Journal of Molecular Catalysis B: Enzymatic 67 (2010) 231–235

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Effects of alkyl chain lengths of gallates upon enzymatic wool functionalisation

Kh.M. Gaffar Hossain, María Díaz González, José María Dagá Monmany, Tzanko Tzanov*

Group of Molecular and Industrial Biotechnology, Department of Chemical Engineering, Technical University of Catalonia, C. Colom, 11, 08222 Terrassa, Barcelona, Spain

ARTICLE INFO

Article history:
Received 9 March 2010
Received in revised form 13 August 2010
Accepted 18 August 2010
Available online 24 August 2010

Keywords:
Alkyl-gallate
Laccase
Wool
Antimicrobial
Water repellent
Antioxidant

ABSTRACT

The covalent grafting of alkyl gallates on wool through a laccase catalysed reaction in 80/20 (v/v, %) aqueous–ethanol mixture provided in a one-step process a multifunctional textile material with antioxidant, antibacterial and water repellent properties. Gallic acid and its alkyl esters ethyl, propyl, octyl and dodecyl gallate have been enzymatically grafted on wool fibres in order to study the effect of alkyl chain length on wool functional modification. The capacity of laccase to oxidise these phenolic compounds in an aqueous–organic medium has been verified by electrochemical techniques. The increase of CH_2, CH_3 groups in the FTIR spectra, together with the XPS analysis of the enzymatically modified fabrics confirmed the covalent grafting of ester gallates on wool. The result obtained in this work for antibacterial, water repellent as well as antioxidant properties show that the length of the alkyl chain of gallates molecule play an important role on wool functionalisation.

1. Introduction

Wool is a natural protein material largely used for clothing due to its lightness, softness, warmness, and smoothness. However, the complex chemical composition and architecture of the wool fibres is responsible for some undesirable properties such as high water absorption, felting in mechanical wet operations, and limited UV protection during use. Under proper temperature and humidity the wool materials could be a good medium for generation and propagation of microorganisms [1] that could cause fibre damage, skin irritations, and infections [2]. Microbial growth on textiles leads to odour development, discolouration, and even loss of elasticity and tenacity [3]. The infestation can be removed only by washing at boiling temperature where structural changes of the wool goods might occur. Moreover, the protein fibres are difficult to keep clean and are easily damaged by conventional cleaning agents.

For these reasons, the wool materials of high-grade clothes must be protected against microbial attack to prevent damage of the fibres and growth of pathogenic microorganisms. In addition to antibacterial finish, nowadays there is a great demand to produce textile goods with multifunctional properties such as antioxidant activity and self-cleaning properties. Hydrophobicity (water repellence) of textiles is frequently associated with self-cleaning properties. The self-cleaning action of hydrophobic coatings stems from their high water contact angles. Water on these surfaces forms almost spherical droplets that readily roll away carrying dust and dirt with them [4,5].

Gallic acid and its alkyl esters are natural compounds [6] used as food additives due to their antioxidant activity [7,8]. The alkyl gallates, having a molecular structure composed of a hydrophilic head (phenolic ring) and a hydrophobic alkyl chain, have also been reported to have antifungal and antibacterial activity [9,10]. Though different studies investigated the relation between the hydrophobic chain lengths and alkyl gallates' properties, there is no clear consensus regarding the effect of the chain length upon the antioxidant and antibacterial properties of gallates [11–15].

In addition to the antioxidant and antibacterial activities, the aliphatic chain present in the chemical structure of these compounds would provide hydrophobic properties as well. Therefore, it could be expected that the modification of wool with alkyl gallates would provide a combination of self-cleaning, antimicrobial and antioxidant properties.

A proof-of-concept one-step enzymatic process using laccase in aqueous–organic media for covalent grafting of dodecyl gallate (DG) on wool has been recently described [16]. Laccase is able to catalyse the oxidation of a wide range of aromatic compounds using molecular oxygen as electron acceptor and has been reported to retain activity in some water miscible organic solvents, e.g. ethanol [17,18]. The reaction was carried out in an 80/20 (v/v, %) aqueous–ethanol mixture to balance the need for dissolving DG with the need for maintaining the catalytic activity of laccase. The enzymatic grafting of dodecyl gallate on wool provided a multifunctional textile material with antioxidant, antibacterial and water repellent properties.

* Corresponding author. Tel.: +34 937398761; fax: +34 937398225.
E-mail address: tzanko.tzanov@upc.edu (T. Tzanov).

1381-1177/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2010.08.011

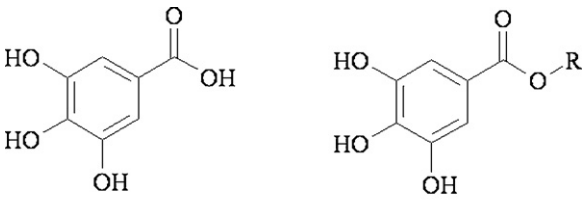

Fig. 1. Chemical structures of gallic acid and gallates compounds.

In the current work gallic acid and its alkyl esters with different aliphatic chain length, such as ethyl (EG), propyl (PG) and (OG) octyl gallates were tested as laccase substrates for enzymatic modification of wool targeting increased hydrophobicity, and enhanced antioxidant and antimicrobial properties. The objective was to elucidate the influence of the alkyl chain length on the functional properties of wool fibres.

2. Materials and methods

2.1. Reagents

Laccase (EC 1.10.3.2 *Trametes* sp. laccase, Laccase L603P; 0.125 g protein/g solid; 0.14 U/mg protein) was provided by Biocatalysts, UK. Enzyme activity (U) was defined as μmol of guaiacol oxidised per min at pH 4 and 40 °C (ε_{max} 6400 M^{-1} cm^{-1}). Methanol, sodium tartrate, hydrochloric acid, 1-diphenyl-2-picrylhydrazyl radical (DPPH•)were purchased from Sigma–Aldrich. Gallic acid (GA), ethyl gallate (EG), propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), (chemical structures of all these phenolic compounds in Fig. 1), baird parker agar and egg yolk tellurite emulsion were provided by Fluka. Ethanol 96% was purchased from Panreac. All chemicals used in this work were of the highest grade commercially available.

2.2. Electrochemical experiments

Voltammetric measurements were performed using a μAutolab Type III (EcoChemie) potentiostat/galvanostat controlled by Autolab GPES software version 4.9. All the experiments were carried out in a 20 mL Metrohm cell with a three-electrode configuration. The working electrode was a glassy carbon (GCE) with a surface diameter of 3 mm (Metrohm). The counter and reference electrodes were platinum (Metrohm) and Ag/AgCl (Metrohm) electrode, respectively. The renewal of the glassy carbon surface was achieved by polishing with 1.0 and 0.3 μm alpha-alumina (Micropolish, Buehler) on a microcloth polishing pad (Buehler), followed by washing in an ultrasonic Selecta bath for 2 min. For the experiment with laccase a 5 μl sample of a 0.02 g protein/mL enzyme solution was dropped onto the polished surface of the GCE and allowed to dry for 15 min at room temperature.

2.3. Wool preparation

Woven 100% wool fabric supplied by Lokateks (Slovenia) was washed previously to the enzymatic treatment with 1 g/L non-ionic surfactant Cotemol NI (Colorcenter, Spain) in liquor to good ratio 20:1 in a laboratory winch machine (0.1 M Na_2CO_3/$NaHCO_3$ buffer pH 9) at 40 °C for 30 min. Thereafter the fabric was bleached at the same bath ratio with 0.1 mL/L of 30% H_2O_2 (0.1 M Na_2CO_3, $NaHCO_3$ buffer pH 9) at 55 °C for 1 h.

2.4. Enzymatic treatment of wool

Samples of wool fabric (5 g) were incubated in a 80/20 (v/v, %) buffer/EtOH solution with 14 U/mL laccase and 1 mM GA, EG, PG, OG and DG according to the optimised procedure described in our previous work [16]. The reaction was allowed to proceed for 2 h, at 40 °C, 30 rpm in a laboratory dying machine Ahiba (Datacolor). Control samples without enzyme and phenolic compound were also treated at the above condition.

After the enzymatic treatment the samples were washed extensively in an 80/20 (v/v, %) buffer/EtOH solution for 1 h at room temperature, then with tap water and with distilled water at 80 °C for 30 min. After the hot wash every sample were washed in cold distilled water and dried at 50 °C for 2 h.

2.5. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectra of the wool fabrics before and after treatment were collected using a Perkin Elmer Paragon 1000 FT-IR spectrometer, performing 100 scans for each spectrum over the 400–4000 cm^{-1} range. All spectra were normalised against the peak at 1230 cm^{-1} corresponding to the amide I group.

2.6. X-ray photoelectron spectroscopy (XPS) analysis

XPS experiments were performed in a PHI 5500 Multitechnique System (from Physical Electronics) with a monochromatic X-ray source (Aluminium Kalfa line of 1486.6 eV energy and 350 W), placed perpendicular to the analyser axis and calibrated using the 3d5/2 line of Ag with a full width at half maximum (FWHM) of 0.8 eV. The analysed area was a circle of 0.8 mm diameter, and the selected resolution for the spectra was 187.5 eV of Pass Energy and 0.8 eV/step for the general spectra and 23.5 eV of Pass Energy and 0.1 eV/step for the spectra of the different elements. All measurements were carried out after cleaning by sputtering the surface with an Ar+ ion source (4 keV energy) in a ultra high vacuum (UHV) chamber at pressure between 5×10^{-9} and 2×10^{-8} Torr. The analysis were carried out in triplicate.

2.7. Water repellence of wool

The contact angles (θ) of water on the unmodified and modified wool fabric were measured using a 1/2 CCD camera, DSA 100, Kruss, GmbH, Germany. The measurements were carried out with distilled water as test liquid at 24 °C and 65% relative humidity [19]. The contact angles were followed during up to 600 s after the drop deposition on the surface of each sample. Three independent measurements were recorded on each sample.

2.8. Antioxidant activity

The radical scavenging activity of alkyl gallates modified wool was determined measuring the decrease in absorbance of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) at 515 nm [20]. This method, applied for both solid and liquid samples, is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors [21]. Samples of modified wool (200 mg) were incubated with 3 mL of a 3×10^{-5} M DPPH• solution in MeOH at room temperature in the dark for 1 h. Solution containing unmodified wool was used as a control. The experiment was carried out in triplicate and the results expressed as % inhibition of DPPH accord-

ing to the following formulae [22]:

$$\text{Inhibition of DPPH}\,(\%) = \left(1 - \frac{A_{\text{Sample}}}{A_{\text{Blank}}}\right) \times 100 \quad (1)$$

2.9. Antimicrobial activity

The most commonly used quantitative method for testing the antimicrobial activity of textiles is the Shake Flask method according to ASTM-E2149-01. This standard method was used to measure the reduction rate in the number of colonies formed and provided quantitative data, which could then be converted to average colony forming units per millilitre (CFU/mL) of buffer solution in the flask. Alkyl gallates modified and unmodified fabric swatches (0.5 g each) were incubated in 50 mL suspension of Gram-positive *Staphylococcus aureus* at 37 °C for 1 h. The suspension both before and after contact was diluted and cultured at 37 °C for 24 h to determine the number of surviving bacteria. The antimicrobial activity is reported in terms of percentage of bacteria reduction calculated by comparing the number of surviving bacteria before and after contact. The reduction rate in the number of colonies was calculated using the following formula:

$$\text{Bacteria reduction}\,(\%) = \left(\frac{A - B}{A}\right) \times 100 \quad (2)$$

where A and B are the average number of bacteria at time 1 h for unmodified and GA, EG, PG, OG and DG modified wool, respectively.

3. Results and discussion

3.1. Alkyl gallates oxidation by laccase in aqueous–ethanol mixture

Due to the insolubility of alkyl gallates in aqueous media, an appropriate aqueous/organic mixture to balance the need for dissolving the substrate with the need for maintaining catalytic activity of laccase is required to develop the enzymatic grafting of these compounds on wool. In our previous work a process in 80/20 (v/v, %) aqueous–ethanol mixture was optimised for enzymatic modification of wool with dodecyl gallate (DG). At this solvent to water ratio laccase retained 75–80% of its activity [16]. Using the same reaction condition the laccase oxidation of gallates with varying alkyl chain length was studied here by means of cyclic voltammetry. UV/vis spectrophotometry was not suitable for studying the enzymatic oxidation of gallates because no differences in the UV/vis spectra of these compounds were observed during the reaction. Alternatively, electrochemistry can be easily applied to study this reaction since the phenolic compounds usually exhibit an electrodic process, while the suitability of electrochemistry to study enzymatic reactions in aqueous–organic media was previously reported.

The electrochemical behaviour of all gallate compounds studied can be summarized in the example in Fig. 2, where the cyclic voltammograms (1st and 2nd scan) obtained for PG are presented. All gallates underwent irreversible oxidation processes (E_{pa}) at 374, 378, 374, 344 and 383 mV for GA, EG, PG, OG and DG, respectively. The decrease in the oxidation current recorded after the first scan is most probably due to phenolic compounds polymerisation on the electrode surface.

In the presence of laccase, an important decrease in the anodic peak due to catalytic instead of electrodic oxidation of the phenolic compound was recorded for all gallate compounds (Fig. 3). As the peak current is related to the concentration, if one compound is enzymatically oxidised by laccase, a decrease in the oxidation current related to the minor concentration of non oxidised specie present at electrode surface is expected.

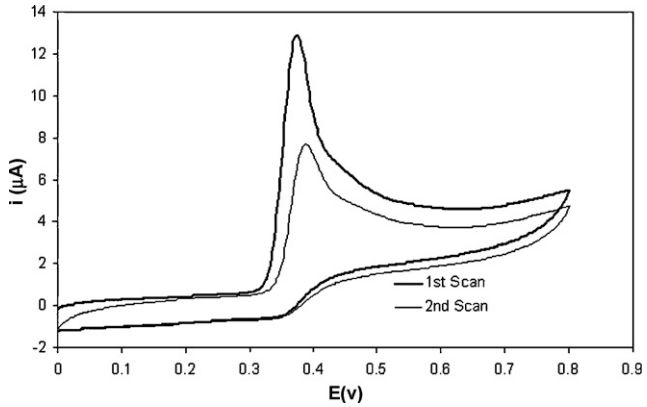

Fig. 2. Cyclic voltammograms of 0.5 mM PG 80/20 (v/v, %) 0.1 M tartrate buffer–EtOH mixture pH 4.

3.2. Enzymatic grafting of GA and alkyl gallates on wool fabrics

Wool fabric was treated with 1.0 mM concentration of the different gallate compounds and laccase (14 U/mL) in 80/20 (v/v, %) buffer/EtOH. The concentration of phenolic compound was previously optimised for the enzymatic modification of wool with dodecyl gallate (DG) [16]. Unfixed phenolic compounds on wool surface were removed in an 1 h cleaning step with 80/20% (v/v) H_2O/EtOH followed by extensive washing with tap and distilled water at room temperature.

Changes in the surface chemistry of the enzymatically modified wool fabrics were studied using FTIR spectroscopy. The FTIR spectrum in Fig. 4 shows a significant difference between modified (lines GA, EG, PG, OG and DG) and unmodified wool fabrics (line C). The increasing absorption at 1456 cm^{-1} and 1474 cm^{-1}, corresponding to the bending vibrations of CH_2 and CH_3 respectively, is consistent with the grafting of alkyl gallate moieties on the fabrics [23]. Furthermore, the peak intensity at 1456 cm^{-1} increased gradually increasing the length of the alkyl chain of the gallates grafted. Similar to previously reported DG, which FTIR spectrum is also included, during the enzymatic treatment laccase oxidises these phenolic compounds into reactive quinones that can undergo either Schiff base or Michael's-type addition reaction with amine groups in wool [24].

The modified and unmodified wool samples were further characterized by XPS spectroscopy and the data given in weight percentages for the chemical composition change on the surface of wool after enzymatic modification are shown in Table 1.

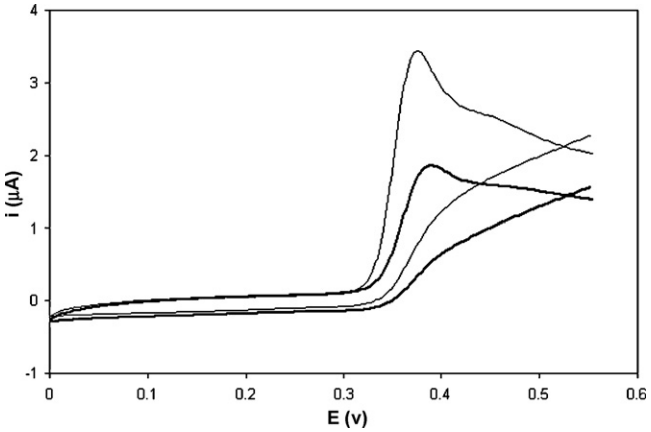

Fig. 3. Cyclic voltammograms of 0.5 mM PG alone (—) and in the presence of laccase (▬). Scan rate 5 mV/s.

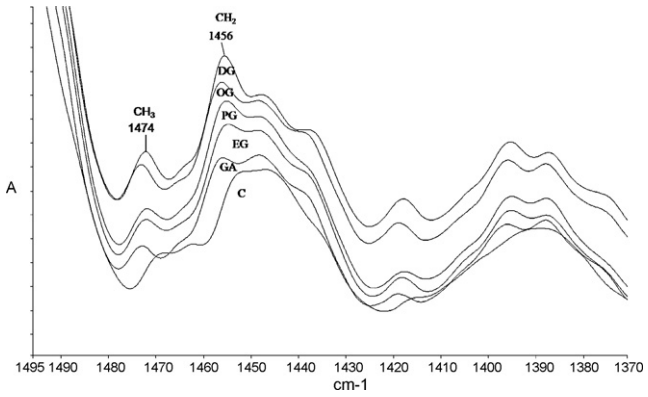

Fig. 4. Fourier transform infrared spectroscopy (FTIR) spectra in the range of 1370–1495 cm^{-1} of: (C) untreated wool; (GA) laccase and 1 mM gallic acid treated wool (EG) laccase and 1 mM ethyl gallate treated wool; (PG) laccase and 1 mM propyl gallate treated wool (OG) laccase and 1 mM octyl gallate treated wool and (DG) laccase and 1 mM dodecyl gallate treated wool. Laccase, 14 U/mL.

The surface composition of treated wool samples confirmed the laccase-catalysed grafting of alkyl gallates. Comparing the weight percentages in the unmodified wool fabric with those determined in GA and EG treated samples, it can be observed that they are very similar for the four elements measured (C, O, N, S), meaning that the grafting for GA and EG must be very limited. However, in the cases of PG, OG and DG modified fabrics the changes in carbon and oxygen composition correlate directly with the amount of carbon and oxygen coming from the PG, OG and DG molecules grafted on fabric surface. It should be noted that XPS is a surface analysis and an absolutely uniform grafting/coating of an inherently non-uniform surface (textile fabric) is virtually impossible. Also, we could not assume that the level of grafting on fabric surface is exactly the same for PG, OG and DG. Nevertheless, it seems logical that if PG, OG and DG grafting on the surface occurred, the overall percentages in mass for N and S only encountered in wool, should decrease compared to the amount of N and S on the surface of the unmodified fabric. The percentages of all four elements measured on the DG modified sample agree perfectly in variation in respect to the untreated sample. The percentages of carbon and oxygen are greater in the DG-treated fabric than in the control sample, because the DG brought to the fabric surface additional carbon and oxygen, while the content of N and S remained unchanged. This is not a decrease in the absolute content of N and S, but rather a decrease of their proportion in the area analysed by XPS on expense of the increased content of carbon and oxygen.

Table 1
Elemental composition in weight percentages determined by XPS for GA, alkyl gallates and the samples as described in Fig. 4.

		C	O	N	S
Ester gallates	GA	49.92	47.02		
	EG	54.55	40.37		
	PG	56.6	37.7		
	OG	63.81	28.33		
	DG	67.43	23.64		
Wool fabrics	C	64.73	17.05	9.80	5.79
	GA	63.53	16.68	10.28	6.88
	EG	64.64	16.41	8.81	5.63
	PG	61.59	18.51	8.83	5.35
	OG	63.12	18.81	9.14	5.58
	DG	65.33	17.36	8.44	5.22

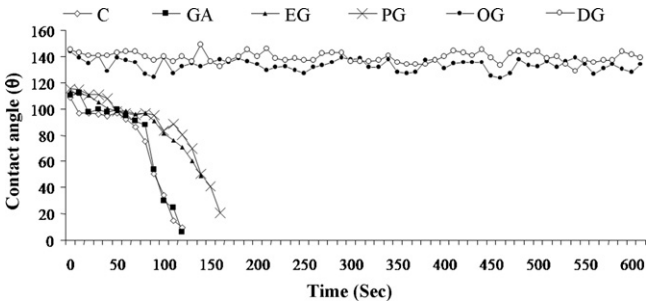

Fig. 5. Contact angles over the time for samples as describe in Fig. 4.

3.3. Water repellence of modified wool

The water contact angle (θ) reflects the surface hydrophilicity by measuring the water droplet spreading on a surface. The higher the contact angle, the more hydrophobic the surface is. The time-dependent spreading of a water drop on wool samples is shown in Fig. 5. Both untreated (C) and GA treated wool (GA) showed contact angles (θ) of about 109°, and wetting time about 100 s. These results reveal that wool is hydrophobic in nature even after scouring due to the strong influence of the fatty layer on its surface properties. As expected, the presence of the alkyl chain of gallates had a direct effect on the hydrophobic properties of wool after the enzymatic modification. All samples modified with alkyl gallates exhibited an increase in wetting time and contact angle values compared to those obtained for unmodified and GA modified wool. Furthermore, a clear effect of alkyl chain length on both parameters was observed. The incorporation of OG and DG with longer chain lengths (8 and 12 carbons, respectively) showed a pronounced hydrophobisation effect on wool. For both compounds wetting times higher than 600 s and contact angle values around 146° were obtained. The enhanced hydrophobic properties of the alkyl gallates treated fabrics indirectly confirm the enzymatic grafting of these phenolic compounds on the fibres.

3.4. Antioxidant activity

The results obtained for the antioxidant activity of wool samples enzymatically modified with GA, EG, PG and OG are shown in Fig. 6 including also the results for the previously studied phenolic compound dodecyl gallate (DG). As previously reported [7] all gallates tested, regardless of their alkyl chain length, showed similar scavenging activity on DPPH in solution indicating that the alkyl chain length was not directly related to this activity. However, after incorporation on wool the antioxidant activity of gallates increased with the increase of the length of gallate alkyl chain. As the antioxidant efficiency of gallates depends upon the reactivity of the phenolic groups, the results obtained suggest that during the grafting reaction on solid surface (wool fibres) more phenolic groups remained

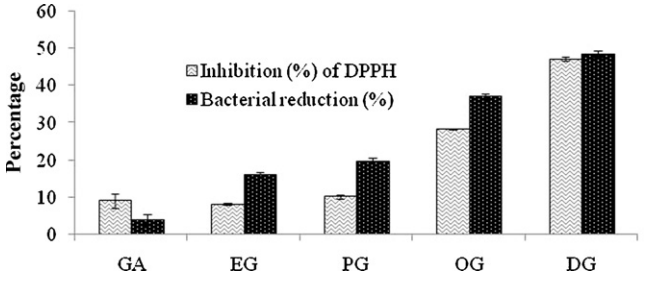

Fig. 6. Antioxidant activity as inhibition (%) of DPPH and antibacterial effect (bacteria reduction %) of the wool samples as described in Fig. 4.

active/non-oxidised for the compounds with longer alkyl chain, compared to the experiments carried out in solution. This might be related to some diffusion constrains imposed by the fibre surface to the enzyme for oxidation of all hydroxyl groups available in the corresponding gallate already grafted on the fibres, in addition to the hydrophobisation effect of the alkyl chains.

3.5. Antimicrobial activity

As can be seen in Fig. 6, all modified wool fabrics exhibited antimicrobial activity against Gram-positive *Staphylococcus aureus*. Antimicrobial activity similar to that obtained for untreated wool was observed for gallic acid modified fabric, confirming inefficient grafting of this compound on the fabric. By contrast, all the other gallates modified samples (EG, PG, OG and DG) showed a marked improvement in their antimicrobial activity. The percentage of bacterial growth reduction increased with the increase of hydrocarbon chain length grafted on wool up to 37% and 50% for the wool modified with OG and DG, respectively. These results are in agreement with previously reported hypothesis for antifungal [9] and antimicrobial activity [25–28] for head-tail structure chemicals having a molecule composed of two parts—a hydrophilic head and a long chain hydrophobic tail. The hydrophilic head of these chemicals uses an intermolecular hydrogen bond to bind with the hydrophilic portion of the microorganism membrane, while the nonpolar hydrophobic tail aligns into the membrane lipid bilayer by dispersion forces and disturbs the lipid–protein interface of integral proteins, disrupting their conformation.

4. Conclusions

Covalent grafting of otherwise insoluble alkyl gallates (EG, PG, OG and DG) on wool fabrics using laccase in aqueous–organic medium provided a textile material with antimicrobial, antioxidant and water repellent properties. These functional properties of wool revealed to be strongly influenced by the alkyl chain length of the gallates. The longer the hydrocarbon chain of alkyl gallate grafted on wool is, the higher is the hydrophobicity, antimicrobial and antioxidant activity. Contact angles of the modified wool fabrics increased from 109° for GA to 146° for both OG and DG, indicating excellent water repellence for these two compounds. The enzymatic modification of wool with alkyl gallates provided also antioxidant activity expressed in about 55% inhibition of DPPH for DG. Reduction of bacteria growth increased with the increase of hydrocarbon chain length grafted on wool and reached up to 37% and 50% for wool modified with OG and DG, respectively. The laccase-catalysed covalent grafting of alkyl gallates on wool was supported by FTIR and XPS analysis. Cyclic voltammetry measurement of gallates oxidation with laccase demonstrated the feasibility of the process in aqueous–organic medium.

Acknowledgements

We gratefully acknowledge the Agency for Administration of University and Research Grant (AGAUR) providing the PhD grant of Kh.M. Gaffar Hossain, and the Universitat Politècnica de Catalunya providing the Postdoctoral research contract of Dr. María Díaz González.

References

[1] J. Kuwana, S. Nakamura, H. Sugiyama, Textile Res. J. 33 (1963) 489–500.
[2] E. Heine, H.G. Knops, K. Schaefer, P. Vangeyte, M. Moeller, Multifunctional Barriers for Flexible Structure Textile, Leather and Paper. Chapter: Antimicrobial Functionalisation of Textile Materials, Springer Verlag 97 (2007) 23–38.
[3] M.S. Masri, F.W. Reuter, M. Friedman, Textile Res. J. 44 (1974) 298–300.
[4] A. Marmur, Langmuir 20 (2004) 1317–1320.
[5] C.W. Extrand, Langmuir 18 (2002) 7991–7999.
[6] M. Inoue, R. Suzuki, N. Sakaguchi, Z. Li, T. Takeda, Y. Ogihara, B.Y. Jiang, Y. Chen, Biol. Pharm. Bull. 18 (1995) 1526–1530.
[7] A.M. Campos, E. Poncea, E.A. Lissi, J. Phys. Org. Chem. 22 (2009) 1208–1211.
[8] I. Kubo, N. Masuoka, P. Xiao, H. Haraguchi, J. Agric. Food Chem. 50 (2002) 3533–3539.
[9] I. Kubo, P. Xiao, K. Fujita, Bioorg. Med. Chem. Lett. 11 (2001) 347–350.
[10] N. Togashi, A. Shiraishi, M. Nishizaka, K. Matsuoka, K. Endo, H. Hamashima, Y. Inoue, Molecules 12 (2007) 139–148.
[11] C.W. Sheu, E. Freese, J. Bacteriol. 115 (1973) 869–875.
[12] A. Mates, J. Appl. Bacteriol. 37 (1974) 1–6.
[13] I. Kubo, H. Muroi, A. Kubo, J. Agric. Food Chem. 41 (1993) 2447–2450.
[14] N. Kabelitz, P.M. Santos, H.J. Heipieper, FEMS Microbiol. Lett. 220 (2003) 223–227.
[15] Y. Tanaka, S. Fukuda, H. Kikuzaki, N. Nakatani, ITE Lett. Batter. New Technol. Med. 1 (2002) 777–780.
[16] Kh.M. Gaffar Hossain, M. Díaz González, G. Rocasalbas Lozano, T. Tzanov, J. Biotechnol. 141 (2009) 58–63.
[17] T. Chen, G. Kumar, M.T. Harris, P.J. Smith, G.F. Payne, Biotechnol. Bioeng. 70 (2000) 564–573.
[18] F. Guoning, Z. Jingchan, Z. Yongke, G. Zhian, Polym. Bull. 56 (2006) 507–515.
[19] N. Ayrilmis, T. Dundar, Z. Candan, T. Akbulut, BioResources 4 (2009) 1536–1544.
[20] M.S. Blois, Nature 181 (1958) 1199–1200.
[21] A. Serpen, E. Capuano, V. Fogliano, V. Gökmen, J. Agric. Food Chem. 55 (2007) 7676–7681.
[22] F. Sousa, G.M. Guebitz, V. Kokol, Process Biochem. 44 (2009) 749–756.
[23] V.L. Furer, I.I. Vandukova, E.A. Tatarinova, A.M. Muzafarov, V.I. Kovalenko, Acta Part A 70 (2008) 692–699.
[24] K. Yamada, T. Inoue, Y. Akiba, A. Kashiwada, K. Matsuda, M. Hirata, Biosci. Biotechnol. Biochem. 70 (2006) 2467–2475.
[25] I. Kubo, P. Xiao, K. Fujita, Bioorg. Med. Chem. Lett. 12 (2002) 113–116.
[26] I. Kubo, H. Muroi, H. Masaki, A. Kubo, Bioorg. Med. Chem. Lett. 3 (1993) 1305–1308.
[27] I. Kubo, H. Muroi, A. Kubo, Bioorg. Med. Chem. Lett. 3 (1995) 873–880.
[28] I. Kubo, K. Fujita, K. Nihei, A. Kubo, J. Appl. Microbiol. 96 (2004) 693–699.