

Laccases for enzymatic colouration of unbleached cotton

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Abstract

The concept presented in this paper is the utilisation of the natural flavonoids present in cotton as anchors to attach other phenolic compounds to the fiber surface. Laccase can catalyze the oxidation of flavonoids in solution producing quinones that can be further polymerised and grafted onto surface of the cotton providing yellow to brown colouration, depending on the external flavonoids used and on the reaction conditions. Factors such as temperature, time of reaction, pretreatment of cotton, mechanical agitation and the role of an organic solvent were studied in order to improve this laccase colouration reaction. After dyeing, colour measurements and fastness tests (washing, friction and weathering fastness) were performed. A strong mechanical agitation, an increased reaction temperature (from 30 to 50 °C), and the addition of an organic solvent improved dyeing.

The natural flavonoids present on cotton were found to play an important role on the grafting reaction, improving dyeing and colour fastness. Since the traditional bleaching pretreatment of cotton removes these natural flavonoids from cotton, the proposed laccase colouration reaction could be carried out without a previous bleaching treatment resulting in a more environmentally friendly process.

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1. Introduction

The flavonoids consist of a large group of low-molecular weight polyphenolic substances, naturally occurring in fruits and vegetables, and are an integral part of the human diet [1,2]. They are usually present almost exclusively in the form of β -glucosides and they can be divided on the basis of their molecular structure into four main groups, flavones, flavonols, flavonones and isoflavones [3,4].

The flavonoids have been the object of extensive studies their biological, pharmacological and chemical properties [5].

Cotton's natural colour is due to flavonoid compounds, with the types and quantities of these flavonoids depending on the growing environments [6]. When cotton is used in textiles the flavonoids are eliminated in the bleaching process, usually with hydrogen peroxide. This bleaching agent can damage fibers via radical reactions. There are many areas of researches focusing on new cotton bleaching methods, based on laccase action, that can prevent such damage [7].

The flavonoid polyphenols can be oxidized by some enzymes in a very important biochemistry reaction, since the subsequent coupling reactions are involved in some biosynthetic pathways such as melanin and tannin formation [8,9].

Enzymatic processes have been developed for wet processing of textile goods in a wide range of operations from cleaning preparations to finishing processes. The applications of enzymes in textile production provides alternative options of processing conditions such as milder temperature, pressure and pH condition, and water savings [10].

The synthetic process of polymeric materials by oxidoreductive enzymes, for instance, laccases, peroxidases and tyrosinases is widely reported with environmental friendly concept as "green polymer chemistry" [11,12]. Recently, the potential use of laccases for polymerizing, crosslinking and functionalizing various compounds was studied. Thus, increasing interest has been focused on the application of this enzyme as a new biocatalyst in organic synthesis [13].

Laccases (EC 1.10.3.2) are multicopper polyphenol oxidases capable of oxidizing phenols by hydrogen atom abstraction resulting in the formation of the corresponding phenoxy radicals [14,15]. These reactive species can perform non-enzymatic reactions in further steps. By this way laccase can oxidize flavonoids forming *o*-quinones that can perform further polymerization, as

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has previously been reported [16]. This oxidation reaction was already used to improve the whiteness of cotton by an enzymatic decolouration process of its natural flavonoids [7,17].

In this work, the oxidation and polymerization of some flavonoids by laccase from *Trametes hirsuta* was applied as a concept of environmental friendly dyeing of cellulosic fibers by grafting these flavonoid polymers onto the cotton surface. Furthermore some reaction factors were studied and the colour fastness was measured via washing, rubbing and weathering.

2. Material and methods

2.1. Enzyme, chemicals and fabrics

Laccases from *Trametes hirsuta* (100 U/mL, 693 mg/mL) were used at pH 5 (0.1 M Na–acetate buffer) to perform the enzymatic experiments. The flavonoids (Fig. 1) were purchased with high purity from Aldrich and used as supplied.

The bio desized woven cotton fabric having 80/65 warp/weft cm^{-1} was supplied from project ‘ViaBio’. The enzymatically desized 100% cotton was scoured with 1% NaOH and 0.2% Lutensol AT 25 solution for 1 h at boiling temperature. [18]. Then it was bleached with 5% hydrogen peroxide, 1% commercial sodium hydroxide, 3% sodium silicate (w/w of fabric) and 0.1% Lutensol AT 25 solution for 100 min at boiling temperature. The other chemicals were purchased from Aldrich at degree of purity higher than 95%.

2.2. Colouration on the cellulosic fibers

The colouration was done on differently pretreated cotton (scoured cotton, scoured and bleached cotton) using procedure detailed in 2.1.

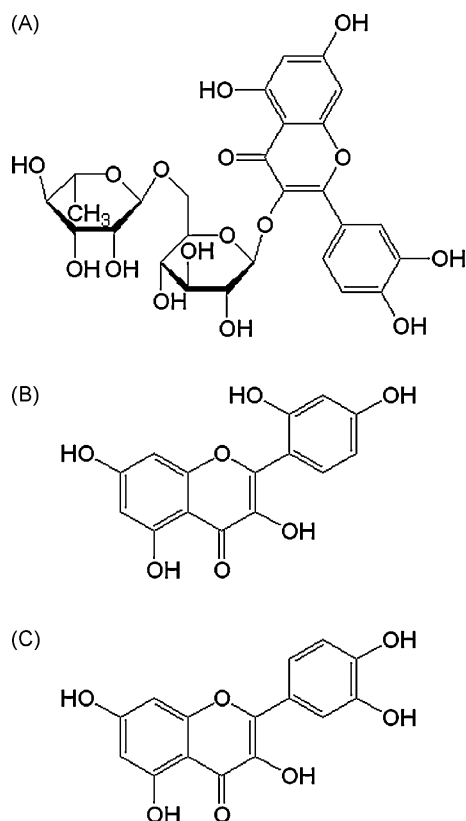


Fig. 1. Chemical structure of flavonoids used ((A) rutin, (B) morin, (C) quercetin).

Essentially, 100 mL reaction solutions containing 30 μL of laccase, flavonoid suspensions (10 mM final concentration) and buffer (0.1 M Na–acetate, pH 5) were introduced in reaction mixture pots with 5 g of fabric samples at 50 °C. No laccase addition was performed on control pots.

The washing and rinsing processes following colouration were carried out with cold tap water for several times until no more colour was washed from the samples or with boiling tap water for 40 min with stirring. The fibers were thoroughly washed with 0.1% Lutensol AT 25 solution to remove unlinked flavonoid compounds from the cotton surface.

To provide different power of mechanical agitation, two kinds of machines were used: Rota Wash MK II, purchased from SDL International Ltd., to provide a strong level of agitation (40 rpm, vertical agitation) and a normal shaker bath was chosen for providing mild agitation (80 rpm, orbital agitation). To see the effects of reaction time on colour strength, scoured cotton fabric samples were taken after 1, 2, 3, 5 and 24 h.

All treatments were processed with 20:1 of liquor to fabric ratio.

2.3. *K/S* measurements

2.3.1. Colour strength determination

The colour strength was evaluated using *K/S* values obtained spectrophotometrically by a Datacolor apparatus at standard illuminant D65. *K/S* is represented by the equation of Kubelka–Munk, where *K* is the sorption coefficient, and *S* is the scattering coefficient. The data presented are the mean value of five measurements, obtained automatically from the machine.

2.3.2. Colour fastness estimation

2.3.2.1. Washing fastness. Treated cotton pieces (4 cm × 10 cm) and bleached 100% cotton fabric were used to estimate colour migration. The standard test method for colour fastness to domestic and commercial laundering (ISO 105-C06) was followed [19].

2.3.2.2. Friction fastness. Wet and dry friction fastness was measured following the standard test method colour fastness of Zipper tapes to crocking: ASTM D2054-99 [20].

2.3.2.3. Weathering fastness. For weathering fastness tests, Accelerated Weathering Tester (QUV) Spray LU-0819 from Q-PANEL, equipped with UVA 340 lamp, at 70 °C and 77 W/m² of irradiation was used. This QUV test chamber simulates weather conditions. Exposure cycles of 8 or 24 h were applied on samples until remarkable colour degradation was observed. Each flat plaque was holding two samples of standard sizes (75 mm × 150 mm). The change of colour was measured as colour difference.

2.3.2.4. Evaluation of fastness test. Tests of colour fastness were conducted using spectrophotometric analysis and estimated by *K/S* values.

3. Results and discussion

3.1. Colouration of cotton

The oxidation of different flavonoids (rutin, morin and quercetin) was accomplished by laccase treatment in 0.01 M aqueous flavonoids suspension. The flavonoids reacted with laccase showing a dark brown colouration. This is due to the presence of oxidative polymerization reactions [21], since laccases are capable to catalyze the *o*-quinones formation from phenolic compounds. The quinones are highly reactive compounds and can polymerize spontaneously to form high molecular weight compounds or brown pigments (melanins) or may suffer nucleophilic attack by water [22–24].

This enzymatic oxidative polymerization of flavonols has been studied by several researchers using HPLC analysis and

UV–vis spectroscopic studies [9,22,25,26]. In this research, the polymerization of morin was confirmed by HPLC. The analysis was performed with a size exclusion chromatography test of a 1 mM morin solution treated with laccase for 1 h (50 °C, pH 5). A Superdex 200 column was used with phosphate buffer (0.05 M, pH 6.5, 0.1 M KCl) as mobile phase (flow rate 0.5 mL/min). This revealed a peak of 8000 kDa with an UV detector (280 nm) demonstrating the formation of polymer compounds.

This polymerization reaction was applied to develop a new system to coat and dye cotton fibers. This reaction assisted by laccase produces flavonoid polymers that provide new colour properties to cellulosic fibers. The colouration experiments on cellulosic fibers by laccase were performed at different conditions such the type of flavonoid, strength of mechanical agitation, temperature, reaction time, pretreatment of cotton fabrics and temperature of washing and rinsing of dyed fabrics. Then the effect on the colouration of cotton fibers was analyzed in order to analyze the dyeing process.

As a general rule, the cellulosic fibers were coloured with all flavonoids tested, although there were no remarkable changes on colour difference of samples treated with rutin. This is an expected effect as rutin has a much lower rate of oxidation [23] than the other flavonoids used (morin and quercetin), even when a higher concentration of enzyme is used. Besides a low reaction rate, poly(rutin) is a water soluble compound, which can stay in solution instead of coupling to cellulosic fibers surface. In contrast, quercetin showed the highest values of K/S observed (Fig. 2). The chemical structure of flavonoids may play an important role in this behavior, since the glycosylation of rutin probably have a negative effect in laccase reaction rate compared with other flavonoids. The substitution pattern can determine the reaction pathway of flavonoids [22] so the slight structural difference between morin and quercetin may explain the different results obtained with them.

The K/S values of flavonoids colourized cellulosic fibers tended to increase when reaction time and strength of mechanical agitation also increase. During the first hour of treatment the enhancement of K/S value was from 3 to 7-fold in respect to the control experiment depending on the flavonoids used, whereas during the rest of treatment the increase of K/S was less (Fig. 2). This was probably due to the thermal inactivation of laccase. Testing of the laccase solution stability at pH 5 and 50 °C showed a continuous laccase activity, with a residual laccase activity of less than 5% after 24 h of incubation.

The strength of mechanical agitation was the most important factor on colouration results, since a strong mechanical agitation (vertical agitation with Rotawash machine) permitted us to improve colour absorption on cotton fabrics due to a stronger beating effect than mild agitation (performed by an orbital shaker).

The stronger beating effect caused by vertical agitation probably increase the superficial area of fabric that is accessible to flavonoid polymers to attach on cotton.

In our experiments laccase performed two main roles in flavonoids solution: first, this enzyme catalyzes the formation of mainly semiquinones and reactive electrophilic *o*-quinones and then the oxidative polymerization of these intermediate com-

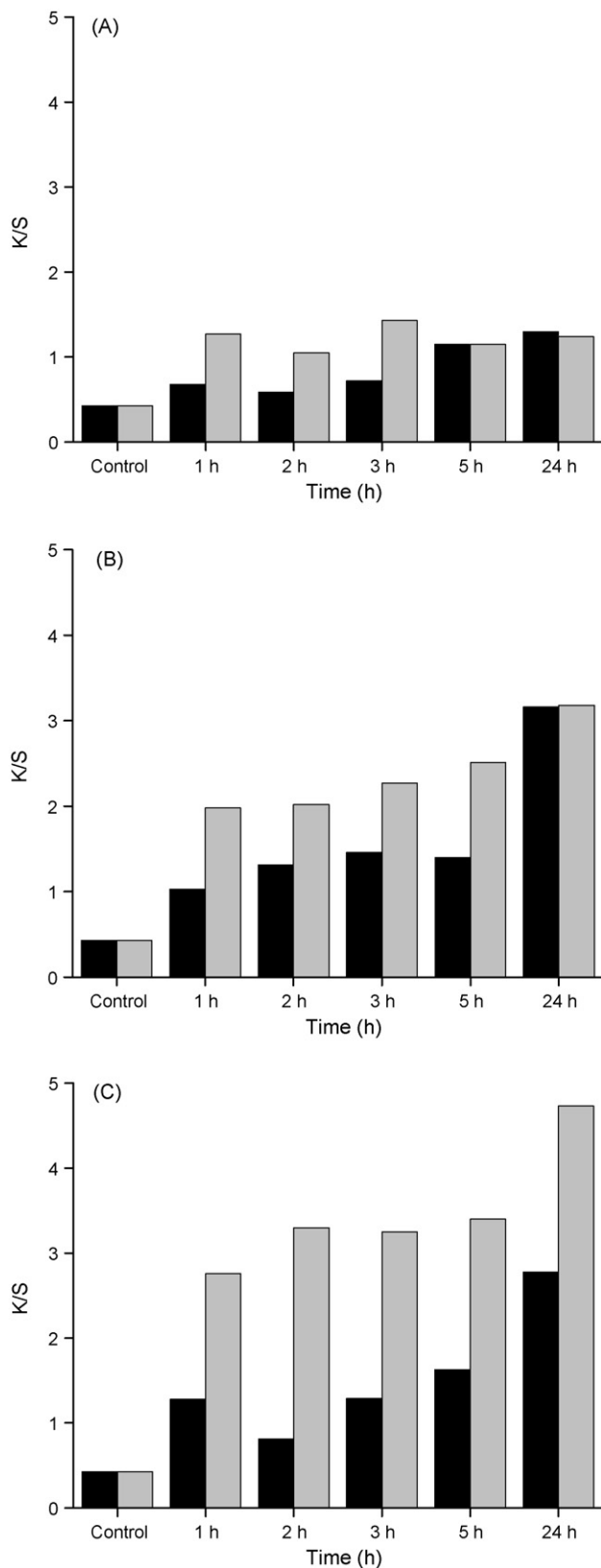


Fig. 2. Colour strength of laccase-assisted dyed cotton in presence of 10 mM flavonoid suspensions: rutin (A), morin (B) and quercetin (C) under orbital (■) and vertical (□) agitation.

pounds previously formed [23]. As it was reported, the reaction of laccase with rutin showed the formation of an *o*-quinone intermediate, which may be further polymerized by laccase [16].

The low water solubility of flavonoids has been regarded as a long standing problem in their application [18]. An organic co-solvent was required to solvate rutin, morin and quercetin [1]. Therefore, methanol (10%, v/v) was added to the reaction mixture to improve the solution of flavonoids, although it was not enough to solve them completely. These new suspensions of flavonoids were tested to colourize cotton in comparison with non-methanol added suspensions.

Fig. 3 shows the colour difference values obtained depending on reaction conditions such as composition of reaction solution and mechanical agitation (vertical, orbital). Furthermore, the washing and rinsing were done with cold or boiling water to investigate colour fading. As logically expected, washing in boiling water resulted in a lower colour difference values in consequence of the loose of some non-fixed flavonoid polymers gone with warm but not with cold washing. This effect was easy to detect by a simple visual inspection. The use of methanol allowed the use of increased concentration of flavonoids, with a higher flavonoid availability to laccase, consequently the colour difference should be higher if methanol was added in reaction solution, however, this effect was only clearly observed when quercetin was used. In the experiments using morin only the washing temperature had an important effect, whilst rutin showed different colouration properties depending on the mechanical agitation: under mild agitation no effect of methanol and washing temperature was easily observed whereas under strong agitation a positive effect of methanol addition and boiling washing was detected. Nevertheless rutin provided a poor colouration grade, much lower than morin or quercetin.

Consequently the optimum colouration conditions change depending on the flavonoids and only the washing temperature seems to produce a predictable effect on colour retention. As we confirmed by washing and rinsing experiments, the colour permanent of the fabric with cold water washing was regarded as transient.

In this research, we also tried to find the role of the natural flavonoids present on cotton surface that are responsible for its original colour [6]. These flavonoids can affect the reaction with flavonoid polymers formed by laccase. Therefore we performed new experiments at 30 and 50 °C, with scoured cotton (with natural flavonoids) and with scoured and bleached cotton (without natural flavonoids). Colour difference (ΔE^*) values were obtained by comparison of scoured or scoured/bleached cotton before and after laccase assisted dyeing to estimate the colour

Table 1
Results of washing fastness tests of flavonoids colourized scoured and bleached cotton at 30 °C (A) and 50 °C (B)

	Scoured cotton ^A	Bleached cotton ^A	Scoured cotton ^B	Bleached cotton ^B
Rutin	2	2	4	4
Morin	2	2	4–5	4
Quercetin	1–2	1–2	4	3

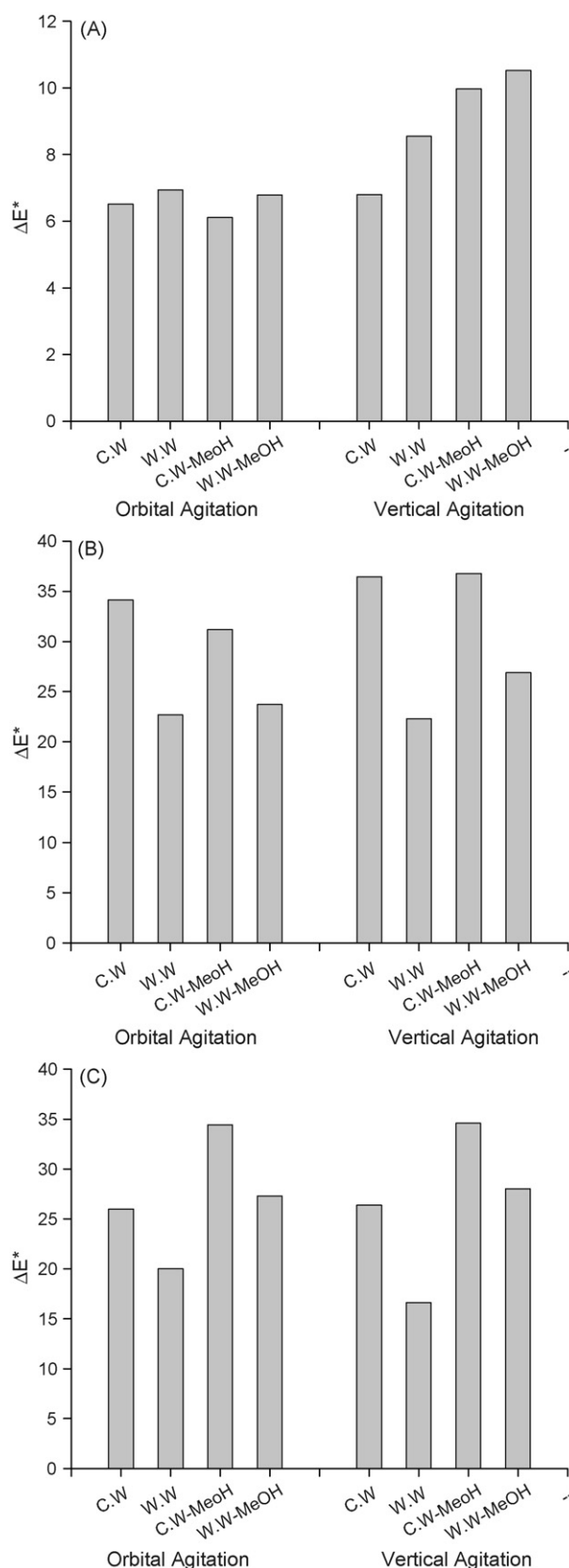


Fig. 3. Colour difference of flavonoids ((A) rutin, (B) morin and (C) quercetin) dyed cotton under several agitation conditions and methanol addition (MeOH), combined with different washing temperature after dyeing treatment (C.W.: cold water, W.W.: warm water).

Table 2

Results of wet and dry friction fastness tests of flavonoids colourized scoured and bleached cotton at 30 °C (A) and 50 °C (B)

	Scoured cotton ^A		Bleached cotton ^A		Scoured cotton ^B		Bleached cotton ^B	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Rutin	5	5	5	5	5	5	5	4–5
Morin	5	4–5	5	4–5	5	5	5	4–5
Quercetin	5	5	5	4–5	5	5	5	4–5

visibility (Fig. 4). A higher increase of colouration was detected if the cotton is only scoured, showing that natural flavonoids of cotton have a positive effect on dyeing. These flavonoids may function like grafting points where the intermediates (quinones) formed during laccase oxidation reaction [22,23] could bind.

In any case the difference is slight, probably because the concentration of natural flavonoids is not enough to produce a high increase of ΔE^* . The most interesting consequence of better colouration results on scoured rather than on bleached cotton is that bleaching step is not necessary.

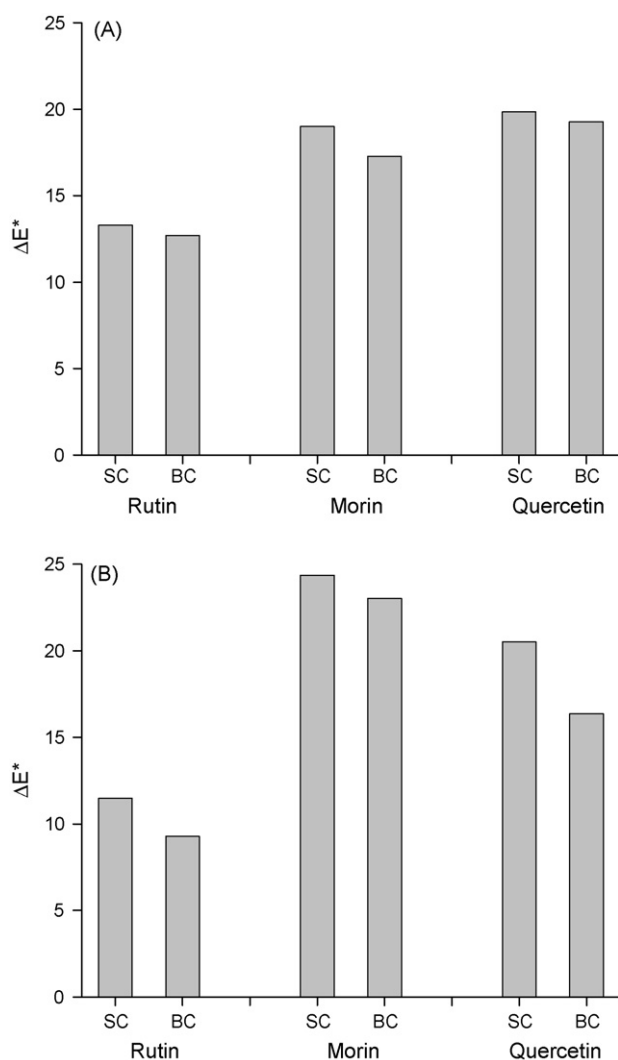


Fig. 4. Colour difference of “only scoured” (SC) and “scoured and bleached” (BC) cotton dyed with flavonoids at 30 °C (A) and 50 °C (B).

The reaction temperature used is a very important factor on enzyme catalyzed processes and was also a main factor on flavonoids colouration of cotton and greatly affected the final colour difference. The higher temperature (50 °C) used enhanced the colouration of cotton, especially cotton treated with morin. This is an expected result since the laccases used in this experiment have a documented optimum activity at 50 °C.

3.2. Colour fastness measurements

The colour resistances of washing, friction and light are essentially requested conditions in textile dyeing process.

3.2.1. Washing fastness

The colourized cotton samples were tested to see the colour resistance after washing processing. The *K/S* values of colourized samples were measured before and after washing test and the degree of colour change was obtained (Table 1). The results show different washing fastness dependant on reaction temperature. The oxidised flavonoid compounds seem to couple strongly on cotton at higher temperature, consequently the colour stability increase against a washing treatment.

In morin and quercetin treated samples, different washing fastness values were observed on scoured or scoured and bleached cotton. The scoured samples showed better washing fastness, again illustrating the positive effect of natural flavonoids present on cotton and the possibility to avoid the bleaching pretreatment on cotton.

3.2.2. Friction fastness

The friction fastness tests were done as wet and dry tests. Most of the samples showed high values of wet and dry friction fastness (Table 2). The results showed that flavonoid colourized fibers have strong colour resistance against friction stress.

Table 3
Weathering fastness test results

	Scoured cotton ^A (%)	Bleached cotton ^A (%)	Scoured cotton ^B (%)	Bleached cotton ^B (%)
Rutin	62	56	53	69
Morin	48	62	66	61
Quercetin	46	64	60	66

Colour degradation after 64 h of test on enzymatic dyed cotton at 30 °C (A) and 50 °C (B).

3.2.3. Weathering fastness

Colour degradation of flavonoids coupled with cellulosic fibers was tested using QUV-Spray tester at 70 °C. The results of weathering fastness were not satisfactory since more than 50% of colour was degraded in almost all the samples after 64 h of test (Table 3). The pretreatment of cotton, the type of flavonoid or the reaction temperature are factors that did not have a predictable effect on light resistance of colour.

4. Conclusions

This study shows the possible application of flavonoids (rutin, morin and quercetin) to dye cotton by an enzymatic process catalyzed by laccase. The cellulosic fibers coloured with oxidised flavonoids by laccase had different colour strength depending on the flavonoid used, temperature of reaction, mechanical agitation, pretreatment of cotton and after treatment conditions, etc. The colouration is probably due to the polymerization and linkage of the quinones formed by laccase.

The washing and friction fastness test of flavonoids coloured cotton showed good results confirming the feasibility of this new and promising colouration technique. However more knowledge is needed about the colouration process to improve the weathering fastness.

Cotton has natural flavonoids that help to obtain a better performance of the colouration reaction assisted by laccase. If the bleaching pretreatment of cotton is avoided, better colouration and even superior resistance of colour is obtained permitting us to conclude that the usual bleaching pretreatment of cotton is not necessary at all, consequently important chemical, water and energy savings could be obtained by using this colouration process catalyzed by laccase.

In future work, it is intended to improve the reaction conditions to make this process a suitable one for industrial application.

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