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Original Article

Engineering spray-dried rosemary extracts with improved physicomechanical properties: a design of experiments issue

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ABSTRACT

A 3^3 Box–Behnken design and Response Surface Methodology were performed to evaluate the influence of extract feed rate, drying air inlet temperature and spray nozzle airflow rate on the process yield, stability parameters (moisture content and water activity) and on several physicomechanical properties of spray-dried rosemary extracts. Powder yield ranged from 17.1 to 74.96%. The spray-dried rosemary extracts showed moisture content and water activity below 5% and 0.5%, respectively, which indicate their chemical and microbiological stabilities. Even without using drying aids, some sets of experimental conditions rendered dried products with suitable flowability and compressibility characteristics for direct preparation of solid dosage forms. Analysis of variance and Response Surface Methodology proved that studied factors significantly affected most of the spray-dried rosemary extract quality indicators at different levels. The main processing parameter affecting the spray-dried rosemary extract characteristics was inlet temperature. The best combination of parameters used to obtain a reasonable yield of stable dry rosemary extracts with adequate technological properties for pharmaceutical purpose involves an extract feed rate of 2 ml/min, 80 °C inlet temperature and 40 l/min SA. The design of experiments approach is an interesting strategy for engineering spray-dried rosemary extracts with improved characteristics for pharmaceutical industrial purpose.

Introduction

Rosemary (*Rosmarinus officinalis* L., Lamiaceae), native to the Mediterranean, is a household specie used worldwide as a food preservative and food-flavoring agent. Over the last decades, the valuable medicinal properties of this herb have been in the spotlight of the scientific community. An evidence-based systematic review of rosemary's therapeutic use has been published (Ulbricht et al., 2010). Recently, preclinical surveys have contributed to evidence that rosemary has remarkable anti-inflammatory (Rocha et al., 2015), anti-proliferative (Cattaneo et al., 2015), and antibiotic (Barreto et al., 2014) activities, as well as antidepressant-like, anxiolytic, and antinociceptive effects (Abdelhalim et al., 2015).

Moreover, a clinical trial in humans reported the beneficial effects of rosemary for treatment of opium withdrawal syndrome during addiction treatment programs (Solhi et al., 2013). This broad range of relevant therapeutic properties is likely attributed to the antioxidant activity of this herb, which is a powerful source of antioxidant compounds *e.g.*, rosmarinic and carnosic acids, camphor, and 1,8-cineol (Barreto et al., 2014; Cattaneo et al., 2015; Lemos et al., 2015; Rocha et al., 2015; Li et al., 2016).

To become suitable for further therapeutic applications, herbal raw material typically undergoes several pharmaceutical processing technologies *e.g.*, the extraction of bioactive compounds and drying of plant extracts. In the latter, the dryer type and set of operating conditions used in the drying process of a liquid feed extract play important roles in determining the properties of co-processed products (Oliveira et al., 2006; Souza et al., 2008). As spray drying presents several advantages over other drying techniques such conventional air drying, freeze-drying and spouted bed drying, it is the most commonly employed in the pharmaceutical, food and flavor industry (Patel et al., 2014).

In brief, spray drying is a three-step unit operation (*i.e.*, atomization, dehydration, and powder collection) in which dry particulate products are recovered from a liquid solution or dispersion (suspension or emulsion) by spraying the liquid into a stream of drying gas under determined set of conditions (Oliveira and Petrovick, 2010). Due to its remarkable operational flexibility, spray drying offers a very accurate control over powder particle properties such as physicochemical stability, solubility, morphology and

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flowability (Ameri and Maa, 2006; Vehring, 2008; Cortés-Rojas and Oliveira, 2012).

The equipment's operating variables have been widely studied during the spray drying of phytopharmaceuticals. Such variables include solution or dispersion feed rate, drying air inlet temperature, drying air outlet temperature, spray nozzle airflow rate, spray nozzle diameter, spray nozzle type, drying air flow rate, aspiration rate, drying air inlet humidity, drying air outlet humidity, compressed air flow, compressed air pressure, *etc.* (Oliveira and Petrovick, 2010).

Currently, our research group has struggled greatly to develop rosemary's phytopharmaceuticals intermediate products with greater aggregate value by spray drying (Couto et al., 2012a). The operating variables set up demonstrated an effect on the chemical profile and *in vitro* antioxidant activity of spray-dried rosemary extracts (SDRE). From a phytopharmaceutical technology point of view, besides the physicochemical quality control and biological activity assessments, determining the effects of spray drying process variables in the drying performance and physicomechanical properties of the products, is a starting point for the full and accurate validation and scale-up of rosemary's powder technology process.

Improving both chemical and microbiological stabilities, as well as their flowability and compressibility during the preformulation studies, may turn the SDRE into more inviting intermediate products for developing solid dosage forms (SDF), which represent the majority of medicines marketed worldwide. Multifactorial chemometric tools such as the design of experiments approach and Response Surface Methodology (RSM) may be useful strategies in this pursuit (Matero et al., 2013).

In this paper, we report the effect of extract feed rate (EF), drying air inlet temperature (IT) and spray nozzle airflow rate (SA) in several physicomechanical properties of SDRE. A 3^3 Box–Behnken design and RSM were performed. The correlations between the process adequacy indicators were also assessed. By using a determined set of experimental conditions, we engineered products with improved levels of stability, flowability and compressibility, which may enable the direct manufacture of tablets and/or capsules filling.

Materials and methods

Herbal drug

Samples of rosemary leaves (Rosmarinus officinalis L., Lamiaceae) were collected from specimens located in the medicinal plants garden of the Hospital de Medicina Alternativa da Secretaria Estadual da Saúde do Estado de Goiás (863 m, 16°43′50.3″ South, 49°14′32.9″ West/Goiânia, GO, Brazil). Once identified, a voucher specimen was prepared and deposited in the Universidade Federal de Goiás (UFG) Herbarium under the registration identification UFG – 43206. The leaves were dried at room temperature (25 ± 2 °C) and ground in a TE-625 knife mill (Tecnal Ltda, Piracicaba, SP, Brazil). A mean powder size of 437.94 ± 7.00 μm was achieved. Powdered material (herbal drug) was stored sheltered from light and moisture for subsequent use in the extraction procedure.

Feed extract

The hydroalcoholic feed extract was obtained at room temperature by percolation of the herbal drug, using as solvent an ethanol:water solution (80:20, v/v) as previously reported (Couto et al., 2012a). The extract was stored in closed flasks protected from light at a temperature between −2 and 8 °C prior to characterization and further use in the drying experiments. Extract density and alcoholic content were determined according to the methods described in the Brazilian Pharmacopoeia 5th edition (Farmacopeia Brasileira, 2010). Solid content (C_S, w.b.) of a 1 g sample was measured with a gravimetric method in an MB 35 halogen lamp analyzer (Ohaus Inc., Pine Brook, NJ, USA). The extract viscosity was measured at room temperature using a DV–III+ viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). All experiments were performed in triplicate.

Table 1
Coded factors and their levels in the 3^3 Box–Behnken factorial design matrices.

Run	EF (ml/min)	IT (°C)	SA (l/min)
1	−1 (2)	−1 (80)	0 (40)
2	+1 (6)	−1 (80)	0 (40)
3	−1 (2)	+1 (140)	0 (40)
4	+1 (6)	+1 (140)	0 (40)
5	−1 (2)	0 (110)	−1 (30)
6	+1 (6)	0 (110)	−1 (30)
7	−1 (2)	0 (110)	+1 (50)
8	+1 (6)	0 (110)	+1 (50)
9	0 (4)	−1 (80)	−1 (30)
10	0 (4)	+1 (140)	−1 (30)
11	0 (4)	−1 (80)	+1 (50)
12	0 (4)	+1 (140)	+1 (50)
13	0 (4)	0 (110)	0 (40)
14	0 (4)	0 (110)	0 (40)
15	0 (4)	0 (110)	0 (40)

−1, 0 and +1: low, average and high coded values, respectively, in the factorial design matrices.
EF, extract feed rate; IT, drying air inlet temperature; SA, spray nozzle airflow rate.

Design of experiments

The spray drying experiments followed a 3^3 Box–Behnken design with three factors in three levels and three central point replicates as presented in Table 1, which shows the design matrices with the coded and non-coded values of each factor studied. The critical process parameters investigated (factors) were: (i) extract feed rate (EF, ml/min); (ii) drying air inlet temperature (IT, °C) and (iii) spraying airflow rate (SA, l/min). Their selection was based on our previous experiences.

The drying processes were performed at room temperature in a laboratory-scale spray dryer model MSD 1.0 (Labmaq do Brasil Ltda., Ribeirão Preto, SP, Brazil) with concurrent flow regime. The liquid extract feed system was composed of a peristaltic pump and a pneumatic (two fluid) spray nozzle with an inlet orifice diameter of 1.2 mm. The cylindrical drying chamber was made of borosilicate glass, 160 mm in diameter and 645 mm in height. The drying air was supplied by a blower (nominal flow rate of 1 m^3/min) and electrically heated. The temperature was maintained by a digital PID controller.

The following set of conditions remained fixed for all experiments: nozzle air pressure of 0.4 MPa; mass of extract portion feed (W_E) of 300 g and aspiration airflow of 100%. Before the extract was fed into the chamber, the drier was started with distilled water in order to reach thermal equilibrium. Then, if the inlet and outlet temperatures were constant, the feed extract was sprayed into the chamber. Drying air outlet temperatures (OT, °C) in each run were recorded in order to better understand the relations between factors studied and powder properties.

The SDRE were separated from air by a stainless steel cyclone and collected in a glass flask. The SDRE were weighed, stored in closed flasks protected from light and kept in a desiccator at room temperature for further characterization.

Characterization of the SDRE

The powder recovery or process yield (PY) was calculated immediately after the drying experiments based on the ratio of the mass

of powder (dry basis) collected in the flask (W) to the W_E and its solid content (C_S, % w.b.) by Eq. (1).

$$PY = \frac{W}{W_E \times C_S} \times 100\%, \quad (1)$$

The moisture content of powder (MC, % w.b.) was measured from a 0.5 g of sample employing an MB 35 halogen lamp analyzer (Ohaus Inc., Pine Brook, NJ, USA). Water activity (WA) was measured using a Testo 650 thermo hygrometer (Testo AG, Lenzkirch, Germany) and a hermetic chamber.

Bulk (ρ_b, g/cm^3) and tapped (ρ_t, g/cm^3) densities, Hausner's ratio (HR) and Carr's or Compressibility Index (CI, %) were determined according to the methods described in the American Pharmacopoeia 30th edition (USP, 2007). The product ρ_b was determined by pouring 5 g of dry powder into a 25 ml graduated cylinder and measuring its volume. The ρ_t was determined by controlled tapping of the cylinder using a sieve shaker (Bertel Ltda, Caieiras, SP, Brazil) until a constant volume was achieved. Hence, HR and CI were calculated according to their definitions:

$$HR(-) = \frac{\rho_t}{\rho_b} \quad (2)$$

$$CI(\%) = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \quad (3)$$

Angles of repose (θ,°) were determined according to the method described elsewhere (Araújo et al., 2010). The mean powder particle diameter (D_{50}, μm) was measured from the cumulative size distribution, by sieving 20 g of powder with Tyler series sieves (710, 355, 300, 250, 180, 106 and 53 μm) and an electro-magnetic sieve-shaker (Bertel Ltda, Caieiras, SP, Brazil) for 20 min (Brazil, 2010).

RSM

In Table 1, the factors levels were coded to allow the Analysis of Variance (ANOVA) by RSM following the coding rule given by Eq. (4):

$$\text{Coded.value} = \frac{(\text{uncode.value} - 0.5 \times (\text{high.value} + \text{low.value}))}{0.5 \times (\text{high.value} - \text{low.value})} \quad (4)$$

ANOVA/RSM on the experimental data was performed using the Statistica 7 software (Statsoft Inc., Tulsa, OK, USA). Only the factors with $p \leq 0.05$ were considered significant. The response function applied was a quadratic polynomial equation, given by Eq. (5):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (5)$$

In Eq. (5), Y is the predicted response (dependent variable); β_0 is the model constant; X_1, X_2 and X_3 are independent variables; β_1, β_2 and β_3 are linear coefficients; β_{12}, β_{13} and β_{23} are cross product coefficients; and β_{11}, β_{22} and β_{33} are the quadratic coefficients.

Scanning electronic microscopy (SEM)

The morphology of the spray-dried powder that presented the most improved set of physicomechanical properties was evaluated using a scanning electron microscope (JEOL, JSM – 6610) equipped with energy dispersive X-ray spectroscopy (Thermo Scientific NSS Spectral Imagging). Before SEM analysis, the sample was mounted on a metal stub with double-sided adhesive tape and recovered with a gold layer of 200 Å thickness under vacuum using a metallisator (Denton Vacuum, Desk V). Photomicrographs with magnifications of 5500×, 15,000× and 25,000× were recorded at 5 kV.

Results and discussion

Characterization of the feed extract

The concentrated hydroalcoholic extract presented a solid content of 9.7 ± 0.1 (% w.b.), density of 0.964 ± 0.002 g/cm^3, viscosity of 5.2 ± 0.1 mPa s and alcoholic content of 38.2 ± 0.5 (% v/v). Generally, in a spray drying process the characteristics of the dried particles depend on the characteristics of the atomized droplets. They are closely related to the PY, as to the packaging and flowing characteristics of the dried powders. It has been reported that the solid content, density, viscosity and surface tension of the feed material influence the droplet size and shape and consequently the size, shape and the density of the dry particles (Goula et al., 2004; Goula and Adamopoulos, 2004; Huntington, 2004). Accordingly, such feed extract characteristics must to be considered during the spray drying experimental design since they may drive the choice of the dryer's operational parameter levels in order to obtain products with improved physicomechanical, physicochemical and pharmacological properties.

Critical process parameters affect the physicomechanical properties of the SDRE

The spray drying process adequacy was estimated in terms of several physicomechanical characteristics of the dried products. Product recovery (yield) may indicate the success and affordability of the process. Powder moisture content provides information as regards to the solvent removal efficiency and may influence the cohesiveness and size distribution of the dried particles. WA correlates with the product's chemical and microbiological stabilities and consequently its shelf life. The HR, CI, angle of repose, and mean powder particles diameter were used to indirectly estimate the compressibility and flowability (cohesiveness) characteristics of the dried powders.

Evaluating all these particulate material properties is a prerequisite for the rationale of developing intermediate products for further SDF manufacturing by applying specific unit operations *e.g.*, mixing, granulation (powder agglomeration), tableting, coating and capsule filling (Matero et al., 2013). Additional information concerning the drying behavior as a result of the binomial feed material/drier's set up was supplied by the OT. The results of complete product characterization are displayed in Table 2, which shows that the SDRE had diverse characteristics when different sets of conditions were applied in the drying process.

ANOVA and correlation analyses were performed by RSM in order to accurately determine the effects of the critical process parameters studied in the dried product properties. The tables with complete ANOVAs for each powder property are overlooked, but a summary of the main effects (bold values) and their significance are listed in Table 3. Table 3 also displays comments on the types of the effects as represented by positive (directly proportional) and negative (inversely proportional) signs.

RSM also enabled the fitting of polynomial equations of the process adequacy indicators (dependent variables) as a function of the significant factors (independent variables) for predicting product characteristics. Their predictive ability is expressed by the determination coefficient (R^2) as given in Table 3. Complementarily, the response surface plots of the adequacy indicators, as functions of the factors that were shown to be significant, are shown in Figs. 1–4.

The OT results varied between 45 °C and 90 °C (Table 2). The design of the dryer used is such that the OT, contrary to the IT,

Table 2
Results of spray-dried products characteristics.

Run	OT (°C)	PY (% w/w)	MC (% w/w)	WA (−)	HR (−)	CI (%)	θ (°)	D_{50} (μm)
1	50	52.81	2.85	0.22	1.08	8.16	25	387.07
2	45	63.58	2.74	0.23	1.32	24.53	42.33	330.62
3	90	17.1	1.26	0.24	1.38	27.5	45	157.63
4	79	33.92	1.17	0.26	1.35	26.09	43.87	262.1
5	77	64.98	2.19	0.23	1.27	21.05	39.33	300.65
6	62	74.96	2.61	0.21	1.42	29.54	47	342.43
7	70	26.28	1.38	0.26	1.14	12.5	28.67	163.81
8	60	59.86	2.21	0.22	1.33	25	42.67	348.03
9	49	69.33	3.46	0.23	1.37	26.83	44.03	354.62
10	87	37.32	1.91	0.23	1.17	14.63	33.67	335.23
11	50	51.92	2.51	0.24	1.18	15.09	35	262.7
12	80	21.67	1.49	0.24	1.23	18.6	38.32	133.82
13	64.3	62.63	2.18	0.22	1.28	22.22	39.93	277.79
14	65	63.54	2.11	0.24	1.29	22.92	40.72	240.8
15	66.7	57.53	2.11	0.22	1.29	22.45	40.17	235.61

OT, drying air outlet temperature; PY, process yield; MC, moisture content; WA, water activity; HR, Hausner Ratio; CI, Carr's Index; θ, angle of repose; D_{50}, mean powder particles size.

Table 3
Summary of RSM analysis.

Factor	OT (°C)	PY (% w/w)	MC (% w/w)	CI (%)	θ (°)	D_{50} (μm)
Intercept	**65.33**[c]	**61.23**[c]	**2.13**[c]	**22.5**[c]	**40.27**[c]	**251.4**[c]
EF	**−5.13**[c]	**+8.89**[b]	0.13	**+4.49**[a]	**+4.73**[a]	34.25
EF^2	0.71	−3.96	−0.19	1.14	0.22	25.05
IT	**+17.75**[c]	**−15.95**[c]	**−0.72**[c]	1.53	1.81	**−55.78**[a]
IT^2	−0.04	**−15.42**[b]	0.06	−2.1	−1.44	7.91
SA	**−1.88**[a]	**−10.86**[c]	**−0.32**[a]	−2.61	−2.42	**−53.07**[a]
SA^2	1.21	−0.75	0.15	−1.65	−1.08	12.28
EF × IT	−1.5	1.51	0.01	−4.45	−4.62	40.23
EF × SA	1.25	5.9	0.1	1.00	1.58	35.61
IT × SA	2.0	0.44	0.13	3.93	3.42	−27.37
R^2	0.99	0.98	0.95	0.78	0.77	0.87

Bold values represent the effects of the fitted polynomial equations that proved to be significant at $p < 0.05$.
[a] $p < 0.01$.
[b] $p < 0.001$.
[c] Positive (+) and negative signs (−) indicate directly and inversely proportional effect, respectively; EF, extract feed rate (ml/min); IT, drying air inlet temperature (°C); SA, spray nozzle airflow rate (l/min); OT, drying air outlet temperature; PY, process yield; MC, moisture content; CI, Carr's Index; θ, angle of repose; D_{50}, mean powder particles size; R^2, determination coefficient for the fitted prediction models.

cannot be set with a temperature regulator. Hence, it results from a combination of the in-process parameters *i.e.*, feed extract properties, pump setting, IT, atomizer pressure, spray nozzle inlet orifice diameter, compressed airflow rate in the atomizer, as well as the flow rate of drying air. ANOVA/RSM proved that EF, IT and SA all affected OT with p values of 0.0007, 0.000002 and 0.041, respectively (Table 3). Increased IT resulted in increased OT because of increased supply of heat energy (Table 3, Fig. 1a and c). On the other hand, increased EF and SA resulted in decreased OT due to increased solvent evaporation rate (Table 3, Fig. 1 a–c). The fitted prediction model, with $R^2 = 0.99$, is given by:

$$\mathrm{OT}(^\circ\mathrm{C}) = 65.33 - 5.13\left(\frac{\mathrm{EF}-4}{2}\right) + 17.75\left(\frac{\mathrm{IT}-110}{30}\right) - 1.88\left(\frac{\mathrm{SA}-40}{10}\right) \quad (6)$$

The PY values ranged from 17.1 to 74.96%. Even without using any drying aid only two (15.4%) of the thirteen different experimental conditions studied lead to powder recoveries lower than 20%. Powder recovery depended on the EF parameter at $p = 0.003$ (Table 3). It can be observed in the response surface shown in Fig. 2a and b that increasing EF had a positive linear influence on PY, which means that the higher the EF, the higher the PY. Both IT and its squared term (IT^2) exerted negative nonlinear effects on powder recovery (Table 3, Fig. 2a and c), with p values of 0.0002 and 0.0014, respectively. Conversely, SA exerted a negative linear effect on PY with $p = 0.0012$ (Table 3, Fig. 2b and c). The fitted prediction model, with $R^2 = 0.98$, is given by:

$$\mathrm{PY}(\%) = 61.23 + 8.89\left(\frac{\mathrm{EF}-4}{2}\right) - 15.95\left(\frac{\mathrm{IT}-110}{30}\right) - 15.42\left(\frac{\mathrm{IT}^2-110}{30}\right) - 10.86\left(\frac{\mathrm{SA}-40}{10}\right) \quad (7)$$

Increasing the feed rate of the spraying material and decreasing both IT and SA presumably lead to an increase in the droplet size and viscosity and, subsequently, particle size and density. In turn, the loss of fine and light particles in the exhausted air that passes through the cyclone decreases, which raises the powder recovery along the drying process. These promising product recoveries achieved may also be attributed to low adherence of the powders on the drying chamber and cyclone walls. A valid explanation to this is that the resultant drying temperature stayed lower than the extract's glass transition temperature in most sets of operating conditions.

Due to the inherent complexity of a multifactorial unit operation such as the spray drying process, there is a broad range of in-process variables that are assumed to affect the PY during the development of pharmaceutical products. Besides the effects of critical processing parameter adjustments (Jangam and Thorat, 2010; León-Martínez et al., 2010; Couto et al., 2013), there are reports concerning the impact of design and size of the drying

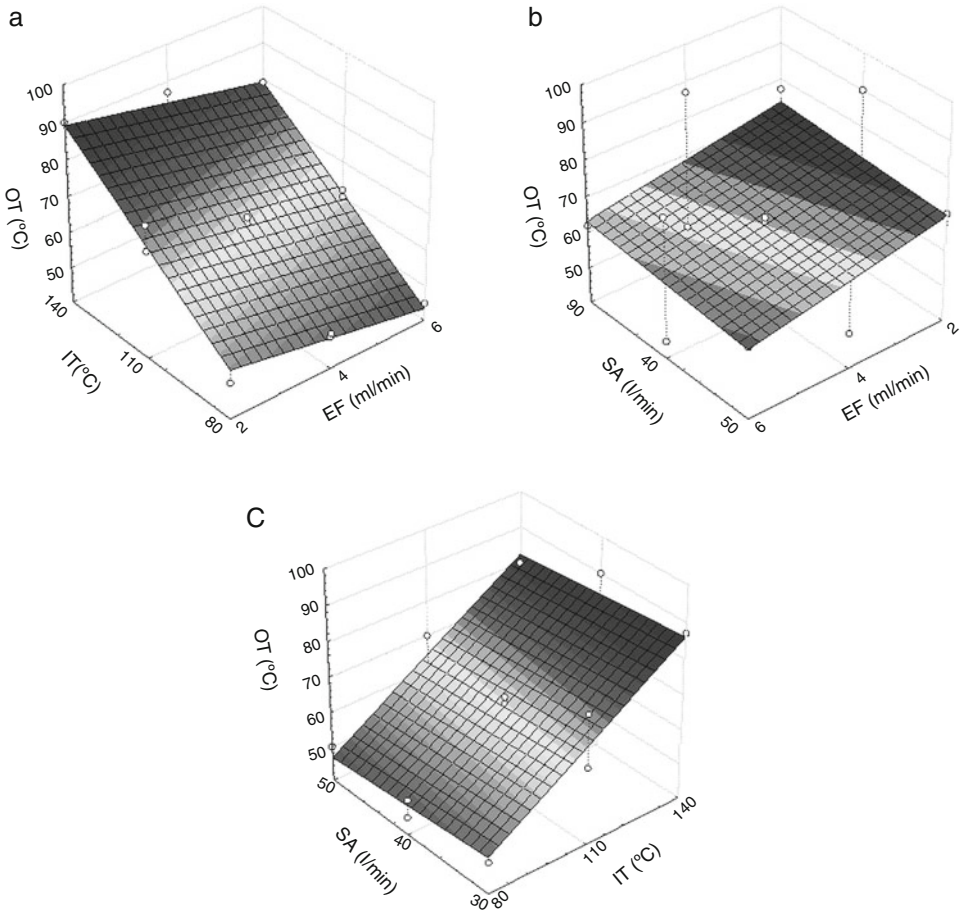

Fig. 1. Surface plot of drying air outlet temperature as a function of: (A) extract feed rate and drying air inlet temperature; (B) extract feed rate and spray nozzle airflow rate; (C) drying air inlet temperature and spray nozzle airflow rate.

chamber and cyclone of lab-scale spray dryers (Ameri and Maa, 2006).

The type, amount and incorporation time of drying aids (Couto et al., 2012b, 2011), and even the environmental factors (Ciesielski and Zbicinski, 2010) have also been proved to exert a key role in the PY. Despite the efforts in evaluating the impact of these parameters in spray drying performance, the literature does not present a consensus regarding the acceptable range of powder recovery for both bench-top and industrial spray drying process so far.

In our spray drying runs, the MC ranged from 1.17% to 3.46% (w/w), and for all SDRE the WA values were below 0.3 (Table 2). Residual moisture and WA values below 5% (w/w) and 0.5, respectively, meet the pharmaceutical requirements for dry materials at particulate level (USP, 2007). Therefore, it is possible to assert that all the products shown in Table 2 presented satisfactory levels of MC and WA.

According to ANOVA/RSM, none of the studied factors significantly affected WA. However, both IT and SA had linear negative influence on MC with p values of 0.0005 and 0.02, respectively (Table 3). In other words, an increase in both IT and SA resulted in reduced values of moisture content. The surface response of the MC as a function of IT and SA is shown in Fig. 3. The fitted prediction model, with $R^2 = 0.95$, is given by:

$$\mathrm{MC}(\%) = 2.13 - 0.72\left(\frac{\mathrm{IT} - 110}{30}\right) - 0.32\left(\frac{\mathrm{SA} - 40}{10}\right) \quad (8)$$

In a spray drying system, removal of moisture from the sprayed particles depends upon the temperature of the drying air. The greater the drying air temperature, the greater the temperature difference between the particle and the drying air, the more efficient is the heat transfer phenomena and, therefore, the greater the evaporation rate. Synergistically, an increase in drying airflow rate helps in removal of moisture at a higher rate. This happens since for a given amount of feed extract, at any given time, increasing the SA generates smaller atomized droplets into the chamber. Hence, the contact surface between the particle and the air would rise, a larger amount of hot air would be available and less energy would be required for moisture removal.

Although seeming to be very close in name, residual moisture and WA are distinct physical parameters that, but not necessarily, often correlate with each other. Whereas the moisture content represents the whole water composition in a dried system, WA is a measurement of the availability of free water that is responsible for any chemical (hydration interactions) and biochemical (enzymatic) reaction, and also microbiological growth (Quek et al., 2007). Hence, the lower the WA, the lower the chemical potential of water and the driving force in interactions involving water, which not necessarily directly depends on the moisture content in the product.

For example, in our results (Table 2), the SDRE with a greater MC value (3.46%, run 9) had an WA of 0.23, while the SDRE with lower MC (1.17, run 4) exhibited an WA of 0.26. A plausible explanation for this finding is that the gravimetric method used for the determination of moisture content (loss on drying), contrary to that used for WA, did not measure only the water in the sample, but all the volatile compounds existing in it. As rosemary is a great source of volatile oils, and some of them may be extracted by the hydroalcoholic mixture during the extraction procedure, they may

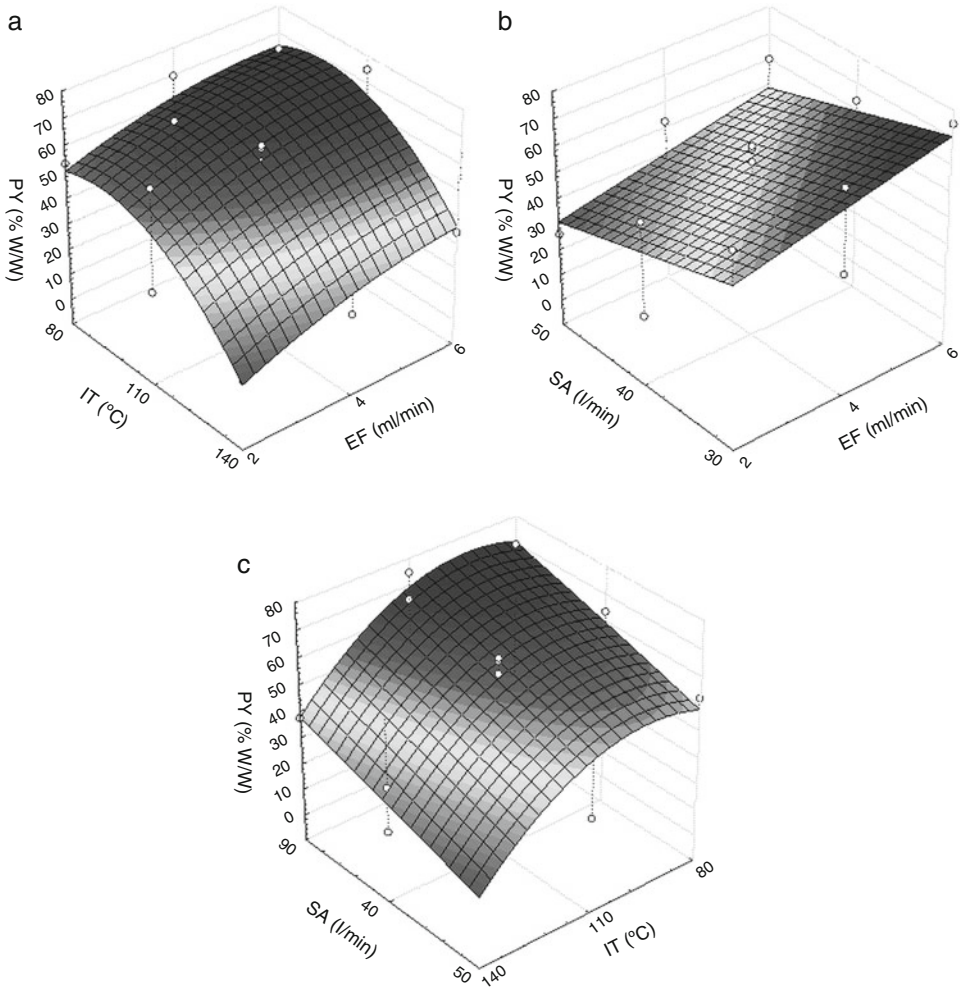

Fig. 2. Surface plot of process yield as a function of: (A) extract feed rate and drying air inlet temperature; (B) extract feed rate and spray nozzle airflow rate; (C) drying air inlet temperature and spray nozzle airflow rate.

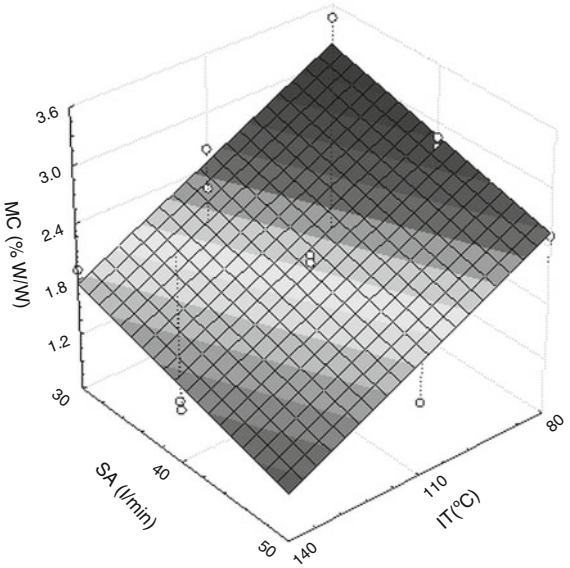

Fig. 3. Surface plot of moisture content as a function of drying air inlet temperature and spray nozzle airflow rate.

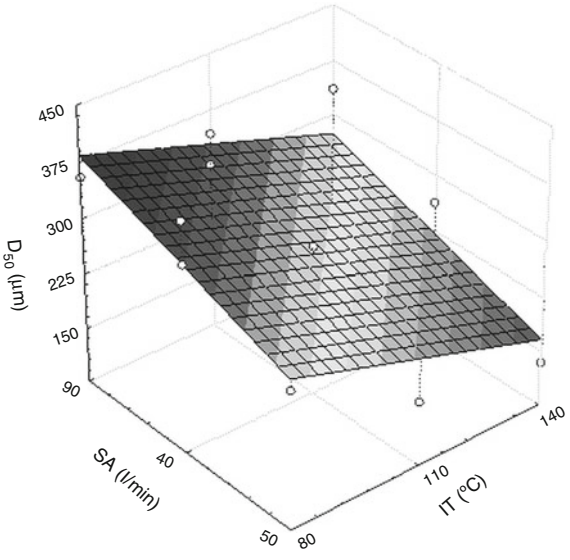

Fig. 4. Surface plot of mean powder particles size as a function of drying air inlet temperature and spray nozzle airflow rate.

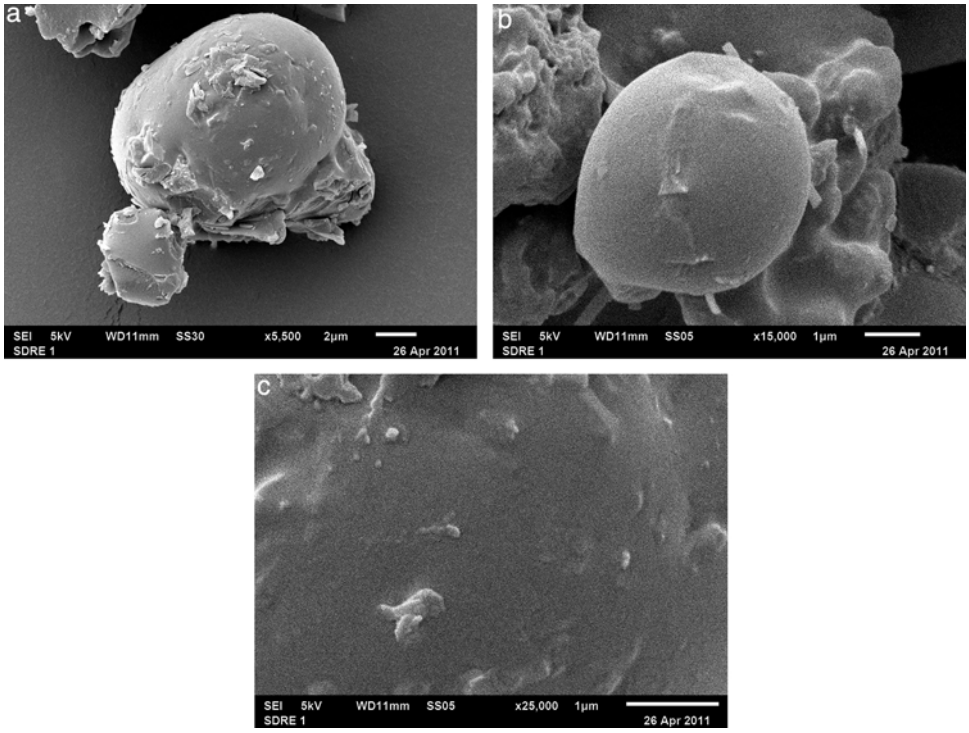

Fig. 5. Micrographs of spray-dried rosemary extract recorded at 5 kV with magnification of: (A) 5500×, (B) 15,000× and (C) 25,000×.

be maintained in the dried powder and considered during the mass loss determination.

The HR varied between 1.08 and 1.42. Since HR lower than 1.25 indicates non-cohesive and low internal friction powders (USP, 2007), satisfactory rheological properties where achieved in five (38.5%) of the thirteen different experimental runs. None of the studied factors significantly affected the HR at $p < 0.05$. The CI varied from 8.16% to 29.54%. For this quality indicator, the literature proposes the following classification of flowing properties: excellent (CI < 10%), good (11% < CI < 15%), fair (16% < CI < 20%), passable (21% < CI < 25%), poor (26% < CI < 31%), very poor (32% < CI < 37%) and very, very poor (CI > 38%) (USP, 2007).

Therefore, as observed for the HR parameter, five conditions studied produced powders with suitable flowability (CI < 20%). ANOVA showed that only the EF influenced the CI, with a p value of 0.047. Increasing the EF linearly increased the CI. This may be attributed to an increase in MC in the powders when using upper levels of EF. Raising the residual moisture may increase the cohesive forces between the particles due to hydrogen and/or Van der Waal's interactions and hence impairs the powder flowability. The fitted prediction model, with $R^2 = 0.78$, is given by:

$$\mathrm{CI}(\%) = 22.5 + 4.49\left(\frac{\mathrm{EF}-4}{2}\right) \quad (9)$$

The angles of repose ranged from 25.0° up to 47.0°. Angles of repose in the range of 25° to 40° indicate that the cohesiveness of the powder product is within the satisfactory limit according to the pharmaceutical needs (USP, 2007). The less stable the packed system, the better the flow and smaller the θ value. Moreover, θ values varying within 41° and 45° are passable for a particulate bed, but adding a lubricant is required to improve its flowability (USP 30th Ed., 2007). Therefore it, is possible to assert that seven (53.9%) of our different experimental runs provided intermediate products with valuable flowability ($25° < \theta < 40°$), in which two are in the range attributed to excellent ($25° < \theta < 30°$), two others are good ($31° < \theta < 35°$) and three indicate fairly good ($36° < \theta < 40°$) flowing properties. As for the CI, the angle of repose depends only on the EF with a p value of 0.041, in a way that increasing EF increased the θ (Table 3). A direct correlation between CI and θ can be seen and results can be explained along the same line as that of the CI. The fitted prediction model, with $R^2 = 0.77$, is given by:

$$\theta(°) = 40.27 + 4.73\left(\frac{\mathrm{EF}-4}{2}\right) \quad (10)$$

Non-cohesive free-flowing powders are required for SDF manufacturing. Together with exerting a key role in the efficiency of manually or industrially filling of capsules, they are determinant for a successful "die filling" (filling the matrix compression) and compression stages along the industrial production of tablets. Accurately accomplishing these unit operations are compulsory to assure the content uniformity and dosage of the drug contained in each formulation. Furthermore, it can influence the final hardness of tablets, which may affect the physical stability of its dosage form during further coating, packing, transport, storage and handling by the user, as well as in the availability of the drug to be further absorbed and reach its action site.

It is noteworthy that even without using any drying aid most of our SDRE presented better flowability and compressibility than those obtained using colloidal silicon dioxide (Tixosil 333®) and maltodextrin DE 14 as drying carrier (40:20 proportion in relation to the total solids content) in the study of spray and spouted bed drying of rosemary extract (Souza et al., 2008).

The mean particle diameter (D_{50}) ranged from 133.82 to 387.07 µm. In addition to its moisture content and density, the size distribution and shape display a remarkable effect on the compression and flow of a pharmaceutical powder, because it influences the particle packing geometry and the particle-to-particle interaction levels (cohesion). Typically, the smaller and more spherical are the particles, the greater will be the stability of the packed system, which decrease the particles bed compressibility and flowability.

On the other hand, smaller and more spherical particles have increased solubility due to the increased surficial area to interact with surrounding solvent during the dissolution phenomena. The D_{50} directly correlate to the content uniformity and

Table 4
Correlation matrix between the quality indicators of the spray-dried products.[a]

Quality indicator	OT (°C)	PY (% w/w)	MC (% w/w)	WA (−)	HR (−)	CI (%)	θ (°)	D_{50} (μm)
OT (°C)	1	−0.45	**−0.68**	0.13	0.04	0.01	0.01	−0.31
PY (%)	−0.45	1	**+0.65**	−0.45	0.09	0.09	0.08	**+0.52**
MC (%)	**−0.68**	**+0.65**	1	−0.39	0.002	0.001	0.001	**+0.58**
WA (−)	0.13	−0.45	−0.39	1	−0.04	−0.03	−0.03	−0.38
HR (−)	0.04	0.09	0.002	−0.04	1	**+0.99**	+0.96	0.01
CI (%)	0.01	0.09	0.001	−0.03	**+0.99**	1	+0.97	0.001
θ (°)	0.01	0.08	0.001	−0.03	**+0.96**	**+0.97**	1	−0.001
D_{50} (μm)	−0.31	**+0.52**	**+0.58**	−0.38	0.01	0.001	−0.001	1

[a] Values are the determination coefficients (R^2) from the correlation analysis; positive (+) and negative signs (−) indicate directly and inversely proportional effect, respectively; bold values represent the more relevant correlation; OT, drying air outlet temperature; PY, process yield; MC, moisture content; WA, water activity; HR, Hausner Ratio; CI, Carr's Index; θ, angle of repose; D_{50}, mean powder particles size.

dissolution rate and, indirectly with the bioavailability (because it also depends on drug permeability) and clinical efficacy of a drug from a pharmaceutical dosage form. Accordingly, during the rationale development of SDF, controlling the particle size distribution is required for reaching equilibrium between their physicomechanical and biopharmaceutical properties in order to guarantee the quality and efficacy of the medicine.

The surface response plot of D_{50} as a function of IT and SA is presented in Fig. 4. The surface shows that the mean powder diameter increased with decreasing both IT and SA, which is a result of the increase in the size of droplets sprayed into the drying chamber. The effect of IT and SA on the mean particle diameter was confirmed by ANOVA (Table 3), which demonstrated p values of 0.02 and 0.024, respectively for these in-process parameters. The fitted prediction model, with $R^2 = 0.87$, is given by:

$$D_{50}(\mu m) = 251.4 - 55.78\left(\frac{IT - 110}{30}\right) - 53.07\left(\frac{SA - 40}{10}\right) \quad (11)$$

The results arising from this investigation demonstrate the remarkable impact of the process parameters on the drying performance during the rosemary hydroalcoholic extract spray drying. The high determination coefficients observed in the fitted models confirm their ability to describe the experimental results and predict responses (Table 3). From the results displayed in Table 3, it can be seen that IT is the main factor affecting the quality indicators of the SDRE, because the effect values (coefficients) for this independent variable are greater than the others that have shown to be significant.

Our findings emphasize that the comprehension and accurate selection of the drying parameter levels are the key to guarantee satisfactory levels of processing performance while developing SDRE. Under the ranges studied, the experimental run number 1 (Tables 1 and 2), with a low EF (2 ml/min), low IT (80 °C) and intermediate SA (40 l/min), provided spray-dried powders with improved properties for direct preparation of tablets and capsules filling.

These results, together with the fact that the process can be adjusted to attain optimized performance, suggest that the spray drying technique may be attractive and suitable for developing novel intermediate phytopharmaceutical products of rosemary aiming at the manufacture of SDF. To the complete development and validation of a pilot spray drying process it would be necessary to extend the validity of these results by further performing complementary preformulation studies. Our further investigations will be targeted to evaluate other processing parameters *e.g.*, spray nozzle inlet orifice diameter, nozzle air pressure and drying airflow rate, and their interactions with the properties of dry extracts of *R. officinalis*.

How do the process adequacy indicators correlate with each other?

To simplify interpretation of the relationships between the process quality indicators, a correlations matrix was prepared and is presented in Table 4. The determination coefficients (R^2) values that we obtained for the more relevant interactions are highlighted (bold).

In summary, the OT had an appreciable negative correlation with the residual moisture content ($R^2 = 0.68$). This means that increasing the OT proportionally decreases the MC in the powders. In turn, the MC positively correlated with both the process yield ($R^2 = 0.65$) and mean particles size ($R^2 = 0.58$) *i.e.*, an increase in MC leads to an increase in PY and D_{50}. A reasonable interaction was evidenced within these parameters, which also positively correlated with each other ($R^2 = 0.52$).

The major interdependencies ($0.96 \leq R^2 \leq 0.99$) were observed between the HR, CI and the angle of repose. These correlations were expected, because the HR, CI and θ were all related to powder mechanics. None of the other matrix terms significantly correlated with each other. Overall, these correlations lend further support for the validity of the comments addressed for the trends presented in the previous section, and reinforce the hypothesis that the variables of the spray drying process must be analyzed altogether during the preformulation studies for engineering products with improved properties.

Morphological aspects of the most promising product

The SEM images of the SDRE produced by experimental run number 1, are shown in Fig. 5a–c, with a magnification level of 5500×, 15,000× and 25,000×, respectively. By SEM the SDRE particles were mostly observed as spherical (Fig. 5a and b) and showed a smooth surface, but some depressions and cracks were also present (Fig. 5c). These characteristics meet those observed for spray-dried particles of other industrial crops such as soybean (Georgetti et al., 2008), açaí (Tonon et al., 2008), chicory (Toneli et al., 2010), *Bindens pilosa* (Cortés-Rojas and Oliveira, 2012), clove (Cortés-Rojas et al., 2014) and *Achillea millefolium* (Vladic et al., 2016). As the majority of the feed materials have singular drying behavior, determining the effect of process variables upon particle morphology in general terms has been reported to be a difficult task. This issue has been extensively revised elsewhere (Walton, 2000).

Conclusion

The spray drying technique has made it possible to engineer intermediate products of rosemary with improved physicomechanical characteristics for further developing SDF. The design of experiments approach and RSM were useful strategies for the selection of the proper drying set of conditions. The main factor affecting

the process quality indicators is the IT. The best set of conditions to achieve a greater recovery of dry powder of *R. officinalis* extract with low levels of moisture content and WA, as well as suitable flowability and compressibility, requires a low RF (2 ml/min), low IT (80 °C) and intermediate SA (40 l/min).

Authors' contributions

LTC (PhD student) contributed in obtaining the herbal drug, running the laboratory work, analysis of the data and drafted the paper. JRP contributed in plant identification, herbarium confection and to critical reading of the manuscript. MTFB contributed to critical reading of the manuscript. ECC and ROC designed the study, supervised the laboratory work, analyzed the data and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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