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Spray Drying of Coloring Extracts Produced by Fungi Isolated from Brazilian Caves

Patrícia Nirlane da Costa Souza[1]
https://orcid.org/0000-0002-0724-4636

Dérica Gonçalves Tavares[2]
https://orcid.org/0000-0002-3727-0173

Cláudia Regina Fernandes Souza[3]
https://orcid.org/0000-0002-4152-8640

Marcelo Luis Lombardi Martinez[3]
https://orcid.org/0000-0001-7699-5356

Wanderley Pereira Oliveira[3]
http://orcid.org/0000-0003-4356-2508

Luís Henrique Souza Guimarães[4]
http://orcid.org/0000-0002-2921-3929

Patrícia Gomes Cardoso[2]
https://orcid.org/0000-0002-0797-2502

[1]Federal University of Jequitinhonha and Mucuri Valleys – Institute of Engineering, Science and Technology, Janaúba, Minas Gerais, Brazil; [2]Federal University of Lavras, Department of Biology, Lavras, Minas Gerais, Brazil; [3]University of São Paulo, Faculty of Pharmaceutical Sciences of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil; [4]University of São Paulo, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, Department of Biology, Ribeirão Preto, São Paulo, Brazil.

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*Correspondence: patricia@ufla.br; Tel.: +55-35-38291883 (PGC).

HIGHLIGHTS

- Fungal pigments were spray-dried in presence of maltodextrin, modified starch or gum arabic.
- Yellow fine powders with low moisture content and water activity were obtained.
- The dried products have enhanced stability and potential to might be used as a natural colorant.

Abstract: Pigments produced by submerged fermentation of three filamentous fungi isolated from Brazilian caves, namely *Aspergillus keveii*, *Penicillium flavigenum*, and *Epicoccum nigrum*, were submitted to spray drying in presence of the adjuvants maltodextrin, modified starch or gum arabic. Yellow fine powders with low moisture content and water activity, and high color retention (> 70%) were successfully generated with a high product recovery ratio (> 50%), independently of the adjuvant used. The dried products have enhanced stability and potential to might be used as a natural colorant in food and pharmaceutical applications.

Keywords: adjuvants; Filamentous fungi; microencapsulation; submerged fermentation.

INTRODUCTION

Currently, there is a growing interest in replacing synthetic colorants with natural alternatives. The chemically synthesized pigments have been verified to be poisonous and those substances had been placed under strict control for practical use [1]. The use of microorganisms for the production of natural colorants is a viable option, since their pigments have a predictable and controllable yield, and can be produced continuously. Among the microorganisms, filamentous fungi have been considered promising sources of dyes, because their pigments exhibit a wide range of color and are more soluble in water [2]. Pigments synthesized by fungi are already being used as natural colorants in the food sector. Applications in textile industries, pharmacology, medicine, and cosmetics have also been reported [3]. Filamentous fungi isolated from Brazilian caves have shown biotechnological potential for producing natural colorants. Among them, some species with the potential for pigment production have been identified. Some of the pigments, which give color to fungal extracts, also exhibit antioxidant activity [4-7].

However, one of the disadvantages of using natural colorants is their low stability. The drying of the natural colorants has been proposed by several authors as a way to increase their stability during storage and to facilitate their transport, processing, and increasing market value [8-11]. Spray drying, a method that transform liquid compositions into dry particles through atomization of the fluid in a current of air at elevated temperature, is one of the most commonly used technologies for drying of natural colorants [12].

Microencapsulation using spray drying is an economical method for the preservation of natural colorants by entrapping them within a wall material or adjuvant that protects the core material against factors that may cause its deterioration, by enhancing its stability [8]. Carbohydrates of high molecular weight such as starch and modified starches, maltodextrins, solid corn syrups, gum Arabic, cyclodextrins, gums and proteins are typical examples of adjuvants commonly used for drying and microencapsulation by spray drying. The adjuvant would protect the core material from various factors that may cause its deterioration, e.g. oxygen, light, and moisture, thereby enhancing the stability [13]. The correct selection of the adjuvants affects drying performance and physicochemical product properties; and has been the focus of many studies linked to microencapsulation of dyes.

The aim of this study was to investigate the spray drying of natural pigments produced by filamentous fungi isolated from Brazilian caves in presence of the adjuvants maltodextrin, modified starch, and gum arabic, by evaluating the properties of the produced powders, such as moisture content, water activity, color, and process yield (product recovery). These adjuvants are widely used as food additives generally recognized as safe (GRAS) for direct addition to humans. They are cheap, stable, easily available, and commonly used in spray drying and microencapsulation of fruits juices, pigments, herbal extracts and so on, generating good-quality powders [10,11,13,14].

MATERIAL AND METHODS

Three yellow pigment-producing fungi isolated from Brazilian caves and belonging to the Mycological Collection of Lavras (CML) of the Department of Phytopathology at the Federal University of Lavras, Brazil, were used. The fungal isolates were previously identified by Souza and coauthors [6] *Aspergillus keveii* (CML2968) belongs to the *Usti* section, presents gray-colored colonies with regular and white borders, with beige to light yellow reverse in PDA (Potato Dextrose Agar) medium and abundant conidiation. *Penicillium flavigenum* (CML2965) belongs to the *Chrysogena* section, presents colonies of green collation with regular borders of white coloration. The reverse of the colony shows yellow pigmentation in PDA medium and presents abundant conidiation typical of fungi of this genus. *Epicoccum nigrum* (CML2971) presents typical morphology of the species, characterized by vigorous aerial mycelial growth, colonies with irregular margins, showing yellow to orange coloration and reverse with intense orange pigmentation in PDA medium. It presents conidiophores and conidia variable in size, with ovoid shape, ellipsoidal to oblong, with a brownish to brown color.

The fungal strains were inoculated on Potato Dextrose Agar (PDA) medium [potatoes (200 g/L), glucose (20 g/L), and agar (20 g/L)] and were incubated at 25 °C for 7 days. After this period, 10 mycelial plugs with 9 mm in diameter from the culture were inoculated in 2000 mL Erlenmeyer flasks containing 1000 mL of Potato Dextrose (PD) medium [potatoes (200 g/L) and glucose (20 g/L)]. The incubation was carried out at 30 °C on a rotary shaker at 150 rpm for 7 days. The incubation was carried out in dark, aiming to simulate the light conditions of original caves environment. Later, the cells were harvested under vacuum filtration using Whatman paper Nº 1 and the cell-free filtrates were subjected to spray drying.

The adjuvants maltodextrin (MOR-REX®1910 Ingredion, Brazil), modified starch (Capsul® Ingredion, Brazil) or gum arabic (Fibregum B® Nexira, Brazil) were added to the cell-free filtrate (colorant extract) before spray drying. Each adjuvant was added individually to the cell-free filtrate at a ratio of 5% (w/v) and maintained under agitation for 2 h. The encapsulating compositions were analyzed by a UV-Vis HP 8453 spectrophotometer operated using HP Chem-Station software, in order to quantify the color of each formulation before drying. Each drying formulation was scanned at 350-600 nm to find the wavelength of maximum absorption for each pigment, used to estimate the pigment retention after spray drying.

The microencapsulation process was carried out in a mini spray dryer, model SD-05 (Lab-Plant, UK) consisting of drying chamber of 500 mm in height and 215 mm in diameter. A two-fluid-type atomizing nozzle with internal mixing and a nozzle diameter of 1 mm was used to feed the encapsulating pigment composition.

The drying parameters were: inlet temperature of the drying air (Tge) of 100 °C, atomizing air pressure (Patm) of 2 kgf/cm^2, drying air flow rate (Wg) of 60 m^3/h, atomizing air flow (Watm) of 15 L/min, and feed rate of encapsulating composition (Wsusp) of 4 g/min. The spray dried products were evaluated by determination of the moisture content, water activity (aw), and pigment retention, while the process yield (product recovery) was used to assess the spray drying performance. Triplicates spray drying runs were done for one fixed experimental condition in order to evaluate the reproducibility of the drying process, showing deviation of nearly 5%. Hence, the other drying runs were performed without replicates, and was assumed a maximum deviation of 5%.

Moisture content (%) was determined by the Karl Fischer titration method in a Metrohm 870 Titrino plus equipment. Approximately 100 mg of sample were used for each determination. Measurements were performed in triplicate, and the results were expressed as means and standard deviation. Water activity (a_w) of the powders was measured in triplicate in an Aqua Lab 4 VTE (Decagon, Devices) equipment at 25 °C, using the dew point sensor. The product recovery of the dried powder (process yield) was calculated by equation 1, obtained by mass balance in the dryer.

$$\text{Recovery } (\%) = \frac{\text{Amount of recovered powder after drying}}{\text{Amount of dry matter fed into the system}} \text{ x100} \quad (1)$$

The powders were analyzed by spectrophotometry to determine the color variation caused by the drying process. The color analyses of the pigments were performed at maximum absorption wavelength of each pigment [400 nm for *P. flavigenum* (CML2965) and *A. keveii* (CML2968), 430 nm for *E. nigrum* (CML2971)]. The spray dried products were redispersed in distilled water at the same concentration of the feed composition (5% w/v). Figure 1 show typical UV-spectra from the original feed composition and of the dried product, when maltodextrin was used as drying adjuvant. Color changes were represented in terms of pigment retention percentage, which was defined as the ratio between the absorbance of the reconstituted spray dried pigment composition by the one before drying, the as follows:

$$\text{Pigment Retention } (\%) = \frac{\text{Absorbance after drying}}{\text{Absorbance before drying}} \text{x100} \quad (2)$$

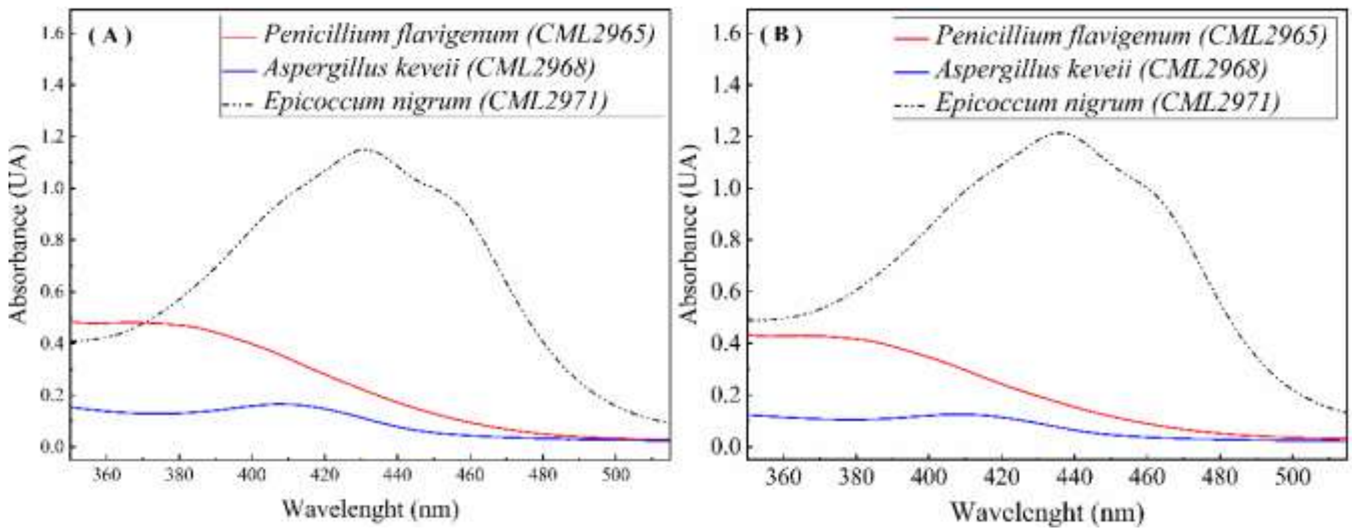

Figure 1. Typical UV-spectra from the original feed composition (A) and of the dried product (B), when maltodextrin was used as drying adjuvant.

RESULTS AND DISCUSSION

Yellow color powdered products (Figure 2) with moisture content lower than 9.4% (Table 1) were successfully produced by the spray drying. Lower moisture content is desirable, since it diminishes the microbial growth [12], increasing product stability.

Seemingly, the moisture content of the dried powders showed dependence on the filamentous fungi pigments. The spray dried pigments of the *P. flavigenum* showed lower moisture contents (~6.0 %), while the highest were presented by the *A. keveii* (from 7.6% to 9.4 %). The addition of maltodextrin or modified starch generated products with similar moisture contents; while the gum arabic tended to increase the product moisture (up to 9.4%), except for the *P. flavigenum* powder. For dried powders stored in non-airtight conditions, acceptable moisture contents are generally between 6% and 7%, within which physical changes are rarely observed [15].

The three adjuvants investigated in this study generated powders with water activity (a_w) in the range of 0.269–0.317 (Table 1). For water activities bellow 0.5 the amount of free water available for biochemical reactions and microbial growth is highly reduced [16]; affecting positively the product shelf life [9, 11]. Therefore, it can be inferred that the spray dried powders attain moisture content and water activities which could enhance their stability for a longer shelf life, with exception of the product loaded with *A. keveii* pigment using gum arabic as adjuvant, which presents moisture content of 9.4%.

The influence of adjuvants on the percentage of pigment retention in the dried powder and powder production during spray drying (product recovery) was also investigated (Table 1). The use of gum arabic as drying adjuvant fully protected the *A. keveii* (CML2968) pigment during spray drying, giving 100% of pigment retention.

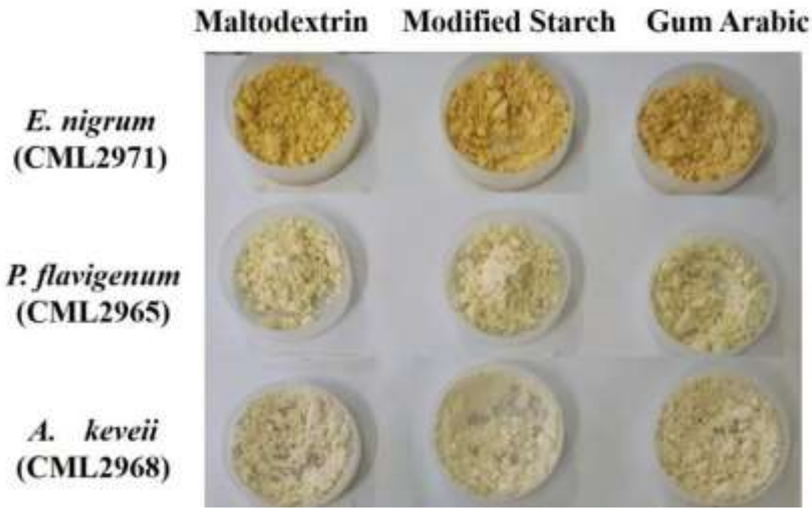

Figure 2. Powders obtained by spray drying process of the colored cell free filtrate from fungi using maltodextrin, gum arabic and modified starch as adjuvants.

Table 1. Selected properties of the spray dried powdered products loaded with pigments produced by filamentous fungi, using different adjuvants.

Strains	Adjuvants	Moisture (%)	Water activity (aw)	Recovery (%)	Pigment retention (%)
E. nigrum (CML2971)	maltodextrin	6.8 ± 0.2	0.304 ± 0.012	53.8	70.2 ± 0.5
	modified starch	7.3 ± 0.3	0.269 ± 0.005	55.3	100. ± 0.3
	gum arabic	7.9 ± 0.6	0.283 ± 0.023	50.5	82.8 ± 0.7
A. keveii (CML2968)	maltodextrin	7.7 ± 0.2	0.312 ± 0.008	62.4	86.1 ± 0.2
	modified starch	7.6 ± 0.4	0.286 ± 0.003	59.5	86.2 ± 0.3
	gum arabic	9.4 ± 0.3	0.317 ± 0.007	56.3	100. ± 0.6
P. flavigenum (CML2965)	maltodextrin	5.8 ± 0.1	0.313 ± 0.001	56.8	84.2 ± 0.9
	modified starch	6.2 ± 0.2	0.280 ± 0.013	59.8	89.9 + 0.3
	gum arabic	6.1 ± 0.6	0.238 ± 0.006	53.5	87.9 ± 0.4

According to Souza [6] there are no published reports on the production of pigments by *A. keveii*, but other species of the genus *Aspergillus* have been reported as possible sources of pigments. The drying of the *E. nigrum* (CML2971) filtrate in presence of modified starch also resulted in 100% of pigment retention; while maltodextrin showed the worst result, with a pigment retention of only 70.2 %. Conversely, the product recovery was slightly affected by the three distinct adjuvants, showing an average value of 53.2 ± 2.5 %. Figure 1 shows that the *E. nigrum* (CML2971) filtrate showed most intense yellow color, while the *A. keveii* and *P. flavigenum* showed almost similar less intense yellow color. The effects of adjuvants could be associated with the inherent composition of the coloring substance, as well as the extract constituents. It was previously reported by our group [12] that substance orevactaene, responsible for the yellow color, also exhibit good antioxidant activity, and shows high potential to be applied as a food colorant [17]. The color retention and product recovery for the *P. flavigenum* (CML2965) filtrate was similar and almost independent of adjuvant used, showing an average value of 87.3 %. However, the spray drying powder production was slightly lower when the adjuvant gum arabic was used (53.5 %). Dihydrotrichodimerol has also been identified in the *P. flavigenum* (CML2965) filtrate, which is a rare compound with complex chemical structure that shows important biological activities, such as antioxidant and antitumoral [6]. The filtrate obtained from *P. flavigenum* (CML2965) cultivation contains high content of phenolic compounds and exhibited high antioxidant activity [7].

These results reported in the present work showed that the adjuvants maltodextrin, modified starch and gum arabic might avoid the denaturation of the natural pigments from filamentous fungi during spray drying, showing pigments retentions ranging from 70.2 to 100 %. Moreover, the adjuvants also promoted a good spray drying performance, showing slightly effect on product recoveries that ranged from 50.5 to 62.4, depending on the fungal pigment extract and adjuvant type. The experimental results of product recoveries are in agreement with values reported for bench-top spray dryers [18].

Although further studies must be conducted, the present work is relevant and innovative, since it presents a successful spray drying microencapsulation technique for fungal colorants, showing very results for pigments protection. The fungal strains isolated from Brazilian caves might be a novel source of colorants and antioxidant compounds, which can be used in the food, pharmaceutical, and cosmetics sectors.

CONCLUSION

In conclusion, the pigments obtained by submerged fermentation of the filamentous fungi *E. nigrum* (CML2971), *P. flavigenum* (CML2965), and *A. keveii* (CML2968) might be successfully spray dried. The use of maltodextrin, modified starch, and gum arabic as adjuvants led to the generation of yellow fine powders with low moisture content and water activity, which enhanced the product stability during storage. In addition, the use of these adjuvants ensured high product recovery and pigment retention.

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