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Influence of process conditions on the physicochemical characteristics of cumaru (*Amburana cearensis*) powder produced by spray drying

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Abstract: The aim of the work was to study the spray-drying of ethanolic extract from *Amburana cearensis* (Allemão) A.C. Sm., Fabaceae, in order to obtain powders with better pharmacological and technological properties for herbal medicine. A 2^3 fractional factorial statistical design was used to find adequate spray-drying operating conditions (inlet air temperature; feed flow rate and air flow rate) to produce *A. cearensis* powder with adequate concentration of active principles (amburoside and coumarin), low moisture content and high process yield. The HPLC analyses showed that the spray-drying powder of *A. cearensis* production did not cause alterations in the chromatographic profile when related to the fluid extract. The most significant factor that affected the amburoside concentration was air flow rate, while the concentration of coumarin, a thermolabile molecule, was influenced mainly by inlet air temperature. The moisture content of the spray-drying powder of *A. cearensis* varied from 3.72 to 5.85% (w/w), while the maximal process yield was 41.1% (w/w). The present study demonstrates for the first time the best operating conditions to produce *A. cearensis* extract powder that were adequate when related to the coumarin and amburoside concentrations and moisture content. However, additional studies are still needed to improve mainly it technological characteristics.

Introduction

Cumaru (*Amburana cearensis* (Allemão) A.C. Sm., Fabaceae) is a typical tree from "Caatinga", a kind of vegetation found in the Brazilian semi-arid region. Cumaru is frequently used by the traditional medicine for the treatment of respiratory diseases including asthma. Phytochemical studies of its trunk bark allowed the isolation of several compounds such as isokaempferide, kaempferol, afrormosin, coumarin (CM) and other phenols compounds including amburoside A (AMB) (Canuto & Silveira, 2000; Canuto et al., 2006; Canuto, 2007).

Toxicological study carried out with the extract from the trunk bark of *A. cearensis* administered to rats by the oral route did not show any toxic effects (Leal et al., 2003a). The cytotoxicity of kaempferol, isokaempferide, amburoside A and protocatechuic acid from *A. cearensis* were evaluated on tumor cell lines and on the sea urchin egg development, as well as their lytic properties on mouse erythrocytes. The results showed that isokaempferide and kampferol, but not AMB and protocatechuic acid, inhibited the sea urchin egg development as well as tumor cell lines (Costa-Lotufo et al., 2003). Previous studies (Leal et al., 2003b; Leal et al., 2006; Leal et al., 2008) showed that the anti-inflammatory and antioxidant activities of hydroalcoholic (HAE), CM, AMB and/or isokaempferide from *A. cearensis* seem to occur by an inhibitory action on the release of inflammatory mediators, and/or alternatively by interfering with some phase of neutrophil migration into the inflammatory focus. In addition, *A. cearensis*, CM and isokaempferide also have a relaxing activity on rodents' tracheal muscles (Leal et al., 2000; Leal et al., 2006).

Cumaru has a great economical importance in the Northeast of Brazil, where the syrup of cumaru is the pharmaceutical form produced by the industry using liquid extract as the active ingredient. The pharmacological potential of cumaru is related at least in part to the presence of coumarin and amburoside A (chemical markers) in the plant.

The chemical and pharmaceutical food industries have been investing substantially in the last few years in the development of dried products mainly by presenting technological advantages in relation to fluid products. One of the most used drying methods has been the spray drying, which can be used on active pharmaceutical raw material drying, including molecules (Sansone et al, 2009), proteins (Gonnissen et al, 2008) and plant extracts (Couto, 2005; Gallo et al, 2011). In addition, this technique has also been used in the development of microparticles or nanoparticles of active principles (Zhao et al., 2011; Sansone et al., 2009).

The drying process by spray drying involves the generation of droplets from solutions (aqueous or organic), suspensions or emulsion, which are immediately transformed into a powder through the action of hot air, for example (Masters, 1991; Gonnissen et al, 2008). The drying method for spray drying is actually considered one of the most important processes for industry, especially because of some advantages presented by this system such as the capability of production of powders with specific size and moisture content, independent of the dryer capacity and of the sensibility to the heat of the product; applicability to thermo labile products and the production of powders with minimum quantity of residual solvent which specifications remain stable during the entire drying process (Masters, 1991; Chan et al., 2008).

Spray drying technique has been extensively used in the development of dried plant extracts both by pharmaceutical industries and by research centers. In this context, among studied species can be related *Rhamnus purshiana* (Gallo et al, 2011), *Phyllanthus niruri* (Couto, 2005), *Achyrocline satureioides* (Petrovick, 2006) and *Ginkgo biloba* (Zhang et al., 2010), which have been used as active raw material in the production of dried plant extracts. However, the successful development of these products, *i.e.* desired technological characteristics with maintenance of their pharmacological properties, requires a detailed study including selection of suitable drying adjuvant setting and adequate operating conditions. In this context, to meet the requirements of the dried product, close attention must be given to operate variables for spray driers that can significantly influence in the quality of the product such as inlet air temperature, liquid flow rate and air flow rate (Gallo et al., 2011).

Thus, considering the chemical, toxicological and pharmacological characteristics of *A. cearensis*, besides its economic value for pharmaceutical industry, the objective of the present study was to investigate the best operational conditions for the production of *A. cearensis* dried extract in order to obtain a product with better pharmacological and technological characteristics and better process yield.

Material and Methods

Plant material

Trunk bark of *Amburana cearensis* (Allemão) A.C. Sm., Fabaceae, was collected from Quixeramobin, Ceará, Brazil. Exsicates (numbers 837 and 847) of the species were deposited in the Herbário Prisco Bezerra at the Universidade Federal do Ceará. The trunk bark of *A. cearensis* was dried in an oven with circulation and continuous renewal of air during 48 h at a temperature of 35.0±5 °C. After drying, the material was pulverized and the moisture of the moderately coarse powder was of 8.23+0.92% (Araruna, 2008).

Chemicals

Coumarin (Sigma-aldrich; purity: 99.9%); Amburoside A (Cenauremn/UFC; purity: 97.3%); ammonium acetate and acetonitrile were supplied by Merck Specialty Chemicals, Mumbai, India and Mallinckrodt Baker, United states, USA. All the reagents were of analytical and HPLC grade. All the solutions were prepared in Milli Q water (Millipore, Billerica, MA, USA). The excipient used was Colloidal silicon dioxide (Aerosil 200®, Degussa, São Paulo, Brazil).

Preparation of the Spray Dryer Powder (SDP) from the EEtOH of Amburana cearensis

The extract of *A. cearensis* was prepared by maceration/percolation of trunk bark into EtOH in the proportion of drug:solvent of 1:1. The corresponding extractive solution containing 1.8±1.2% (w/v) of dry residue was employed to prepare the SDP according to the method previously described (Araruna, 2008). The SDP has in its composition colloidal silicon dioxide 30% in the final product. The etanol extract (EEtOH) was dried using a mini spray-dryer model LM MSD 1.0 (Labmaq do Brazil Ltda, Brazil) with capacity of drying up to 1.0 L/h. The atomization was carried out with a two-fluid 1.0 mm pneumatic nozzle, and the dryer was operated in concurrent flow.

Experimental design

A 2^3 experimental design with center points was used to optimize the production of the SDP from EEtOH of *A. cearensis*. The influence of three variables was investigated (feed flow rate: 0.6, 0.8, 1.0 L/h; air flow rate: 30.0, 35.0, 40.0 L/h and inlet air temperature: 100, 120, 130 °C) on production of SDP, employing as the responses the concentration of CM and AMB, moisture content and process yield. All the experimental runs were performed in randomized order and by triplicate to eliminate possible sources of bias.

The physicochemical characterization of the SDP of Amburana cearensis

Moisture content (MC)

The SDP of *A. cearensis* moisture content was determined by Karl Fischer method (model DL31, Mettler Toledo). Sample moisture content analysis was performed immediately after the spray-drying step (Farmacopeia Brasileira, 2010). This procedure was repeated three times with three distinct samples and the results are expressed as the mean and coefficient of variation.

High Performance Liquid Chromatography analysis

The simultaneous quantification of CM and AMB (chemical markers) in the SDP from EEtOH of *A. cearensis* was determined according to the method validated previously by our laboratory (Araruna et al., 2011).

Process yield

Spray drying yield was evaluated through the determination of recovered product given by the ratio between the total recovered product mass and the mass of extract initially fed into the system, and it was expressed by the following equation (Léon-Martinez et al., 2010):

$$y = \frac{(W_2 - W_1) - X_{wb}(W_2 - W_1)}{M_v T_s} x100$$

where y is the powder yield (%). X_{wb} is the moisture content in wet basis (wb). M_V is the volume of extract feed (L). T_s is the content of total solids (g dry matter/L), while W_1 and W_2 are the weight (g) of the powder receptacle before and after spray drying, respectively.

Statistical analysis

The data were analyzed with the aid of the program Statistic 6.0. (USA). The results were expressed as mean±SD and coefficient of variation. The means were compared using ANOVA, followed by Tukey for multiple comparisons as a *post hoc* test. The significance level was set at $p<0.05$.

Results and Discussion

The present study showed the influence of some spray-drying operating conditions in the production of SDP from EEtOH of *Amburana cearensis* (Allemão) A.C. Sm., Fabaceae, a species with pharmacological potential for treatment of respiratory diseases such as asthma.

Chromatographic analyses of the EEtOH of *A. cearensis* by HPLC-PDA showed among other peaks, two with retention time of 4.8 and 10.0 min, which were similar for AMB and CM (chemical markers) at the same experimental conditions (Figure 1).

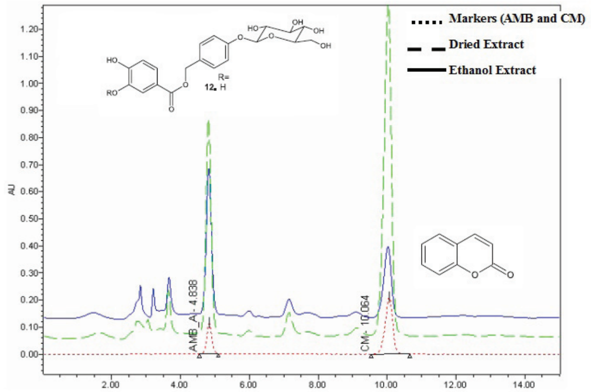

Figure 1. Chromatogram profile of the ethanol extract, SDP, amburoside and coumarin from *Amburana cearensis.*

The HPLC-PDA analysis showed that the SDP of *A. cearensis* production did not cause alterations in the chromatographic profile when related to the EEtOH (Figure 1). The retention time of CM and AMB in the EEtOH or SDP from *A. cearensis* were 10.06 and 4.84 min, respectively. The identification and quantification of these two active principles of *A. cearensis* are very important for quality control and efficacy of this active raw material.

According to the literature, adherence of plant extract in the walls of the drying chamber of spray dryer may be related to the conditions adopted in the drying process, the low efficiency of the cyclone used in the collection of the powder and the excipients used (Masters, 1985; List & Schimdt, 1989). In the present study there was no adherence of particles of solids on the walls of the drying chamber during the production of the SDP of *A. cearensis.* This result is possibly related at least in part with the use of colloidal silicon dioxide as excipient in the formulation of the SDP of *A. cearensis.*

Table 1 and Figure 2 show the effects of the selected factors (inlet air temperature, air flow rate and feed flow rate) in the development of the SDP of *A. cearensis* using as response concentration of markers, moisture content and yield process. Table 1 shows the AMB concentration achieved for each experiment. The lowest AMB concentration was on experiment 6 (63.37±2.68 mg/g) while the highest one was on experiment 2 (82.87±1.10 mg/g). The ANOVA analysis indicated that the most significant factor that affected the AMB concentration was air flow rate, -F-value 40.2, $p<0.0001$

(sample 2: 82.87±1.10 mg/g; sample 6: 63.37±2.68 mg/g). This variable was followed by feed flow rate and inlet air temperature (Table 1; Figure 2). The CM concentration in the SDP was significantly influenced by the all investigated factors. Furthermore, interactions were observed between factors. In this context, CM concentration values in the SDP showed a great variation, from 4.48 to 26.23 mg/g.

Previous studies developed by our laboratory (Leal et al., 2003b; Leal et al., 2008) showed that CM and AMB have anti-inflammatory and/or antioxidant activities in rodents at the dose from 20 to 50 mg/kg, *p.o.*, respectively, besides other pharmacological effects of these molecules. As described in Table 1 the higher concentration of CM in the SDP of *A. cearensis* was obtained in the experiment 7 (CM: 26.23±1.20 mg/g), which was shown in about 75 mg/g of AMB. Therefore, considering the active dose of CM and AMB, the SDP of *A. cearensis* obtained in experiment 7 seems to be most suitable for the development of phytomedicine.

According to Table 1, the moisture content of SDP of *A. cearensis* varied from 3.72 to 5.85%. This response was significantly influenced by all the variables, especially the feed flow rate followed by inlet air temperature and air flow rate. Powder moisture content was observed to increase when the feed flow rates increase and decrease when inlet air temperature increased. However this last result was dependent of the feed flow rate. These results corroborate with previous studies. According to Tonon et al. (2008) at higher inlet air temperatures, there is a greater temperature gradient between the atomized feed and the drying air, resulting in a greater driving force for water evaporation, thus producing powders with lower moisture content. Grabowski et al. (2006) also observed a reduction of powder moisture content with increasing temperatures, studying the spray drying of sweet potato puree.

At the present study the feed flow rate was the variable that showed the greatest influence on moisture content of the SDP of *A. cearensis*. The feed flow rate negatively affected powder moisture content. According to Kurozawa et al. (2009) higher flow rates imply in a shorter contact time between the feed and the drying air, making the heat transfer less efficient and resulting in lower water evaporation.

Table 1. Experimental matrix according to 2^3 factorial design and studied responses.

Assay	Temp (°C)	Feed flow (L/h)	Air flow (L/h)	AMB (mg/g)	CM (mg/g)	Yield (%)	Moisture content (%)
1	100	0.6	30	82.77±1.36	10.48±1.98	19.88±0.96	4.58±0.44
2	140	0.6	30	82.87±1.10	4.52±0.46	33.98±0.50	3.83±1.81
3	100	1	30	72.8±2.85	7.92±2.29	16.75± 1.90	5.05±0.33
4	140	1	30	68.97±1.22	14.98±1.56	13.63±2.15	4.92±0.34
5	100	0.6	40	75.45±1.72	13.20±2.01	24.07±0.91	5.85±0.69
6	140	0.6	40	63.37±2.68	4.48±2.38	19.43±2.77	4.92±0.41
7	100	1	40	74.54±1.45	26.23±1.20	41.10±1.45	5.31±0.92
8	140	1	40	75.61±0.77	6.05±1.16	19.84±3.54	3.72±1.20
9	120	0.8	35	79.21±2.09	9.85±0.96	33.61±1.55	4.92±0.41

The values represent the mean±SD. The analyses were performed in triplicate. AMB: amburoside A; CM: coumarin.

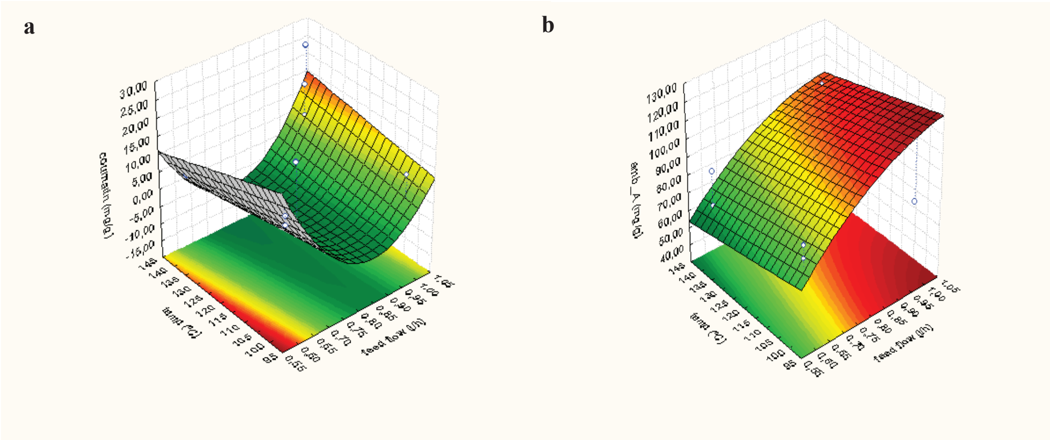

Figure 2. Spray Dryer Powder from *Amburana cearensis*: response surfaces for coumarin (a) and amburoside A (b) for optimization of drying conditions.

Table 1 shows the influence of inlet air temperature, feed flow rate and air flow rate on the spray drying process yield. This response was significantly influenced by all the variables alone, but also by interaction between them. The lowest yield was for experiment 4 (13.63±2.15%) while the highest one was for experiment 7 (41.1±0.6%). The dry process of the fluid extract of *A. cearensis* performed at the present study reaching a yield higher than the previous method developed by our laboratory (Araruna, 2008). However additional studies are still needed to improve the yield of this dry process.

Conclusion

The results of the present study showed that all operating conditions alone or combined influenced the physicochemical characteristics of the SDP of *Amburana cearensis*. Among the parameters investigated, the inlet air temperature and feed flow rate were one of the operating variables with more impact on CM concentration. On the other hand, the inlet air temperature did not show an important influence on AMB concentration.

The present study allowed for the first time the determination of the operating conditions-*e.g.* experiment 7-to produce *A. cearensis* extract powders that were adequate when related to the active principle concentrations (CM and AMB) and moisture content. In this sense, this work opens several investigative perspectives in view to optimize the product and add new technological characteristics.

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