumarppan, F. Halaweish and A. S. Naidu, *Ozone.* In *Natural Food Antimicrobial Systems*, ed. A. S. Naidu, 2000, vol. 1, ch. 29, pp. 788-789.
35. D. Pavia, G. Lampman, G. Kriz and J. Vyvyan, Cengage Learning, 2008, ch. 2, pp. 72.
36. N. Eslahi, F. Dadashian and N. H. Nejad, *Preparative Biochemistry and Biotechnology*, 2013, **43**, 624-648.
37. A. Aluigi, M. Zoccola, C. Vineis, C. Tonin, F. Ferrero and M. Canetti, *International journal of biological macromolecules*, 2007, **41**, 266-273.
38. A. Vasconcelos, G. Freddi and A. Cavaco-Paulo, *Biomacromolecules*, 2008, **9**, 1299-1305.
39. E. Wojciechowska, A. Włochowicz and A. Wesełucha-Birczyńska, *Journal of Molecular Structure*, 1999, **511**, 307-318.
40. R. Al-Itry, K. Lamnawar and A. Maazouz, *Polymer Degradation and Stability*, 2012, **97**, 1898-1914.
41. X. Fan, Ph.D. Thesis, *Value-added products from chicken feather fiber and protein*, ProQuest, Auburn University, 2008.
42. F. Pourjavaheri-Jad, R. Shanks, M. Czajka and A. Gupta, presented in part at the International conference on Advanced Polymeric Materials 2013 (ICAPM 2013), Kottayam, Kerala, India, 11 - 13 October 2013, 2013.

Tables

Table 1: Incubation conditions for the target microorganisms

Target Microorganism	Agar Type	Incubation Conditions	Used Method
General microbial count	SPC	37 °C ± 1 °C for 48 h to 72 h	AS 5013.14.3-2012
E. coli	UTI	37 °C ± 1 °C for 24 h	AS 5013.14.1-2010
***Pseudomonas* spp.**	UTI	30 °C ± 1 °C for 24 h	AS 5013.11.1-2004
Coagulase positive staphylococcus	BPA	37 °C ± 1 °C for 48 h	AS 5013.12.1-2004
Aerobic spore-formers (*Bacillus* spp.)	TSA	37 °C ± 1 °C for 24 h to 48 h	AS 5013.2-2007
Anaerobic spore-formers (*Clostridium* spp.)	WCA	37 °C ± 1 °C for 24 h to 48 h	AS 5013.16-2004
***Salmonella* spp.**	XLD, BSA, NA	37 °C ± 1 °C for 24 h	AS 5013.10-2009

Table 2: Diameter, storage modulus (E'), loss modulus (E") and tanδ values with standard deviation (SD) values and standard error of CFFs barb purified via SEEt (T1) and SLS-ClO_2-SEEt (T7)

Purification treatments	T1	T7
Average Temperature (°C)	18.4	18.5
Average barb diameter (mm)*	0.168	0.119
SD	0.010	0.022
Standard Error (%)	3.04	9.31
Average E' (MPa)	1687	1243
SD	32	22
Standard Error (%)	1.107	1.038
Average E" (MPa)	451	554
SD	6	5
Standard Error (%)	0.705	0.514
Average tanδ	0.268	0.446
SD	0.002	0.004
Standard Error (%)	0.5	0.5

*The mean diameter value of five-point measurements of 20 CFFs barb

Figures

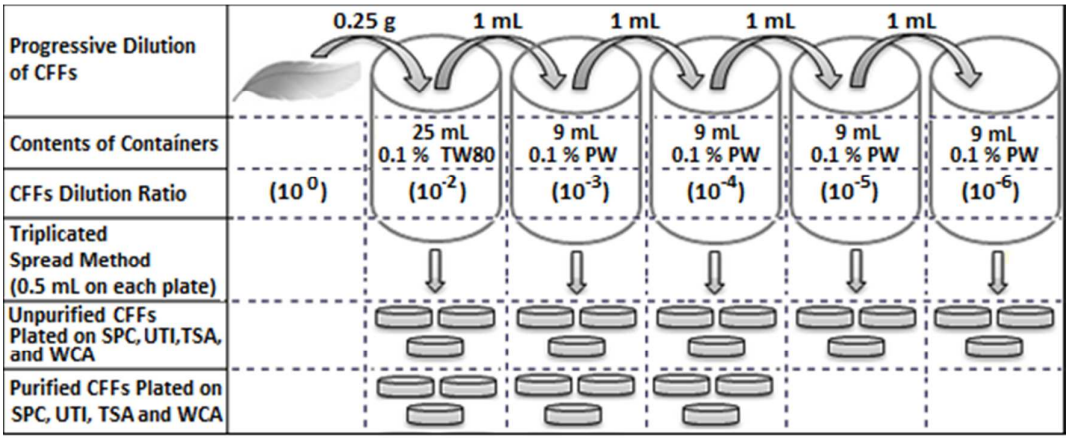

Figure 1: Serial dilutions, plating, and corresponding microbial tests on CFFs

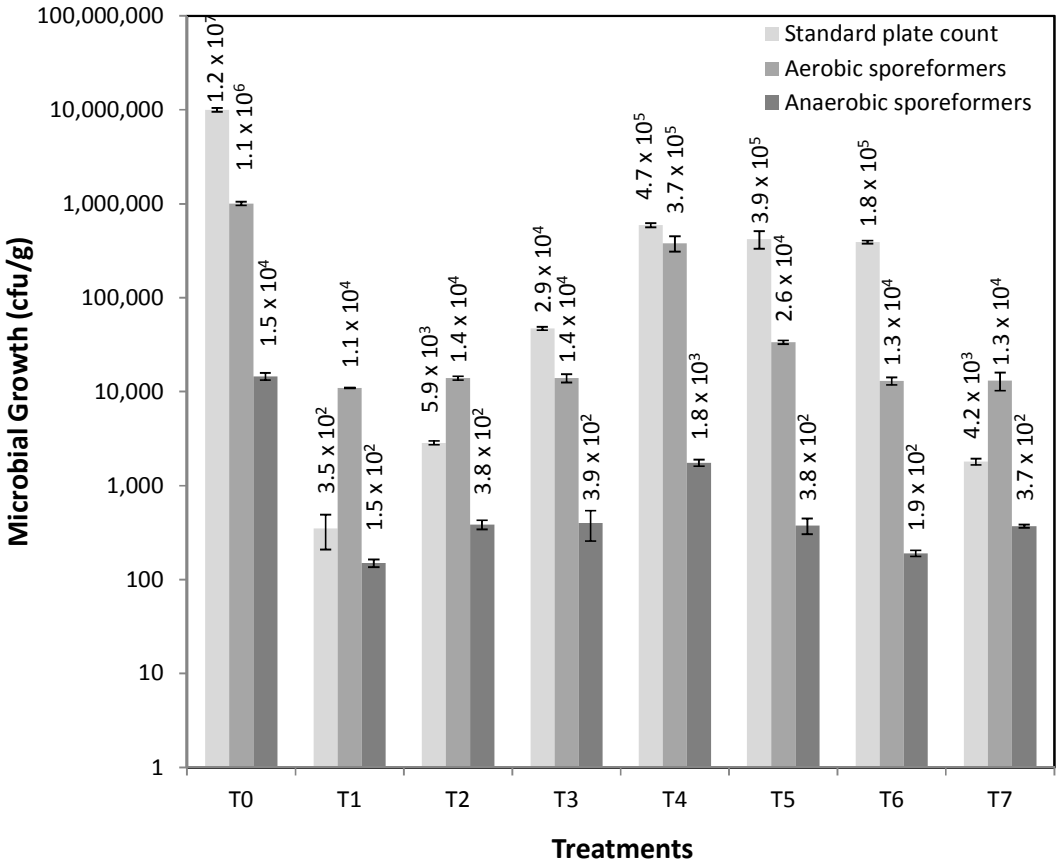

Figure 2: Microbial count (cfu/g) of SPC, aerobic spore-formers and anaerobic spore-formers for (T0) untreated chicken feathers upon receipt, versus chicken feathers purified with (T1) SEEt treatment, (T2) O_3 solution, (T3) ClO_2 solution, (T4) PEG solution, (T5) SLS solution, (T6) CTAC solution and (T7) SLS-ClO_2-SEEt combination

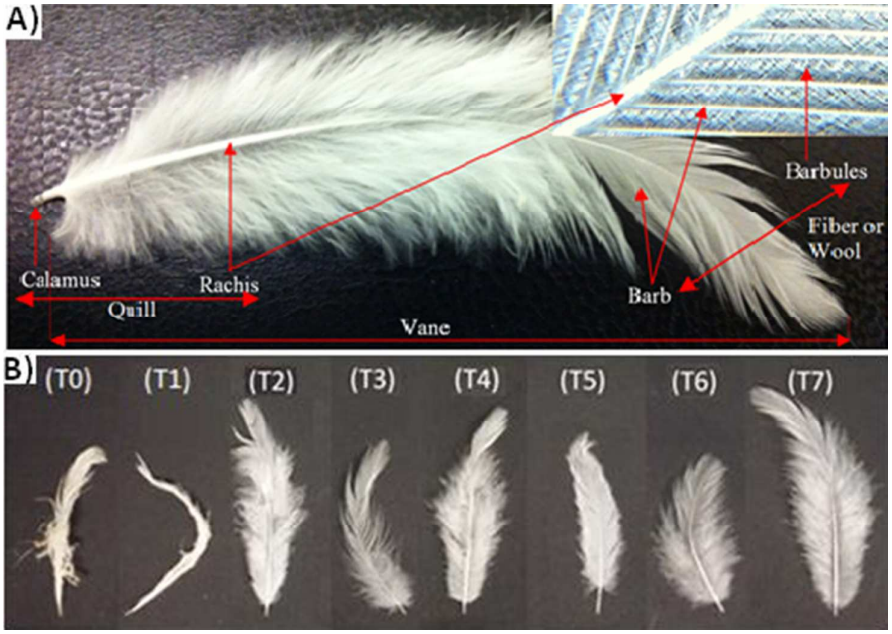

Figure 3: A) The structure of a semiplume chicken feather [42] (fibre or wool: barbs/barbules), (quill: calamus/rachis or shaft), (vane: rachis/barb/barbules) treated with T7; B) Images of the semiplume chicken feathers: (T0) untreated upon receipt, (T1) SEEt treatment, (T2) O_3 solution, (T3) ClO_2 solution, (T4) PEG solution, (T5) SLS solution, (T6) CTAC solution, and (T7) SLS-ClO_2-SEEt combination

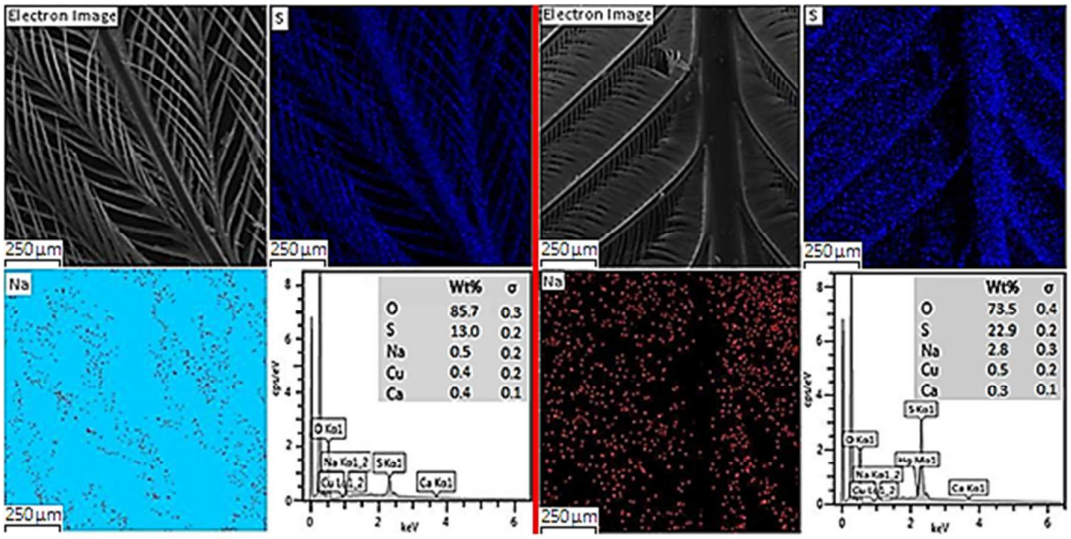

Figure 4: SEM, SEM-EDS, and elemental data derived from the CFFs treated via SEEt (T1) (left quad-image) and SLS-ClO_2-SEEt (T7) (right quad-image)

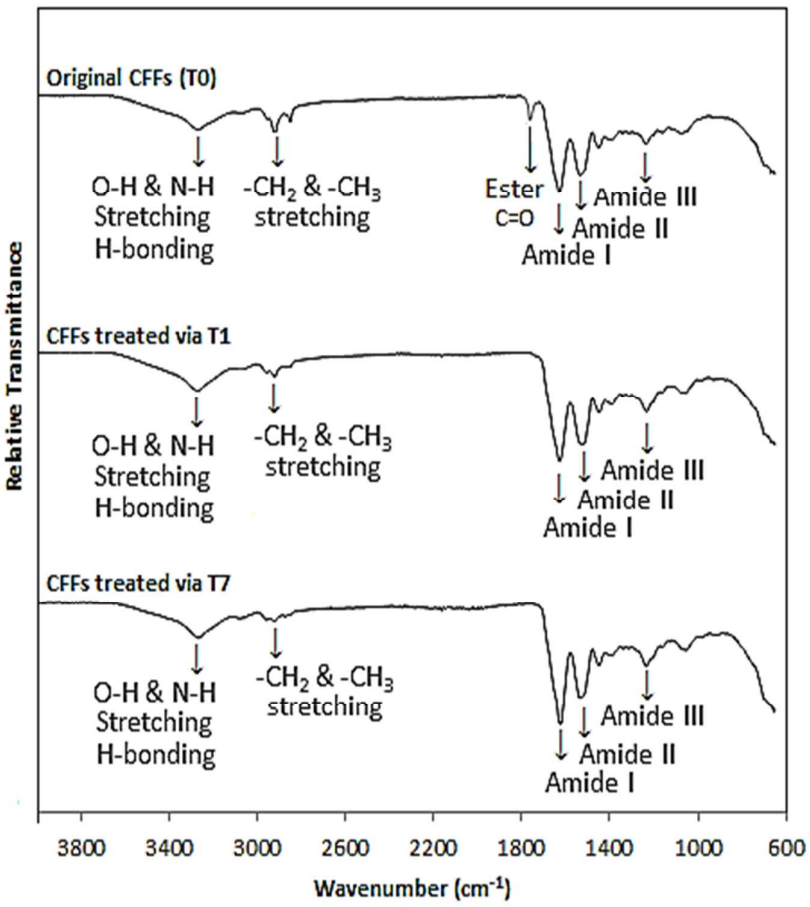

Figure 5: FTIR spectra of original CFFs (T0) and CFFs purified via SEEt (T1) and SLS-ClO_2-SEEt (T7)

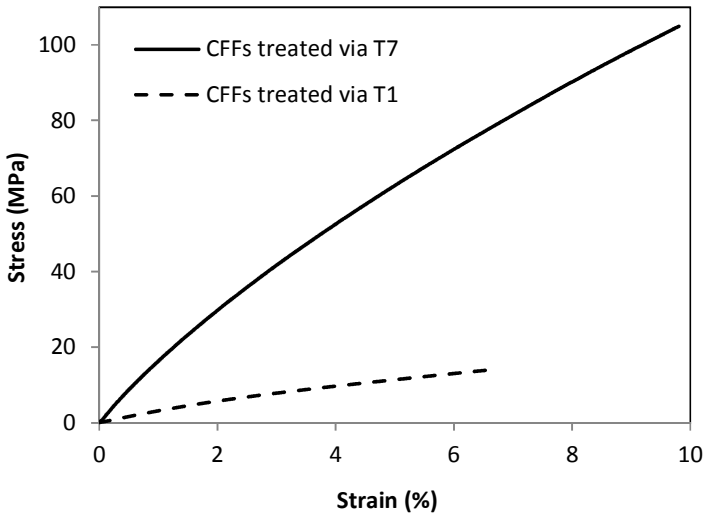

Figure 6: Tensile stress-strain curve of single CFFs purified via SEEt (T1) and SLS-ClO_2-SEEt (T7)