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## A facile and sustainable method for integrating bio-based quercetin into cotton structures to impart multifunctionality: a thorough study on the effects of treatment conditions<sup>†</sup>

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The worsening climate crisis has prompted a call to reduce the use of hazardous chemicals and promote eco-friendly finishing agents for value-added textiles. Therefore, exploring the right biomolecule-based finish with excellent multifunctional properties and wash durability is essential in today's world. Through a sustainable approach, quercetin (a flavonoid) is proposed as a finishing agent on cotton substrates at three different treatment temperatures, *i.e.*, 80 °C, 100 °C, and 120 °C. All treated fabrics show excellent antioxidant and ultra-violet resistance properties. The cotton fabric treated at 120 °C shows antioxidant activity of ~85% and an ultra-violet protection factor (UPF mean value) of ~150 with a 50+ rating after 8 laundering cycles. The best antibacterial performance (~90% against *E. coli* and ~92% against *S. aureus*) is observed when treated at 80 °C. However, there is a decrement in the antibacterial properties against both bacteria with an increase in the treatment temperature. This study provides a detailed analysis of the multi-functional properties of quercetin on cotton fabrics and also systematically presents the variation of functional properties with changes in the treatment conditions. This systematic study is highly focused, as the fragmented and oxidative quercetin by-products formed at different conditions play major roles in the wash durability and multi-functional properties. This novel and simple eco-friendly textile finishing method can certainly facilitate the adoption of biomolecule-based materials to imbue multiple functionalities to cotton fabric with reasonable wash durability.

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### Sustainability spotlight

The growing focus on sustainability has increased the demand for eco-friendly, biocompatible, and biodegradable alternatives to traditional multi-functional finishing agents for textile. This study focuses on the development of multi-functional cotton through a simple approach using only the quercetin biomolecule and water, without any toxic chemicals. The multi-functional properties are also sustained after several machine washes, which is uncommon for many other natural finishing agents. The treated fabric shows UV-protection, antioxidant and anti-bacterial properties. Our study also examined how the treatment temperature and washing conditions impact the functional properties of the treated fabrics, as quercetin can produce fragmented and oxidative byproducts during treatment. This may result in a loss of multi-functional properties and durability. This focused research is necessary for developing wash-durable and sustainable multi-functional quercetin-treated cotton fabric. This study contributes a greener approach by aligning with the UN's sustainable development goals by avoiding the use of toxic chemicals (SDG 3), contributing to the development of eco-friendly products (SDG 9), and increasing the durable use of the textile substrate by enhancing wash durability (SDG 12).

## Introduction

Multifunctional fabrics are currently one of the most sought-after items.<sup>1-3</sup> These fabrics possess several functional properties simultaneously for potential applications. Traditionally, different types of finishing agents and multi-step treatments are required to develop multifunctional properties in a fabric,

which are replaced by using a single finishing agent with minimal-step treatments. Therefore, this change is quite beneficial and cost-effective over traditional methods.<sup>3,4</sup> However, when upgrading to modern methods to prepare multifunctional fabrics, various issues arise due to synthetic multi-finishing agents such as environmental problems, health hazards, and economic crises.<sup>5,6</sup> The most widely used synthetic multi-finishing agents for textile substrates are nano-silica,<sup>7</sup> ZnO nanoparticles with TiO<sub>2</sub>,<sup>8</sup> and silver nanoparticles.<sup>9</sup> Despite their high efficiency, there are major concerns regarding the toxicity and bio-compatibility for the environment. Therefore, in this rapidly climate-changing world, greener approaches are

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more valued and appreciated in the wet processing sector of textile finishing. Owing to increasing concerns, greater attention has been directed toward natural finishing agents. Furthermore, there has been increasing interest in the use of natural extracts, such as chitosan and tea extract,<sup>10</sup> neem,<sup>11</sup> aloe vera extract,<sup>12</sup> to obtain multi-functional properties. However, these natural extracts comprise several biomolecules. Furthermore, the functional properties of one biomolecule may overpower the effects of other biomolecules.<sup>13</sup> Thus, an isolated biomolecule should be explored to obtain the specific desired functional properties. Among all biomolecules, secondary metabolites protect organisms from harsh environments, microbes, and predators. This is the best approach for mimicking multi-functional finishing agents on textile substrates.<sup>14,15</sup> These secondary metabolites, such as flavonoids, phenylpropanoids, benzoic acids, and simple phenols, can be extracted from the waste part of plants and animals and used as multifunctional finishing and dyeing agents for textile substrates due to their diverse range of functional properties.<sup>16</sup> Among the secondary metabolites, a large range of functional properties is covered by flavonoids, consisting of numerous hydroxyl groups and double bonds. The most common flavonoids are catechins, myricetin, and quercetin.<sup>17</sup> Quercetin is one of the most abundantly available flavonoids, and is rich in functional properties.

The structure of quercetin consists of a 3-ring structure, *i.e.*, a pyrene ring having one oxygen and two benzene rings with a total of 5 hydroxyl groups.<sup>18</sup> Despite having many hydroxyl groups, quercetin exhibits a hydrophobic nature, and its structural conformation is responsible for the low water solubility. Quercetin possesses many properties, including antioxidant,<sup>19,20</sup> antibacterial,<sup>21–23</sup> antiviral,<sup>24,25</sup> antifungal,<sup>26</sup> antitumor,<sup>27</sup> anti-inflammatory,<sup>27</sup> anti-diabetic,<sup>28</sup> ultra-violet (UV) protection,<sup>26</sup> and good healing properties.<sup>29</sup> Due to its numerous functionalities, it is widely used in several industries, such as the cosmetic sector,<sup>30,31</sup> pharmaceutical sector,<sup>32</sup> medical sector,<sup>33</sup> drug delivery,<sup>34,35</sup> and as food supplements.<sup>36,37</sup> So, it is a good choice as a multifunctional finishing agent on fabrics due to its significant and numerous functional properties.

Quercetin can be easily extracted from green wastes by ultrasound-assisted extraction, supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, and solid phase extraction.<sup>38,39</sup> These natural extracts consist of several other compounds, which have been further identified and isolated. Thus, the determination, isolation, and purification of quercetin have been carried out by chromatographic analysis and by some electrochemical methods, *e.g.*, High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass spectrometry (GCMS), Thin Layer Chromatography (TLC), voltammetry, spectrophotometry, HPLC-MS, and capillary electrophoresis.<sup>39</sup> Numerous studies were carried out to extract and isolate quercetin from plants, such as *Aesculus indica* fruit by solvent-solvent (methanol) extraction method,<sup>40</sup> and *Actinostemma lobatum* extract by solvent (ethanolic) extraction process.<sup>41</sup> The ultrasound-assisted extraction method was used to extract quercetin from onion

solid waste, where  $11.1 \text{ mg g}^{-1}$  of the total quercetin content was obtained.<sup>42</sup> Quercetin was also isolated from the solid onion extract by solid-phase separation process.<sup>43</sup> A maximum yield of  $10.9 \pm 0.2 \text{ mg g}^{-1}$  of quercetin was obtained from the scales of onion by the single-step liquid-liquid extraction method.<sup>44</sup>

Several research studies have been carried out, where the extracted products from fruits and vegetables are used for multifunctional finishing on textiles. In many cases, quercetin is the major component.<sup>45–49</sup> However, the combination of biomolecules was applied to the textile substrates in these major studies, which generally led to the hampering of the functional properties of a particular biomolecule by other biomolecules. So, multiple biomolecule finishing must be bypassed, and functional properties through a single biomolecule finishing must be a focal point.

There are very few studies in the literature on multifunctional finishing using only the quercetin biomolecule.<sup>50,51</sup> However, no research study has focused on the application of a segregated quercetin biomolecule on cotton fabric to achieve multifunctional properties, whereas cotton is the most consumable textile material worldwide. Despite its excellent properties, quercetin is one of the most unstable biomolecules, and gets easily oxidized under mild conditions.<sup>52,53</sup> There are no detailed studies on the impact of finishing parameters, especially the effect of temperature (having immense influence on the structural integrity of the quercetin, and thus the functional properties) on the functional properties of quercetin-treated fabric. In this study, quercetin is applied to cotton fabric at different temperatures, and the functional properties of the treated fabrics are then assessed in a very systematic way. The study is designed to analyze the effect of temperature in integrating quercetin into the cotton structure, and also the influence of the processing temperature on the functional properties of quercetin on the treated cotton fabric.

## Experimental

### Materials

Cotton fabric ( $70 \text{ g m}^{-2}$ ) of plain weave was collected from a local market situated in New Delhi, India. Quercetin dihydrate (molecular weight 338.3) ( $\geq 98\%$ ) was procured from Central Drug House (Pvt.) Ltd 2,2-diphenyl-1-picrylhydrazyl (DPPH) (95%) was procured from Central Drug House, while both acetonitrile (HPLC grade) and phosphoric acid were collected from Qualigens. The chemicals required for antibacterial testing, *i.e.*, luria broth and agar-agar, were purchased from HiMedia and Qualigens, respectively. Other chemicals such as Optifix were obtained from Clariant (India), ECE non-phosphate detergent (without optical brightening agent) from SDC Enterprises Ltd, methanol from Merck. Ethanol, lissapol-N, and distilled water were also used for treatment and characterizations.

### Application of commercial quercetin on cotton fabric

The procured cotton fabric was washed with Lissapol-N (non-ionic surfactant) to remove dirt and any other temporary



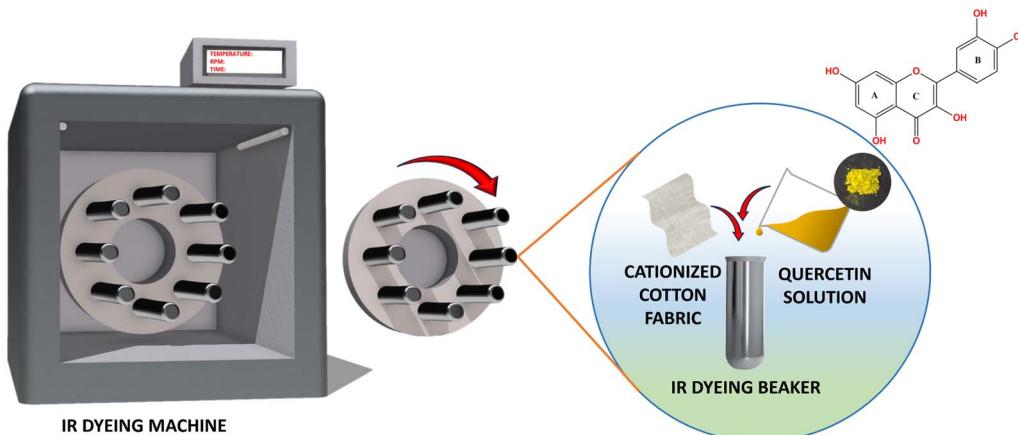


Fig. 1 Schematic representation of finishing cationized cotton with quercetin.

finishing agent present in the fabric. Then, the pre-washed cotton fabric (control 1) was cationized using the Optifix cationizing agent<sup>54</sup> at 6% on weight of fabric (o.w.f) for 40 min (material-to-liquor ratio 1 : 40), followed by drying the fabric at 70 °C. The pretreated cationized cotton fabric (control 2) was used for the integration of quercetin in an IR (infrared) dyeing machine (Starlet DLS-7000) at different temperatures, *i.e.*, 80 °C, 100 °C, and 120 °C, with a 1 : 30 (material-to-liquor ratio) and quercetin concentration of 15% o.w.f in water. The finishing process involved gradually increasing the temperature from 40 °C to the target temperature (2 °C min<sup>-1</sup>, 3 °C min<sup>-1</sup>, and 4 °C min<sup>-1</sup>), maintaining the final temperature for 40 minutes. After completion of the finishing treatment, it was cooled down to 40 °C to collect the finished fabric, followed by water rinse. This quercetin-finished cotton fabric (pretreated with Optifix) was called o-QC fabric. A similar process was followed to develop a quercetin-finished cotton fabric with control 1, which was referred to as QC fabric. The schematic representation of the finishing process is shown in Fig. 1.

### Characterization

The quercetin in aqueous solution was treated at 80 °C, 100 °C, and 120 °C using an IR dyeing machine, maintaining consistent conditions as used for the treatment of cotton fabric with quercetin. After cooling the treated solution, the settled quercetin residue was separated by centrifugation. Both solid residues and supernatant were collected separately, and the solid residues were freeze-dried. The freeze-dried solid residue in methanol solution was used for UV-VIS spectrophotometric and High-Performance Liquid Chromatography (HPLC) analyses, while the filtered supernatant was used for UV-VIS spectrophotometric analysis.

**UV-VIS spectrophotometric analysis.** A UV-VIS spectrophotometer (UV-1900i, Shimadzu) was used to determine the absorbance peaks within the wavelength range of 200 to 800 nm.

**High-performance liquid chromatographic analysis (HPLC).** HPLC (P series model, Shimadzu) was used to detect fragmented or auto-oxidized products of quercetin in solid

quercetin residues. This model consists of a reverse phase C18 column having particles with a diameter of 5 µm. The detailed chromatographic conditions are explained in Note S1.<sup>†</sup> The analysis was performed at 370 nm, with a column temperature of 60 °C and a total run time of 16 min.

**Spectrophotometric analysis (colour value).** The colour depth (*K/S*) and total colour difference ( $\Delta E$ ) value were assessed by the Premier Colour Scan Spectrophotometer (SS 5100H), with a D65 illuminant and observer angle of 10°, following the CIE-LAB (1976) standard. The average colour values were examined by scanning 3 different locations for every individual sample. The detailed calculation of *K/S* and  $\Delta E$  is mentioned in Note S2.<sup>†</sup> In this study,  $\Delta E$  is necessary, as the higher colour difference reflects a significant shift in the colour of the treated fabric compared to the untreated white fabric. For the analysis of the colour values, the integrated wavelength was selected in this case, as the maximum wavelength differs with the changes in the treatment temperatures.

### Functional properties

**DPPH free radical scavenging assay.** The antioxidant property was evaluated by the DPPH free radical scavenging method, where the absorbance of the DPPH solution with the fabric dipped for 30 min was measured at 517 nm wavelength using a UV-VIS spectrophotometer (Shimadzu UV-1900i). The antioxidant efficacy was assessed by the following eqn (1),

$$\text{Antioxidant efficacy}(\%) = \left( \frac{A - B}{A} \right) \times 100 \quad (1)$$

where, *A* and *B* are the absorbance values of the DPPH solution with immersed untreated and treated fabric, respectively.

**Evaluation of the ultraviolet protection property.** The ultraviolet protection factor (UPF) value was evaluated by scanning a fabric sample 5 times from different locations using the LabSphere UPF 2000, following the standard AS/NZS 4399:2017.

**Antibacterial studies.** The antibacterial activity of treated and untreated fabrics was evaluated by quantitative method, *i.e.*, colony-counting method (AATCC 100). The antibacterial properties were studied against both Gram-positive



(*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Firstly, these bacterial stains were cultured for further processes. The cultured bacteria were diluted to achieve an optical density (OD 600) of 0.04 and 0.06 for *S. aureus* and *E. coli*, respectively, *i.e.*,  $2.9 \times 10^7$  CFU mL<sup>-1</sup> of *S. aureus* and  $4.7 \times 10^7$  CFU mL<sup>-1</sup> of *E. coli*, and used as a bacterial suspension. The sample size of 2 cm × 2 cm was suspended in sterilized luria broth solution containing 10 µL bacterial suspension, and incubated for 24 hours at 37 °C. The incubated suspension was serially diluted using sterilized water. A 100 µL volume of diluted suspension was used for agar plating, and the agar plates were further incubated for 24 hours at 37 °C. These experiments were conducted in triplicate. After the incubation of the agar plates for 24 hours, the bacterial colonies were counted by analysing images of the agar plates in ImageJ software. The antibacterial property was evaluated by eqn (2), as follows:

$$\text{Antibacterial efficacy}(\%) = \left( \frac{C - T}{C} \right) \times 100 \quad (2)$$

where,  $C$  = the number of bacterial colonies present in the control sample-agar plate and  $T$  = the number of bacterial colonies present in the treated sample-agar plate.

**Evaluation of the functional durability after washing.** The functional properties and colour value of the treated fabrics were assessed after several machine-washing cycles. A launder-o-meter (R. B. Electronic and Engineering) was used to conduct the laundering cycles of the treated and untreated fabrics by following the ISO 105-C06 standard. Fabric samples were dipped in a beaker containing a 2 gPL ECE non-phosphate reference detergent solution (without optical brightening agent) with 1:50 MLR. Afterward, the beakers were placed in a launder-o-meter for 30 minutes at 40 °C to complete 1 laundering cycle. After the completion of the washing cycles, the samples were taken out and thoroughly rinsed with normal water. These washed samples were used to evaluate the functional properties after certain washing cycles to check the durability of the finishing agent on the fabric.

### Assessment of the flexibility and mechanical strength of the treated fabric

The flexibility and mechanical strength of the treated fabric were characterized by assessing the bending length and tensile strength, respectively. The bending length measurement was carried out by a paramount stiffness tester, following ASTM D1388, while the tensile properties were measured by using the Tinius Olsen testing machine according to ASTM D5035.

## Results and discussion

### Effect of quercetin in aqueous media

Quercetin is insoluble in water at room temperature, and the solubility level of quercetin increases with increasing temperature. However, that partially soluble quercetin again transforms into an insoluble form with decreasing temperature. Fig. 2a represents the solubility behaviour of quercetin in aqueous

solution with changes in temperature. When the quercetin solution remains untouched for a certain period of time, the quercetin aggregates and tends to settle down in the solution. Meanwhile, the heated quercetin aqueous solution appears as a fully or partially transparent solution with no or less quercetin aggregates.<sup>55</sup> Along with the low solubility of quercetin at high temperatures, quercetin in aqueous solution is unstable in alkaline conditions and at higher temperatures, where it undergoes auto-oxidation.<sup>53,56</sup>

Due to deprotonation and auto-oxidation, different fragmented products are produced. There are different pathways that are followed during the auto-oxidation of quercetin, depending on the effective reaction conditions. The fragmented by-products of quercetin are produced either by oxidative decarbonylation by oxygen, quercetin oxidative decarboxylation by oxygen, or keto-enol tautomerisation of quercetin depending on the pH of the solution, and it further breaks down into additional fragmented products.<sup>52,57,58</sup> Among all of the formed by-products, the major products that are most commonly mentioned are protocatechuic acid, phloroglucinol acid, and phloroglucinol.<sup>52,57,59</sup> The structures of the main formed fragmented products are given in Fig. S1.†

### Application of commercial quercetin to cotton fabric

The chances of migration and diffusion of quercetin inside a cotton fiber is almost negligible, as quercetin is insoluble in water at room temperature. The solubility of quercetin in water increases by increasing the finishing temperature.<sup>55</sup> The soluble quercetin penetrates easily into the amorphous zones of cotton fibers at higher temperatures. During the cooling process to room temperature, the soluble quercetin gets converted into the insoluble form and is entrapped within the amorphous zones of the cotton fibers.

The cationization of cotton fabric further improves the affinity of flavonoids toward cotton fabric.<sup>60</sup> There are chances of electrostatic attraction between cationized cotton and the hydroxyl group of the quercetin structure.<sup>61,62</sup> The cotton fabric is cationized using a commercial cationizing agent, *i.e.*, Optifix, as a pre-finishing treatment. There are high chances of electrostatic interaction between the cations present in the pre-treated cotton fabric and the anions present in the functional groups (hydroxyl groups) of quercetin, *i.e.*, the transformation of the O<sup>-</sup> ion from the hydroxyl group at high temperature in aqueous solution, as explained in Fig. 2c.

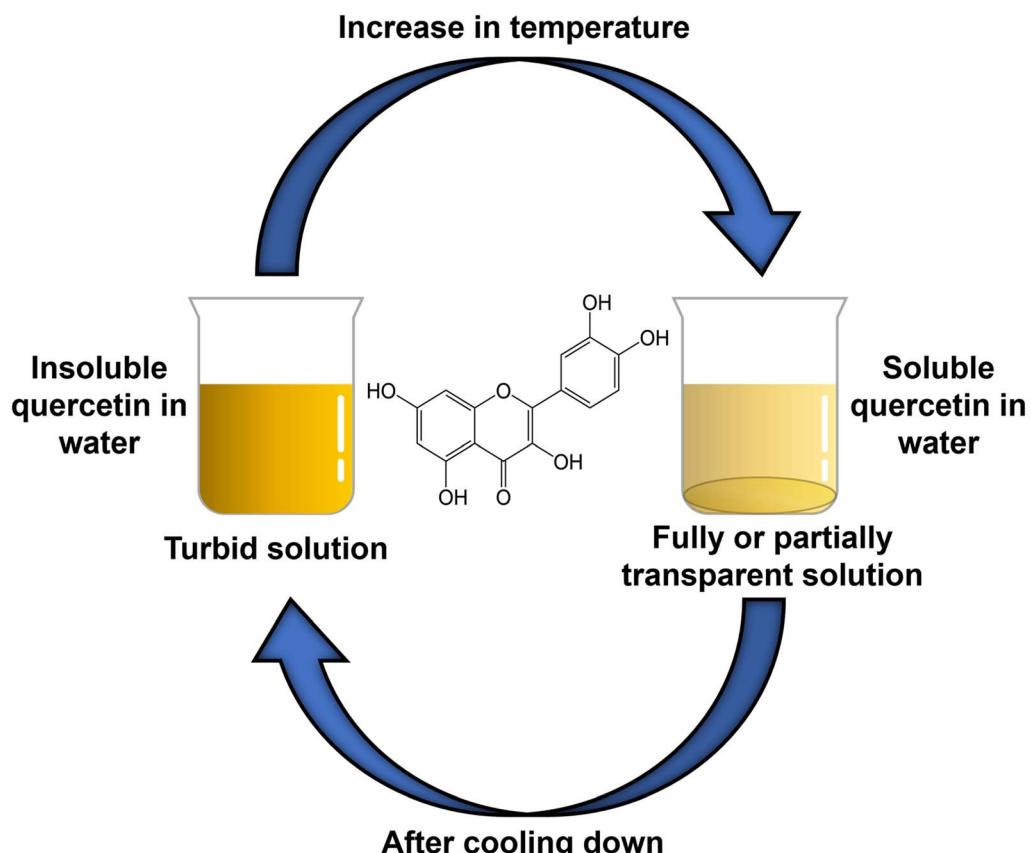
### Characterization

**UV-VIS spectroscopy.** Reports from the literature indicate that the peaks of quercetin are observed at 266 nm and 372 nm.<sup>63</sup> The benzoyl system is responsible for a peak at 260 nm, while the cinnamoyl system is responsible for a peak at 370 nm.<sup>64</sup> Fig. S2† shows that ring A of the quercetin structure contributes to the benzoyl system, and ring B of the quercetin structure contributes to the cinnamoyl system in spectrophotometric analysis.<sup>63</sup>

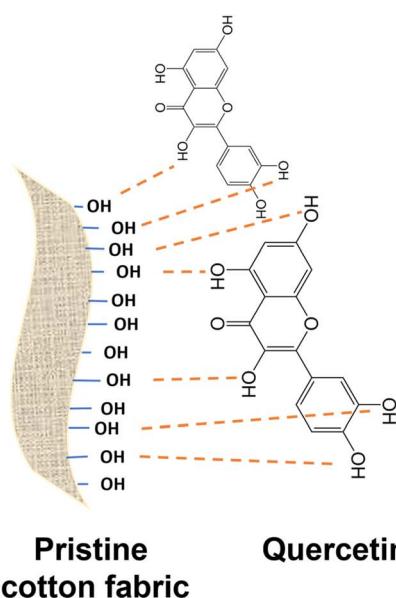
A freeze-dried quercetin powder in methanol solution was used for spectrophotometric analysis. The commercially



(a)



(b)



(c)

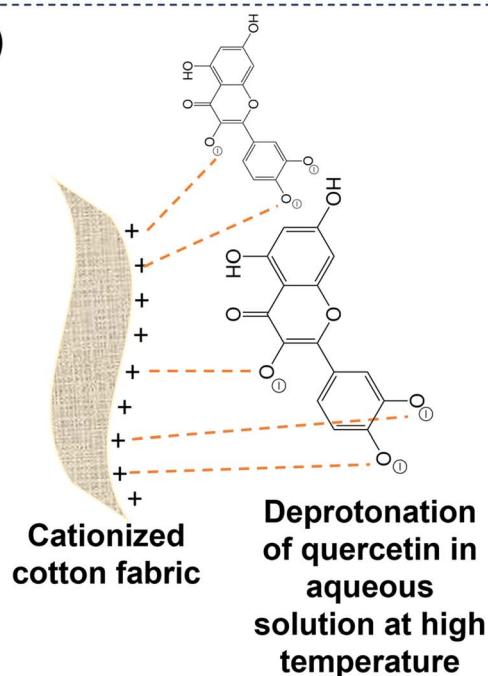


Fig. 2 (a) Schematic representation of the solubility behaviour of quercetin in an aqueous solution. Chemical interaction between the (b) cotton fabric and quercetin biomolecules (hydrogen bonding), and the (c) cationized cotton fabric and anionic quercetin biomolecules (ionic interaction).



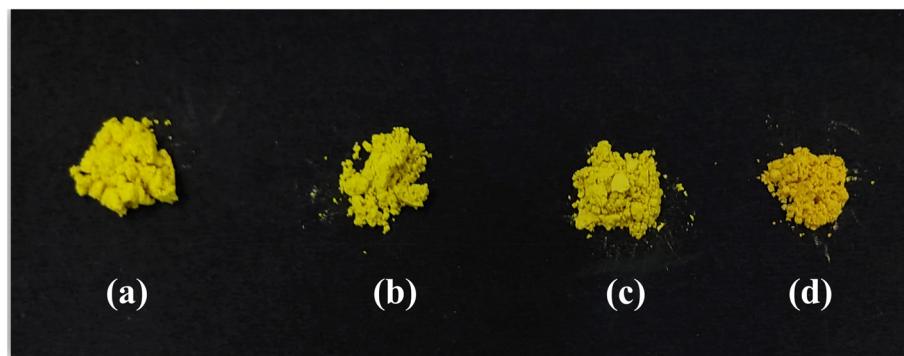


Fig. 3 Images of the (a) commercial standard quercetin and freeze-dried quercetin after treatment in an aqueous solution at (b) 80 °C, (c) 100 °C, and (d) 120 °C.

collected (untreated) quercetin and quercetin sample treated at 80 °C, 100 °C, and 120 °C showed peaks at around 254 nm and 372 nm. There was no change in the peak positions of the treated quercetin compared to the untreated quercetin. This spectrophotometric analysis is illustrated in Fig. 4a. Despite no change in the major peak positions, there was a slight colour change toward a reddish tone in the case of quercetin treated at 120 °C, followed by quercetin treated at 100 °C, compared to quercetin treated at 80 °C (Fig. 3). The dispersed quercetin in an aqueous solution, treated at different temperatures, shows some different peak positions, as observed in Fig. 4b. An extra new peak appeared when quercetin was treated in aqueous solution at higher temperatures compared to the dispersed pure quercetin in aqueous solution at room temperature. In the case of pure quercetin, the peak (due to benzoyl system) is observed at 254 nm, while the peak is visible in the 251–252 nm range for the treated quercetin. The extra peak appeared for the treated quercetin (80 °C, 100 °C, and 120 °C) at 290 nm. However, this peak is missing for the untreated quercetin. The peak intensity at 290 nm is sharper in the case of quercetin treated at 120 °C compared to quercetin treated at 80 °C and 100 °C (Fig. 4b). The peak intensity at 290 nm increases compared to the other two peaks with increasing treatment temperature. The peak position at around 370 nm is observed for the untreated and treated quercetin. The peaks at 370 nm are broader when treated at higher temperatures compared to the pure quercetin at room temperature. The rise of the extra peak in the case of the treated quercetin is an indication of the formation of auto-oxidized or fragmented products due to the high temperature in aqueous media. The higher peak intensity at 290 nm compared to the other peaks with the rise in treatment temperature also proves the increase in the formation of auto-oxidized products. There is a strong possibility of the formation of protocatechuic acid, as there is the presence of extra peaks at around 205 nm and 290 nm. The reported UV-VIS absorbance peaks of protocatechuic acid are 293.7 nm, 258.1 nm, and 218 nm.<sup>65,66</sup> The absorbance peaks show the presence of quercetin, as well as the fragmented part of quercetin (protocatechuic acid). The peaks at 205 nm and 290 nm become more prominent or sharper with the rise in the treatment temperature, which can lead to the increased formation of protocatechuic acid. The peak at 370 nm

becomes broader or less prominent with elevated treatment temperature. This can lead to the dissolution of the cinnamoyl system of quercetin (responsible for peak at 370 nm) with the increased treatment temperature. The spectrometric analysis of only the water-based supernatant of quercetin (treated at 80 °C, 100 °C, and 120 °C) was also carried out, as the colour of the supernatant was different after treating it at three different temperatures, as shown in Fig. 4c(A–C). No peaks are visible in the UV-VIS spectra in the case of the quercetin supernatant after treating it in aqueous solution at 80 °C, and there is a clear, transparent, and colourless supernatant. The peak positions at 252 nm (peak I) and around 288 nm (peak II) are common for the supernatant collected after treating quercetin at 100 °C and 120 °C. In contrast, the peak position for peak III is different for both. The supernatant treated at 100 °C and 120 °C shows peak III at 374 nm and 368 nm, respectively. These peak positions are shown in Fig. 4c. Quercetin is insoluble in water at room temperature, while there is the presence of some soluble and coloured material in the supernatant for quercetin treated at 100 °C and 120 °C. It is also confirmation of the presence of some fragmented or oxidized by-products in the supernatants. The appearance of extra peaks at around 288 nm and 205 nm in the supernatants also reaffirms the same.

**High-pressure liquid chromatography (HPLC).** The confirmation of any changes in the quercetin residues due to treatment temperatures further proceeded through the HPLC chromatogram. The untreated quercetin and treated quercetin residues were dissolved in methanol, and passed through the HPLC column under optimized conditions at 370 nm. The wavelength 370 nm was fixed from the two UV-VIS absorbance peaks of quercetin, *i.e.*, 254 nm and 370 nm. This is because there was no UV-VIS absorbance peak at 370 nm for protocatechuic acid. Fig. 5b–d shows the HPLC chromatograms of the quercetin residues collected after treatment at different temperatures. It can be concluded that the retention time for the major peak, which was around 3.9 min for all treated quercetin samples, is mainly due to the quercetin biomolecule, as it is similar to the peak position for standard commercial quercetin (untreated quercetin). It can be concluded from HPLC analysis and UV-VIS analysis that a negligible amount of auto-



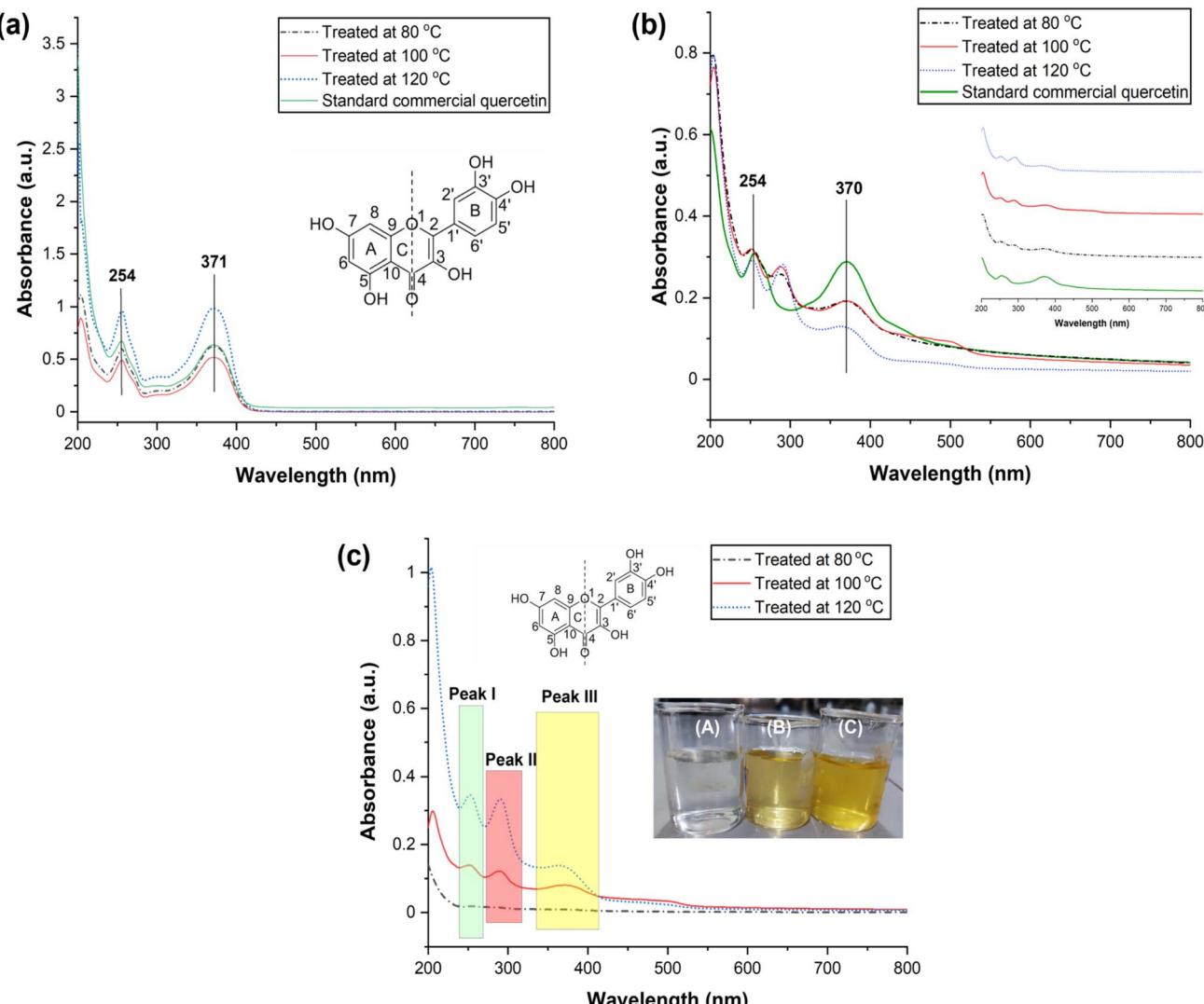


Fig. 4 (a) UV-VIS absorption spectra of the treated solid quercetin residue. (b) UV-VIS absorption spectra of dispersed quercetin. (c) UV-VIS absorption spectra of the quercetin supernatant after treatment at 80 °C, 100 °C, and 120 °C in an aqueous solution, and images of the quercetin supernatant solution after treatment of quercetin at (A) 80 °C, (B) 100 °C, and (C) 120 °C in an aqueous solution.

oxidative and fragmented products is present in the treated solid quercetin residues.

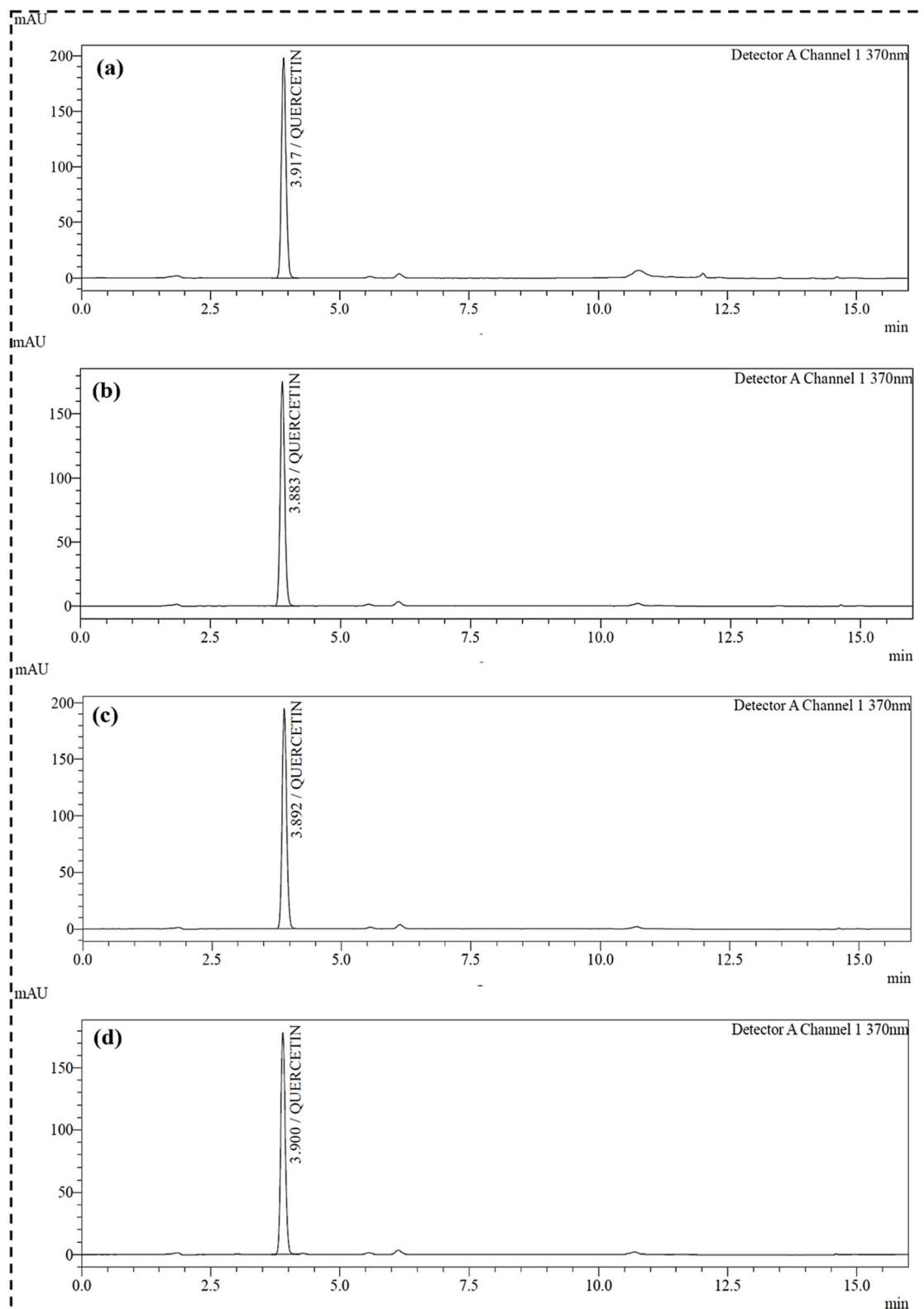
**Colour assessment of quercetin-treated cotton fabric.** It has been observed that the colour shade of the quercetin-treated cotton fabric changes with the rise in temperature. Additionally, the o-QC fabric and QC fabric treated at the same finishing temperature have shown different shades through visual appearance, as represented in Fig. 6d. The QC fabric shows a trend toward a redder tone than the o-QC fabric. This phenomenon is possible due to the higher tendency of the auto-oxidation of quercetin in the QC fabrics compared to the o-QC fabrics. This is because the hydroxyl groups are strongly engaged as ionic bonds with the cationic cotton fabric.

In addition to the colour changes in o-QC at different treatment temperatures, there are also variations in the shades of the fabrics after multiple laundering cycles. The visual appearance of fabrics (Fig. 6c) treated at lower temperatures shows

a yellowish shade, while it changes to a yellow-reddish shade with increased treatment temperature. Furthermore, the shade of the washed fabrics also changes with higher washing cycles, *i.e.*, it changes to a reddish shade from a yellowish shade with a greater number of washing cycles. This change occurs because the degradation of quercetin in water leads to changes in its colour from yellow to reddish tone at higher pH.<sup>55</sup>

Fig. 6a and b show the  $\Delta E$  and  $K/S$  values of treated and washed fabrics, respectively. The treated fabrics show a higher  $\Delta E$  value compared to the washed fabrics. The  $\Delta E$  decreases with an increase in the number of washing cycles. The decrement in colour difference value is higher with more washing cycles for fabrics treated at 80 °C (low temperature). The solubility of quercetin in aqueous media is less at low temperatures, which leads to superficial deposition of quercetin on the cotton surface. This superficial deposited quercetin gets washed out after several washing cycles, resulting in lesser  $\Delta E$  after higher





**Fig. 5** HPLC chromatogram of the (a) standard commercial quercetin and quercetin solid residues after treatment at (b) 80 °C, (c) 100 °C, and (d) 120 °C.

laundering cycles. Unlike the fabrics treated at 80 °C, the quercetin is more soluble at higher temperatures, which results in a greater penetration of quercetin molecules in the

amorphous zone of the cotton fiber and less superficial deposition on the fiber surface. This leads to less  $\Delta E$  with the greater number of washing cycles for fabrics treated at 100 °C and 120 °

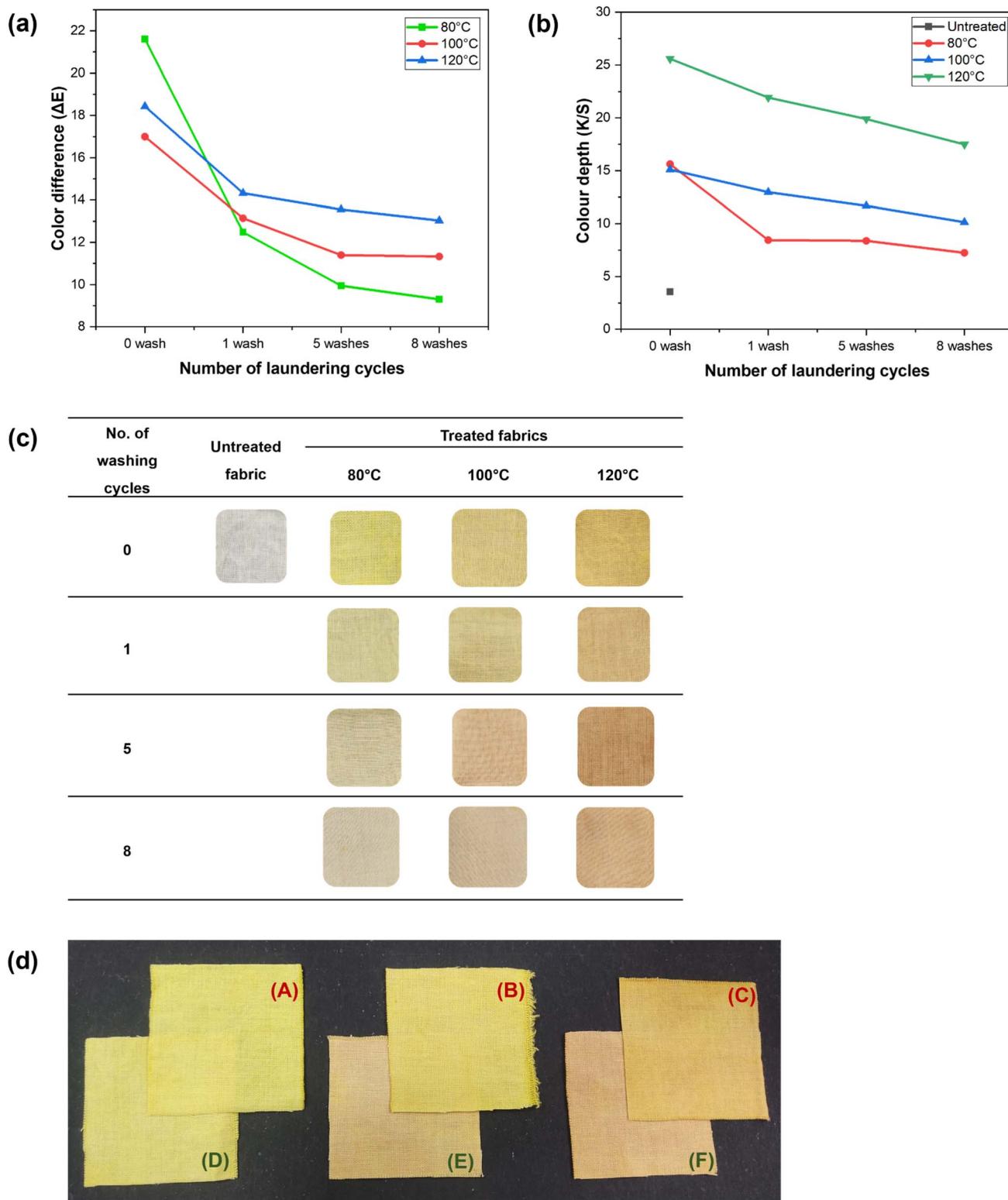


Fig. 6 Graphical representation of the (a) colour difference ( $\Delta E$ ) and (b) colour depth (K/S) of the treated and washed fabrics. (c) Shades of treated fabrics at different temperatures and different washing cycles. (d) Images of o-QC fabrics treated at (A) 80 °C, (B) 100 °C, and (C) 120 °C, and QC fabrics treated at (D) 80 °C, (E) 100 °C, and (F) 120 °C.

C compared to fabrics treated at 80 °C. After the washing cycles, the  $\Delta E$  value increases with elevated treatment temperature. The  $L^*$ ,  $a^*$ , and  $b^*$  values also show a similar trend. Despite the

different treatment temperatures, all unwashed fabrics are lighter (higher  $L^*$  value) than the washed fabrics. The  $L^*$  value decreases with increased washing cycles, while it gets saturated

after 5 laundering cycles. The  $a^*$  value represents the redder tone when it shows a '+' value. Conversely, it represents a greener tone when it shows a '-' value. The fabrics treated at 80 °C show an  $a^*$  value that is negative, *i.e.*, toward a greener tone. However, other fabrics treated at higher temperatures show an  $a^*$  value toward a reddish tone, which increases with a rise in the treatment temperature. All treated fabrics show higher  $a^*$  values with increased laundering cycles, as the interaction of quercetin with the alkali media causes rapid auto-oxidation. Although there is an increase in the  $a^*$  value with the higher laundering cycle, the  $a^*$  value decreases after 8 laundering cycles. This may be due to a loss of quercetin and the auto-oxidized counterpart of quercetin after 8 laundering cycles. The '+' value of  $b^*$  represents a yellower tone, while the '-' value of  $b^*$  represents a bluer tone. The untreated fabrics exhibit the highest  $b^*$  value, which decreases as the number of laundering cycles increases. This is also attributed to the development of a reddish tone with a greater number of laundering cycles. The detailed colour values are mentioned in Table S1.†

The colour depth ( $K/S$ ) value increases with higher finishing temperature. However, there is negligible change in the  $K/S$  value for the cotton fabric that is treated at 80 °C and 100 °C, with a noticeable increase when treated at 120 °C. This is due to the formation of fragmented products at higher temperatures. The  $K/S$  value decreases with the increment of laundering cycles, as there is a loss of quercetin from the cotton fabric after several machine washes. The drop in the  $K/S$  value from 0 washes to 1 wash is greater in the case of fabric treated at 80 °C compared to the other two cases. The solubility of quercetin in aqueous media is higher at the high treatment temperature, which leads to a greater absorption of quercetin to the amorphous region of cotton fiber. During washing, there is a greater loss of superficial deposited quercetin from the cotton surface for the fabric treated at lower finishing temperatures. The cotton fabric treated at 120 °C shows a better  $K/S$  value even after 8 laundering cycles compared to cotton fabric treated at 100 °C and 80 °C.

The variations in the colour value and shade are noted with the increase in the treatment temperature. There is no changes or only a minimal amount observed for the quercetin solid residue. However, major colour changes are observed in the case of the supernatant. The auto-oxidized and fragmented products generated in the supernatant (or quercetin solution) take place during the finishing treatment, along with quercetin residue when cotton fabrics are treated with quercetin in aqueous solution at higher temperatures.

### Assessment of functional properties of quercetin treated fabric

**Antioxidant assay.** The antioxidant properties are abundantly present in flavonoids, including quercetin. The radical scavenging property is predominantly decided by the hydroxyl configuration of the B-ring because of its ability to donate electrons to radicals, and also hydrogen donation. The conjugate effect of the double bond present in the C2 and C3 positions of the C-ring and the keto group present in the C-ring has

the ability of delocalization of uncoupled electrons. The contribution of the hydroxyl groups is present in the A-ring, and the C-ring is also effective for free radical scavenging activity.<sup>67–69</sup>

The DPPH antioxidant assay was carried out to assess the antioxidant properties of the treated cotton fabrics. The antioxidant properties of the QC fabrics and o-QC fabrics are shown in Fig. 7a and b, respectively. The antioxidant property is not significantly varied for the QC fabrics treated at 3 different temperatures. However, there is a significant decrease in the antioxidant properties after 1 laundering cycle. This is due to there being less affinity of quercetin to cotton fiber. Therefore, quercetin comes out from cellulose fibers during washing. After the 1st laundering cycle, the decrease in the antioxidant properties is more pronounced in the case of fabric treated at low temperature, *i.e.*, 80 °C, than the fabric treated at high temperature, *i.e.*, 120 °C. At higher temperatures, quercetin has better solubility, leading to greater penetration into cotton fabric. As a result, there is less quercetin that is washed off from the fibers. The color change in the treatment solution may be due to the formation of fragmented or oxidized quercetin products, which have a stronger affinity for the cotton fibers and are incorporated into the fabric. The antioxidant properties of the o-QC fabric were further explored, and it can be observed that the fabrics treated at 80 °C, 100 °C, and 120 °C show similar trends and excellent antioxidant properties. There is a decrease in the antioxidant efficacy with the higher number of laundering cycles. However, the decrement in the antioxidant properties with increased laundering cycles decreases with higher treatment temperatures. The o-QC fabric treated at 120 °C shows effective antioxidant properties even after 8 laundering cycles. The affinity of quercetin is better with cationized cotton compared to non-cationized cotton due to ionic bonds. As a result, antioxidant properties are sustained in o-QC fabrics even after certain laundering cycles, unlike the QC fabrics. The better solubility of quercetin in aqueous solution and the formation of small, fragmented products show the better penetration of quercetin into the amorphous region of the cotton fiber. This results in a greater amount of antioxidant properties of the o-QC fabric treated at 120 °C than the o-QC fabric treated at 80 °C.

**Evaluation of the ultraviolet protection factor.** Quercetin has efficacious properties to resist ultraviolet rays, where it can efficiently block both UV-A and UV-B. The conjugate structure of quercetin, *i.e.*, the benzoyl and cinnamoyl system of the quercetin structure, is responsible for the UV-resistance property (illustrated in Fig. S2†). The UPF values for both QC and o-QC fabrics are shown in Fig. 7c and d, respectively. The rise in the UPF mean value with higher treatment temperatures for both QC and o-QC fabrics can be attributed to the improved solubility of quercetin in water at elevated temperatures, which allows for better penetration of quercetin into the amorphous region of the cotton fibers. The higher colour depth with increasing treatment temperature also confirms the improved penetration of quercetin into the cotton fiber. The UV-resistance property is also attributed to quercetin, along with its auto-oxidized and fragmented products, as the chemical structures of both quercetin



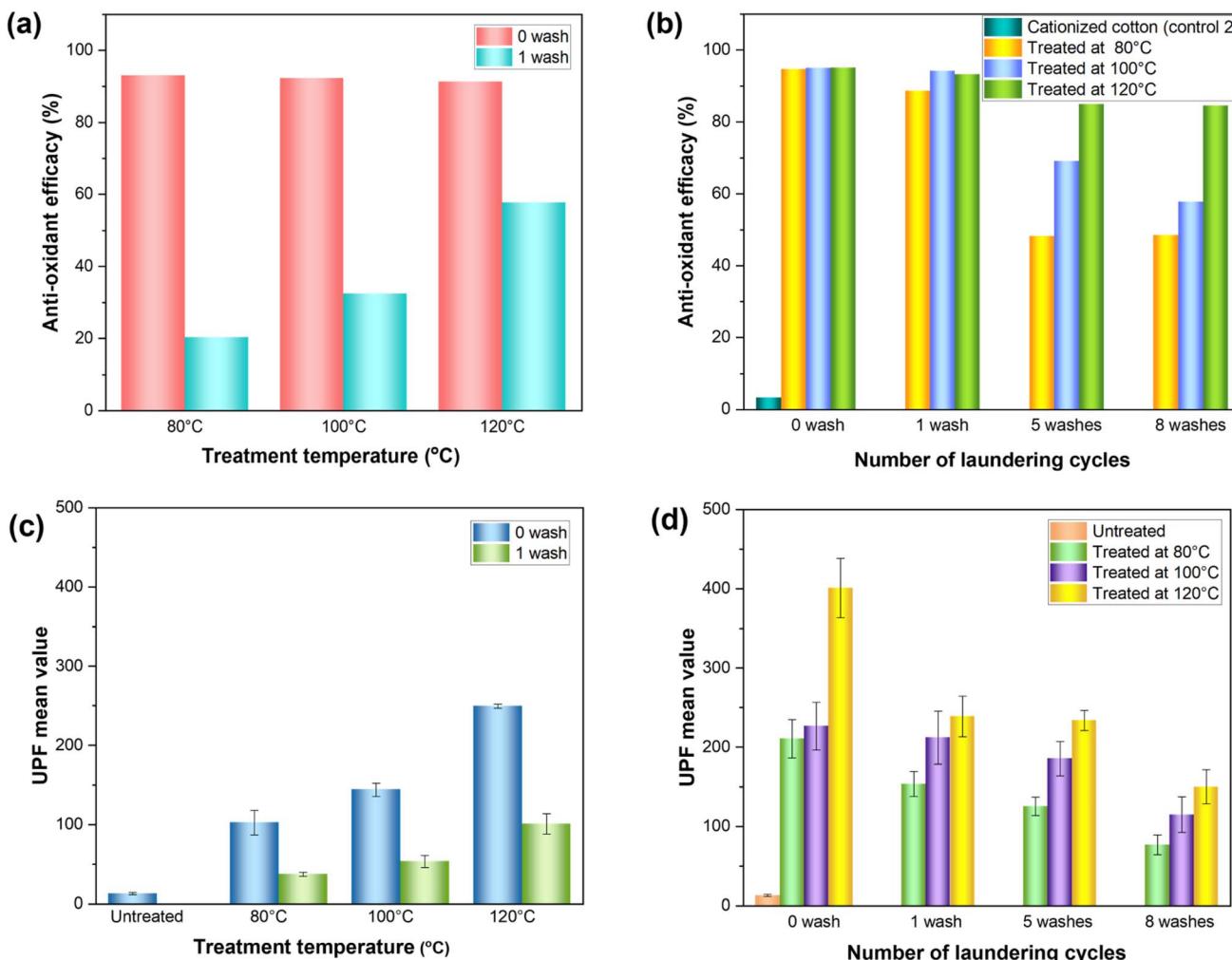


Fig. 7 Antioxidant efficacy of (a) QC fabrics and (b) o-QC fabrics. UPF mean value of (c) QC fabrics and (d) o-QC fabrics after several laundering cycles.

and its breakdown products are responsible for this functional characteristic. Furthermore, a huge decrement in UPF mean value after the 1st washing cycle can be observed in Fig. 7c. The rating of QC fabrics treated at 80 °C and 100 °C also decreases to 30 after the 1st washing cycle, whereas a 50+ rating is maintained for fabric treated with quercetin at 120 °C even after one wash. However, the rating is 50+ even after 8 laundering cycles for o-QC fabrics. Thus, the better affinity of quercetin with the cationized cotton compared to quercetin with non-cationized cotton is again demonstrated here. The UPF rating, along with the UV blocking % of the QC fabrics and o-QC fabrics, are mentioned in Tables S2 and S3,† respectively. The o-QC fabrics treated at 120 °C show the highest UPF mean value even after 8 laundering cycles. It also blocks around 99% of UV-A and UV-B rays. However, there is a slight decrease in the UV-A and UV-B blocking % with the increase of laundering cycles.

**Antibacterial properties.** Quercetin is adequately used in several sectors due to its significant antibacterial properties.<sup>21,70</sup> This functional property is observed in quercetin due to the presence of functional groups, *i.e.*, hydroxyl groups.<sup>22</sup> The

antibacterial properties of the QC and o-QC fabrics were examined by colony-counting method, as illustrated in Table 1, and Fig. S3 and S4.† The QC fabrics show no antibacterial properties against *E. coli* and *S. aureus* despite the different treatment temperatures. However, the o-QC fabrics show better antibacterial efficacy compared to QC fabrics. This is due to the synergistic effect of quercetin and cationizing agent, *i.e.*, Optifix. For confirmation, the bacterial reduction efficacy of Optifix-treated cotton fabric (control 2) is ~83.2% and ~88.2% against *E. coli* and *S. aureus*, respectively. The cationic ions present in Optifix (cationizing agent) are the reason for the better antibacterial properties for the o-QC fabrics. The antibacterial activity declines with the increase in treatment temperature. The oxidation and fragmentation of quercetin in aqueous media take place at higher temperatures, which leads to the formation of fragmentated products and decrease in the presence of the hydroxyl groups. However, the hydroxyl groups in quercetin are responsible for the antibacterial properties. This is the reason why o-QC fabrics exhibit the best antibacterial properties when treated at lower temperatures. The cotton

Table 1 Antibacterial efficacy of cotton fabric treated with quercetin (with and without pretreatment)

Treatment conditions	Treatment temperature	Bacterial reduction (%)	
		<i>E. coli</i> (Gram-negative)	<i>S. aureus</i> (Gram-positive)
Without Optifix	80 °C	~54	~42.5
	100 °C	~54	~42
	120 °C	~52	~36.8
With Optifix	Pristine cotton fabric treated with Optifix (control 2)	~83.2	~88.2
	80 °C	~90.2	~92.3
	100 °C	~71.2	~72.9
	120 °C	~69.5	~66.2

Table 2 Bending length test results of the control and treated cotton fabric

Type of sample	Bending length in cm (standard deviation) [warp way]	Bending length in cm (standard deviation) [weft way]
Control cotton	4.7 (0.11)	3.9 (0.08)
Cotton fabric treated at 80 °C	4.7 (0.07)	4.0 (0.05)
Cotton fabric treated at 100 °C	4.7 (0.10)	3.9 (0.09)
Cotton fabric treated at 120 °C	4.7 (0.14)	3.9 (0.05)

fabric (treated at 100 °C and 120 °C) shows less antibacterial properties. After the laundering cycles, it decreases further (Fig. S6†) and provides unsatisfactory results. However, the fabrics treated at 80 °C show excellent anti-bacterial properties. Due to its superficial deposition on the fibre surface, it gets easily washed off after only 1 wash, which results in a significant decrease in the antibacterial properties.

#### Evaluation of flexibility and mechanical strength of treated fabrics

The flexibility and mechanical properties of treated fabrics are considered as the finishing treatment conditions, and the add-on% can hamper the inherit physical properties of the cotton fabric. In this study, the flexibility is evaluated by measurement of the bending length of treated fabrics. Table 2 shows that there is no significant difference between the untreated and treated cotton fabrics. The add-on% (Table S4†) of quercetin on the treated fabrics is very minimal to hamper the flexibility of the treated fabrics, which can also be observed from the bending length measurement. The mechanical strength, which is evaluated by the tensile strength, shows no impactful changes (both deterioration and improvement) among the control and treated cotton fabrics. This is due to no adjustment of lower pH and significantly higher temperature requirement during the finishing treatment. The tensile strength of the treated fabrics is given in Fig. S5.†

## Conclusions

A multi-functional fabric was successfully developed by eco-friendly quercetin biomolecules on cotton substrates in an IR

dyeing machine. The quercetin-treated cotton fabric shows antioxidant, ultraviolet resistance, and antibacterial properties with eye-catching shades. The changes in the shades, *i.e.*, yellowish to reddish tone, are observed with the increased treatment temperature and higher washing cycles. The shade change is due to the fragmentation and auto-oxidation of quercetin in aqueous solution at different treatment temperatures. The fragmented product formed during the finishing treatment was confirmed by UV-VIS spectrophotometric analysis. It also affects the functional properties of the quercetin-treated cotton fabrics. There is a possibility of the formation of protocatechuic acid during fragmentation at higher treatment temperatures. The o-QC fabrics show excellent antioxidant efficacy of ~95% and UV-resistance efficacy of 50+ rating. The antibacterial properties against *E. coli* and *S. aureus* are ≥90% for the o-QC fabric treated at 80 °C. However, there is a significant decrease in the antibacterial properties when the sample is treated at higher temperatures. The excellent antioxidant properties of ~85% and UV-protective properties of ~150 (UPF mean value) with a 50+ rating is still sustained even after 8 laundering cycles when treated at 120 °C. This study reports on the excellent multi-functional properties of quercetin-treated fabrics, and demonstrates the variation of the functional properties due to the fragmentation with changes in the treatment factors. These findings can boost the market for quercetin-treated fabrics, as it will be an economical-cum-eco-friendly solution against conventional fabrics developed using natural finishing agents. In our next study, further enhancement of the functional properties will be carried out by deeply investigating the identification and quantification of the fragmented products during finishing treatments and correlating their properties with the attained functional properties



on the treated fabric. Additionally, the multifunctional properties of quercetin will be further explored on one of the other types of most sought-after fabric substrates, *i.e.*, polyester.

## Data availability

The data supporting this article have been included as a part of the ESI.†

## Conflicts of interest

There are no conflicts to declare.

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