Development of Fungal Dyes and Application in Leather Dyeing

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Abstract

Certain species of filamentous fungi typically produce colored substances as secondary metabolites, which can be used as dyes for industrial applications to various products such as leather, textiles and food. These natural dyes can be an eco-friendly alternative to synthetic dyes (mainly azo dyes). They are not originated from extractive activities of the natural environment, and no hazardous chemicals are used while they are produced. Moreover, there are not any studies attesting to their toxicity to human health. This study produced and applied fungal dyes extracted from Penicillium spp., Monascus spp. and Fusarium spp. to leather dyeing. These fungi were used in bioprocesses to produce natural dyes by cultivation in liquid medium, extraction and concentration. Finally, they were applied in leather dyeing. The efficiency of the leather dye step was quantified through the remaining dye concentration in the dyeing wastewater by UV-VIS spectroscopy, and the required quality parameters of the dyed leather samples were evaluated by the change in color when they were exposed to light and heat. Each fungus was inoculated at an initial concentration of 10^6 spores/mL in potato dextrose broth under incubation for 21 days at 120 rpm and 30 °C. All these fungi showed promising dye production. The dyed leather samples showed good penetration and color homogeneity.

Keywords: leather dyeing; fungal colorants; *Penicillium spp*., *Fusarium spp*., *Monascus spp*.

1. Introduction

Dyes are applied to many different products in order to attribute desirable coloring, and they are really crucial for market acceptance of these products although many synthetic dyes in extensive use all over the world have negative impact on human health and the environment. However, the development of natural dyes for industry application must satisfy quality and productivity requirements, such as high thermal stability, fastness to UV light and low cost. Natural dyes are produced by living organisms such as plants and animals whereas most well-known natural dyes are extracted from sources with pharmaceutical and nutritional potential. Bordignon *et al.* (2011) tested leather dyeing with cochineal carmine and urucum

natural dyes that showed good surface coating of the leather, good penetration and equalization in the dyeing, and good bath exhaustion.

An alternative route to producing natural colorants is to apply biotechnology tools. Mapari *et al*. (2005) emphasize the need to explore the potentiality of other biological sources such as fungi, bacteria and cell cultures, since the proper selection, mutation or genetic engineering may significantly improve the production and yield of dye from wild organisms. Fungi, particularly ascomycetous and basidiomycetous (most mushrooms) fungi, and lichens (symbiotic association of a fungus with a photosynthetic partner, usually a green alga or cyanobacterium), are known to naturally synthesize and secrete diverse classes of pigments as secondary metabolites of known or unknown function which have an extraordinary range of colors (Maldonado, 2005). Basidiomycetes fungi and microalgae are known to produce a wide range of water-soluble pigments, but low productivity has been limiting their marketability (Bessette, 2001; Hejazi, 2004).

Dyes extracted from filamentous fungi are promising alternatives to natural dyes because they are not dependent on seasonal effects for production and can grow rapidly, which can lead to high yield. Fuck *et al.* (2012) introduced the development steps to produce biodyes from filamentous fungi and use them in leather dyeing, and shared the knowledge and technologies applied in this field.

Studies on the environmental factors that affect the growth and metabolism of filamentous fungi are necessary because they contribute to controlling the cellular metabolism and optimization of certain biosynthetic products. De Carvalho (2011) highlighted the fact that a wide range of microbiological dyes is produced, such as riboflavin, monascus dyes, carotene (astaxanthin, β-carotene, etc.) and phycobiliproteins (such as phycocyanin). Microalgae such as *Chlorella, Dunaliella, Haematococcus, Scenedesmus, Spirulina sp.* are also used for producing dyes and pigments. Previous studies indicate that *Monascus purpureus, Emericella spp.* and *Penicillium spp.* do not produce toxic effects (Youssef El-Maghraby, & Ibrahimm, 2008) and are more compatible with the environment (De Carvalho, 2011).

Monascus can produce six different types of biodyes classified into three groups: orange dyes - rubropunctatin ($C_{21}H_{22}O_5$) and monascorubramin ($C_{23}H_{26}O_5$); red pigment - rubropunctamine ($C_{21}H_{23}NO_4$) and monascorubramine ($C_{23}H_{27}NO_4$); yellow pigment - monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$), as shown in Figure 1. They are widely used in food production as coloring agents of rice, wine, soybean cheese, fish and red meat (Dufossé *et al*., 2005; Hajjaj *et al.*, 2000).

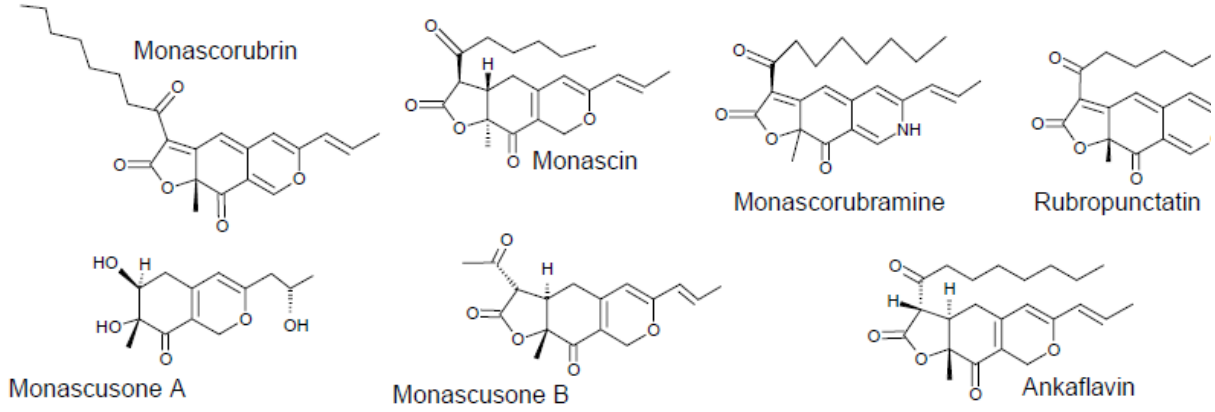

Figure 1: *Monascus* dyes. Source: Mapari, 2005.

In the studies by Velmurugan *et al.* (2010a), the leather dyeing potential was optimized in pre-tanned samples with five dyes extracted and purified from *Monascus purpureus*, *Isaria spp.*, *Emericella spp.*, *Fusarium spp.* and *Penicillium spp.* The results showed that the optimum concentration of the biodyes was 6 % on leather weight. However, the optimum condition for dyeing was 70 °C, at pH 5.0, and 120 min. The maximum uptake of dyes in the leather samples ranged from 40 to 70 %. The changes in shades of the samples were high in *M. purpureus* – red dye and they were compared with visual assessment data. The fungal pigments did not significantly alter the organoleptic properties of the leather sample.

The aims of this study were to investigate the procedures of cultivation, extraction and concentration of fungal biodyes, as well as to test their application to leather dyeing.

2. Materials and methods

2.1 Filamentous fungi

The fungi used for screening purposes belong to the mycology collection of the Laboratory of Biochemistry and Applied Microbiology (UFRGS). The fungal identifications were performed by Lopes (2011), based on genomic DNA extraction, polymerase chain reaction (PCR) using universal primers ITS1 and ITS 4 and part of the β-tubulin gene Bt2a and Bt2b, sequencing, editing and sequencing analysis. The fungi were maintained in Potato Dextrose Agar (PDA) tubes covered with mineral oil at 4 ºC and subcultured periodically.

2.2 Production of biodyes

The first part of this study consisted in producing biodyes. Each fungus was inoculated separately at a concentration of 10^6 spores/mL of potato dextrose broth (Acumedia) and incubated in a rotary shaker at 120 rpm at 30 °C for 2 to 5 weeks. After this period, the incubated solution was centrifuged for 25 min at 6000 rpm and the supernatant was transferred to a suitable vessel. Finally, the dye solution was concentrated in a vacuum rotary evaporator at a temperature of 50° to 70 °C. Optical density (OD) was measured at 494 nm (a wavelength which represents the absorption maxima for the color red) in a VARIAN - CARY 1E spectrophotometer.

2.3 Leather dyeing

The second part of this study consisted in applying the selected biodyes to leather dyeing. Wet-blue leather samples with a thickness of 2.0 – 2.2 mm were used in the tests. The samples were deacidified using sodium hydrogen carbonate, sodium formate and a surfactant. The dyeing step was performed using two additions of 2 % of dye (according to leather weight) at 25 ºC to promote dye penetration into the leather during 3 hours. Then, the temperature was raised to 50 ºC for the fixing step with formic or citric acid. Surfactants, the condensation product of aromatic sulphonic acids and ethoxylated fatty amine sulphate were used as dyeing auxiliaries. To verify the influence of the type of acid on fixation and use of tanning ayxiliaries, pH was adjusted to 5.00. The studied conditions are shown in Table 1.

Table 1: Studied conditions in leather dyeing.

Studied steps	**1**	**2**	**3**	**4**	**5**
Deacidification pH	4.0	5.0	6.0	5.0	5.0
Acid used on fixation	Formic acid	Formic acid	Formic acid	Citric acid	Formic acid
Dyeing auxiliares	Used	Used	Used	Used	Not used

2.4 Qualitative and quantitative analysis

The response variables in this study are described below:

1. The amount of biodye absorbed into the leather was assessed by a VARIAN - CARY 1E UV-VIS spectrophotometer based on the diference between the amount of dye used and the amount that remained in the dyeing bath;
2. Color intensity, equalization and biodye penetration were estimated with an arbitrary visual scale;
3. Color fastness to UV light, thermal stability and fastness to migration into plasticized PVC were measured at TFL Brazil.

Thermal stability was tested by accelerated ageing of dyed leather after 7 days of ageing in an air-circulating oven at 50 °C according to NBR 12830.

Color fastness to UV light was in accordance with NBR 14730:2001 and intended for determining the resistance of the color of leather to the action of a standard artificial UV light source during 24 hours.

Color fastness in respect of migration into plasticised poly(vinyl chloride) - PVC was in accordance with EN ISO 15701 and was based on the transference of color from leather to white plasticised PVC at 50 °C. The side of the leather sample to be tested was placed on a

white pigmented sheet of plasticised PVC and the composite specimen was exposed to heat under pressure in an appropriate apparatus for 16 hours at 50 °C.

The analyses of color fastness were compared to the grey scale. The Grey Scale was used for assessing changes in the color of leather in color fastness tests and consisted of nine pairs of grey color chips, each representing a visual difference and contrast. The fastness rating went step-wise from: Grade 5 = no visual change (best rating) to Grade 1 = a large visual change (worst rating).

3. Results

First of all, the fungi *Aspergillus spp., Penicillium spp, Fusarium spp, Monascus purpureus* were pre-selected as dye producers, as shown in Table 2.

Table 2: The studied fungal dyes and preliminary tests of production.

Fungus	Color	Color intensity*	Amount of dye production**
Aspergillus spp.	Dark brown	High	Good
Fusarium spp.	Purple	Mild	Medial
Penicillium spp. 1	Pale yellow	Mild	Medial
Penicillium spp. 2	Bright yellow	High	Medial
Penicillium spp. 3	Pale red	Mild	Medial
Monascus purpureus	Red	High	Good

* Assessment made with an arbitrary visual scale; ** according to the optical density test.

In the second step of this study, the *Monascus purpureus* biodye was chosen for leather dyeing development because the conditions of fungal cultivation are simple, the required extraction processes are accessible and high yield of dye was produced with intense color. In addition, this biodye has been widely used in foods for centuries without restrictions.

All the dyed leather samples with *Monascus purpureus* biodyes had similar surface coating and penetration into the leather, as shown in Figure 2. Adding the second dye did not improve either the absorption or the superficial color of the leather.

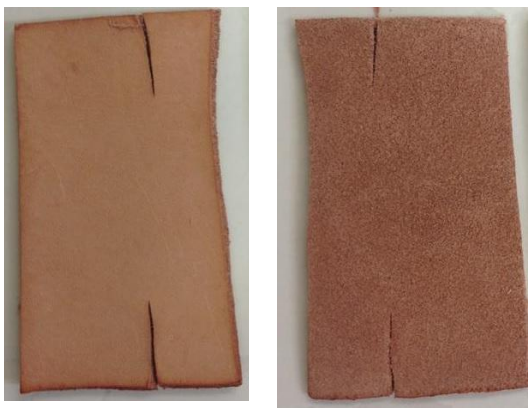

Figure 2: Leather sample dyed with biodye from *Monascus purpureus*.

The influence of pH on the absorption of *Monascus* biodyes into the leather is shown in Figure 3. Biodye absortion by leather was higher than 70 % when the deacidification pH of 4.0 to 5.0 was used, but it decreased at pH 6.0.

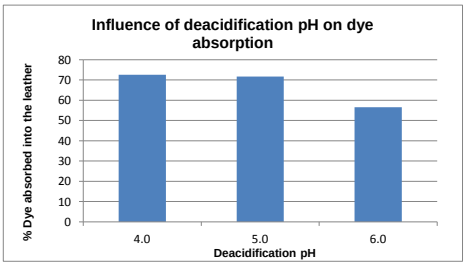

Figure 3: Influence of pH deacidification on the absorption of *Monascus* biodyes (%) into the leather.

Figure 4 shows the influence of dyeing auxiliaries on leather dyeing. The use of dyeing auxiliaries increased biodye absorption by the leather from 67.5 to 71.6 %, and also improved equalization of the surface color.

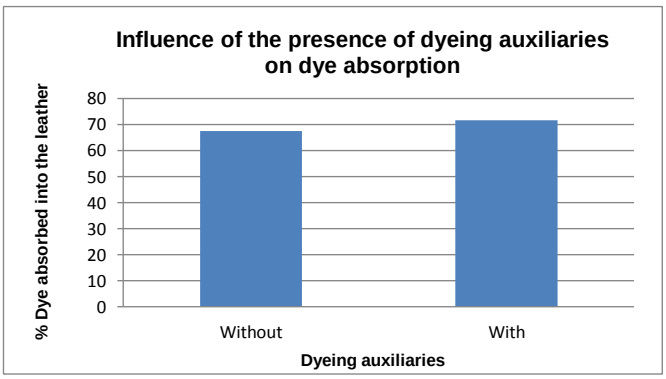

Figure 4: Influence of dyeing auxiliaries on leather dyeing.

The influence of fixation acid on the absorption of *Monascus* biodyes into the leather is shown in Figure 5. Biodye absorption into leather when formic acid used in the deacidification step was higher (71.6 %) than when citric acid was used (64.7 %).

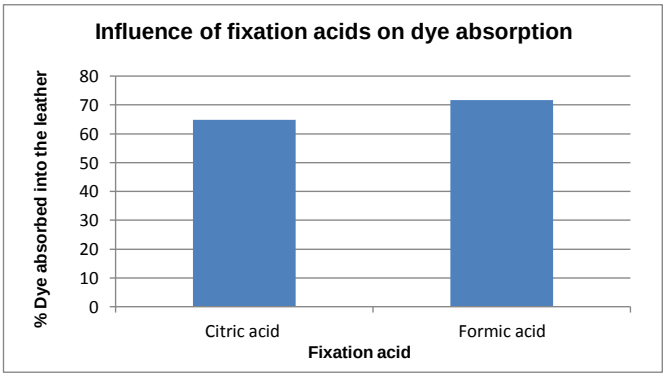

Figure 5: Influence of fixation acids on the absorption of *Monascus* biodyes (%) into the leather.

The results of fastness to UV light, thermal stability and fastness to migration into plasticized PVC are shown in Table 3 below:

Table 3: Results of fastness to UV light, thermal stability and fastness to migration into plasticized PVC

	Fastness to UV light*	**Thermal stability***	**Fastness to migration into plasticized PVC***
Formic acid	**1**	**4**	**3/4**
Citric acid	**1**	**4/5**	**4**

*The analyses of color fastness were compared to the grey scale.

The samples deacidificated with formic acid maintained thermal stability grade 3/4 and fastness to migration into plasticized PVC grade 4, while for those deacidificated with citric acid, these values were increased to grades 4 and 4/5, respectively, according to grey scale values. Fastness to UV light was grade 1 for dyed leather according to the grey scale for assessing change of color.

4. Conclusions

The development of biodyes must associate both the production and the feasibility of application in the leather dyeing step, according to the required quality parameters for different articles. This is not a trivial technology because it involves multidisciplinary knowledge of microbiology / taxonomy, bioengineering and chemical processes of leather.

There is a wide diversity of filamentous fungi to be explored in dye production, as well as techniques to be applied in order to improve their properties.

The best studied leather dyeing conditions with *Monascus* dye are deacidification pH 4.0, use of dyeing auxiliaries in the dyeing step and use of formic acid in the fixation step. In these conditions, the absorption of the *Monascus* dye through the leather is more than 70 %.

The use of citric acid in the fixing step increased thermal stability and fastness to migration into plasticized PVC of the dyed leather.

The fastness to UV light property was not appropriate to leather dyeing; however, *Monascus* can be applied in other products, e.g., food.

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