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Spray-dried rosemary extracts: Physicochemical and antioxidant properties

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ABSTRACT

In this work, spray-dried rosemary extracts were obtained. A 3^3 Box–Behnken design was followed to evaluate the influence of drying conditions on the contents of chemical markers and "*in vitro*" antioxidant activity of the powder. Although the dry products lost some of their polyphenols, they still had antioxidant activities (IC_{50}) ranging from 17.6 to 24.8 $\mu g \cdot mL^{-1}$. Analysis of variance proved that studied factors and some of their interactions significantly affected most of the quality indicators. The best combination of conditions to use for obtaining dry rosemary extracts with adequate physicochemical and functional properties involves an extract feed rate of 6 $mL \cdot min^{-1}$, a drying air inlet temperature of 140 °C and a spray nozzle air flow rate of 50 $L \cdot min^{-1}$.

1. Introduction

In recent decades, industrial manufacturing of phytomedicines has grown considerably and, due to worldwide phytopharmaceutical market trends, is receiving attention from the academic community and pharmaceutical companies in Brazil (Calixto, 2005). For industrial purposes, dried extracts have several advantages over the liquid forms: dried extracts have high stability and are easier to handle, standardise, transport and store (Oliveira, Bott, & Souza, 2006). Moreover, dried extracts allow the manufacture of solid dosage forms, like tablets and capsules, which represent most of the medicines used worldwide (Leuenberger & Lanz, 2005).

Rosmarinus officinalis L. (Lamiaceae), commonly known as rosemary, is a household plant used worldwide as a food-flavouring agent. A preclinical survey confirmed that rosemary has powerful anti-inflammatory (Benincá, Dalmarco, Pizzolatti, & Fröde, 2011), antibacterial (Yesil-Celiktas, Hames Kocabas, et al., 2007), antidiabetic (Bakirel, Bakire, Keles, Ülgen, & Yardibi, 2008), antitumor (Cheung & Tai, 2007), cytoprotective (Yoo, Lee, Lee, Moon, & Lee, 2008) and hepatoprotective (Gutiérrez et al., 2009) properties. Rosemary has one of the highest antioxidant activities of all the herbs and spices that have been investigated (Wojdyło, Oszmiański, & Czemerys, 2007). The antioxidant activity of rosemary justifies its use in a broad range of applications, including food preservatives (Hamre, Kolås, & Sandnes, 2010), cosmetics (Lee et al., 2011), nutraceuticals and phytomedicines (Ibarra et al., 2010). These medicinal attributes can be related to rosemary's high content of polyphenolic compounds, especially rosmarinic acid (Erkan, Ayranci, & Ayranci, 2008), which is considered a chemical marker of this species.

Despite rosemary's medicinal and commercial importance, there is little information on its behaviour during processing and standardisation. Accordingly, undertaking a study to elucidate the effects of processing factors on product properties during the manufacture of standardised dried rosemary extracts is fully justified. In this work response surface methodology (RSM) was used to verify the effect of processing parameters on the chemical markers contents and "*in vitro*" antioxidant activities of rosemary extracts obtained *via* spray drying.

2. Material and methods

2.1. Reagents and chemicals

Rosmarinic acid (98%), rutin (98%), tannic acid (98%) and 2,2-diphenyl-1-picrylhydrazyl (DPPH·) were purchased from Sigma–Aldrich (Sigma–Aldrich Co., Steinheim, Germany). Acetonitrile and methanol were of HPLC grade (Tedia Brazil, Rio de Janeiro, RJ, Brazil). Additionally, anhydrous formic acid (Impex Ltd., Diadema, SP, Brazil), ethanol (Chemis Ltda., São Paulo, SP, Brazil) and ultrapure water from a Milli-Q system (Millipore®, Bedford, MA) were used.

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Nomenclature

AOA	antioxidant activity, as defined in Eq. (3)
E_F	extract feed rate ($mL \cdot min^{-1}$)
HRE	hydroalcoholic rosemary extract
IT	drying air inlet temperature (°C)
RA_C	rosmarinic acid content (% w/w)
S_A	spray nozzle airflow rate ($L \cdot min^{-1}$)
SDRP	spray-dried rosemary products
TF_C	total flavonoid content (% w/w)
TP_C	total polyphenol content (% w/w)
TT_C	total tannin content (% w/w)
X	coded factors in the experimental design
Subscripts	
A	airflow rate
C	content
F	feed rate

All other chemicals were of reagent grade and were used without further purification.

2.2. Herbal material

Samples of rosemary leaves were collected from specimens located in the medicinal plants garden of 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 and ground in a knife mill TE-625 (Tecnal Ltda, Piracicaba, SP, Brazil). Powdered material was stored sheltered from light and moisture for subsequent use in the extraction procedure.

2.3. Feed extract obtainment and characterisation

The hydroalcoholic rosemary extract (HRE) was obtained by percolation of the powdered material (mean particle size of 438 ± 7.00 μm), using ethanol:water solution (80:20 v/v) as solvent mixture. Briefly, 3 kg of powdered material were placed in contact with 1 L of solvent in a glass flask. After an incubation period of 2 h (pre-swelling phase), this material was carefully transferred to a 10L percolator (Revitec Ltda, São Paulo, SP, Brazil) and solvent was added to volume. This system remained in contact with the powdered material for 24 h (intermediate maceration phase). Next, it was extracted exhaustively (0.2 ± 0.05 $mL \cdot min^{-1}$) at room temperature (percolation phase). The extractor solvent was renewed throughout until thin layer chromatography assay no longer detected rosmarinic acid. The obtained extract was evaporated at 40 ± 2 °C using a rotary evaporator MA 120 (Marconi Ltda, Piracicaba, SP, Brazil) coupled to a vacuum pump Te-152 (Tecnal Ltda, Piracicaba, SP, Brazil). The concentrated extract (9 L) was stored in borosilicate flasks protected from light at temperatures from −2 to 8 °C prior to characterisation and further use.

Density, alcoholic content and pH were determined according to the methodologies described in Farmacopéia Brasileira IV (2001). Total solids content of a 1.0g sample was measured with a gravimetric method in a halogen lamp analyser (MB 35; Ohaus Inc., Pine Brook, NJ). Finally, the viscosity was measured using a viscometer (Brookfield DV–III+; Brookfield Engineering Laboratories, Inc., Middleboro, MA).

2.4. Manufacture of dried products

The drying processes were performed in a laboratory-scale spray dryer (MSD 1.0; Labmaq do Brasil Ltda., Ribeirão Preto, SP, Brazil) with a concurrent flow regime and a pneumatic (two-fluid) spray nozzle with an inlet orifice diameter of 1.2 mm. The experiments were carried out following a Box–Behnken design with three factors and three levels (3^3). The factors studied and their levels were: X_1, extract feed rate (E_F), at 2 (−1), 4 (0) and 6 $mL \cdot min^{-1}$ (+1); X_2, drying air inlet temperature (*IT*), at 80 (−1), 110 (0) and 140 °C (+1); X_3, spray nozzle airflow rate (S_A), at 30 (−1), 40 (0) and 50 $L \cdot min^{-1}$ (+1). The factors were coded to allow analysis of variance (ANOVA) by the RSM, following the coding rule given by Eq. (1):

$$\text{Coded.value} = \frac{(\text{uncode.value} - 0.5 \times (\text{high.value} + \text{low.value}))}{0.5 \times (\text{high.value} - \text{low.value})} \quad (1)$$

ANOVA/RSM on the experimental data was performed using the module Visual General Linear Model (VGLM) from the software Statistica 7 (Statsoft Inc., Tulsa, OK). Only the factors with significance higher than or equal to 5% ($p \leqslant 0.05$) were considered. The response function applied was a quadratic polynomial equation, given by Eq. (2):

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

In Eq. (2), *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.

The following set of conditions was kept fixed for all experiments: nozzle air pressure was 4.0 bar; extract mass flow rate was 300 g; drying air flow rate was 1.0 $m^3 \cdot min^{-1}$. The spray-dried rosemary extracts (SDRE) were collected at the dryer outlet, weighed and stored in closed flasks protected from light in a desiccator at room temperature with ambient relative humidity prior to characterisation.

2.5. Determination of contents of chemical markers

2.5.1. Total polyphenol and tannin quantifications

Total polyphenol contents (TP_C) and total tannin contents (TT_C) in HRE and SDRE were determined following previously described methods (Mole & Waterman, 1987a; Mole & Waterman, 1987b), with some modifications. Next, 10 mg (dry basis) of SDRP were dissolved in 10 mL of 20% (v/v) methanol solution. HRE was directly diluted 100 times with this same solution. In both TP_C and TT_C measurements, tannic acid was used to make the calibration curves. In total, 10 mg of tannic acid was dissolved in 20% (v/v) methanol and diluted to 200, 300, 400, 500, 600, 700 and 800 $\mu g \cdot mL^{-1}$.

2.5.2. Quantification of total flavonoids

Total flavonoid contents (TF_C) were measured according to a modified method based on that of Rolim et al. (2005). Ten milligrams (dry basis) of SDRE were dissolved in 10 mL of methanol:acetic acid 0.02 M (99:1). HRE was directly diluted 200 times with the methanol:acetic acid 0.02 M (99:1) solution. The absorbance of 2-mL samples was measured at 361 nm with an SP220 UV/Vis spectrophotometer (Biospectro®, Curitiba, PR, Brazil). Rutin was used to make a calibration curve. Ten milligrams of rutin were

dissolved in the methanol:acetic acid 0.02 M (99:1) solution and diluted to 100, 200, 300, 400 and 500 $\mu g \cdot mL^{-1}$.

2.5.3. HPLC-PDA rosmarinic acid quantification

HPLC analysis was performed on an LC system comprising a quaternary pump (LC-20AT), a degasser (DGU-20A5), an autosampler (SIL 20A) and an SPD-M20A Prominence PDA detector (Shimadzu®, Kyoto, Japan). Chromatographic separation was carried out with a Gemini RP-C18 reverse-phase column (250 × 4.6 mm, 3 μm, 110 Å; Phenomenex, Inc., Torrance, CA). The mobile phase, which was composed of 30% acetonitrile and 70% acetonitrile aqueous solution (2.5% v/v) and formic acid (0.5% v/v), was set in an isocratic mode with a flow rate of 0.5 $mL \cdot min^{-1}$. The detection wavelength was 254 nm. The injection volume was 20.0 μL and the total run time was fixed at 15 min. Data acquisition and analysis were performed by using a Shimadzu® controller module (CBM-20A Prominence) coupled to a computer with Shimadzu® LC Solution software. The HPLC-PDA method was validated following the Agência Nacional de Vigilância Sanitária (ANVISA – Brazilian National Health Surveillance Agency) guidelines (Brazil. Health Ministery. Brazilian National Health Surveillance Agency. Resolution, 2003) (data not shown).

Ten milligrams (dry basis) of SDRE were diluted 100 times with methanol and HRE was diluted 500 times with the same solvent. Rosmarinic acid contents (RA_C) were calculated by comparison with the standard, which was used to make a calibration curve. Ten milligrams of rosmarinic acid were dissolved in methanol and then diluted to 2.5, 5.0, 10.0, 20.0 and 50.0 $\mu g \cdot mL^{-1}$. Prior to injection in the LC system, all samples were filtered through 0.45 μm Millex® (Millipore, São Paulo, SP, Brazil) membranes.

2.6. Assessment of antioxidant activity (AOA)

The scavenging activity of the DPPH· free radical was performed as with a modified method described by Brand-Williams, Cuvelier, and Berset (1995). The samples were first solubilised with 95% ethanol and diluted using the same solution to final concentration ranges of 0.5–500 $\mu g \cdot mL^{-1}$. Aliquots (2.5 mL) of several dilutions of the test materials were mixed with 1.0 mL of a 0.3 mM ethanolic DPPH· solution. After an incubation period of 30 min at 25 °C, absorbance at 517 nm was recorded as A_{sample}. A blank was also performed with the same procedure using a solution without DPPH· and the absorbance was recorded as A_{blank}. A control experiment (antioxidant absent) was performed using a solution without the dilutions of the test materials and the absorbance was recorded as $A_{control}$. The free radical-scavenging activity of each solution was calculated as percent inhibition, according to the following equation:

$$AOA\ (\%\text{inhibition}) = 100 - \frac{(A_{sample} - A_{blank}) \times 100}{A_{control}} \quad (3)$$

AOA was expressed as IC_{50}, defined as the concentration ($\mu g \cdot mL^{-1}$) of the test material required to cause a 50% decrease in initial DPPH· concentration. All of the measurements were performed in triplicate.

3. Results and discussion

3.1. Characterisation of the rosemary extract

The concentrated hydroalcoholic extract possessed a density of 0.964 ± 0.002 $g \cdot mL^{-1}$, a solids content of 9.66 ± 0.07 (% w/w), a pH of 5.106 ± 0.005, an alcoholic content of 38.2 ± 0.53% (v/v) and a viscosity of 5.2 ± 0.09 mPas. The levels of TP_C, TF_C, TT_C and RA_C were, respectively, 30.2 ± 0.24%, 9.13 ± 0.01%, 8.78 ± 0.1% and 10.7 ± 0.43% (w/w). Also, in the *AOA* assessment, the extract possessed an IC_{50} of 17.3 $\mu g \cdot mL^{-1}$.

The feed extract properties provide useful information on experimental planning, since their composition, alcoholic content, solids content and viscosity may affect operational parameters of the dryer chosen. Thus, evaluation of extract properties is essential to obtain spray-dried powders with optimised physicochemical and biological properties under maximised safety conditions.

3.2. Effects of the drying conditions on the product properties

3.2.1. SDRE properties

In general, for phytochemicals, drying is a crucial step since it can lead to different amorphous states for drugs and affects their stability (Araújo, Teixeira, & Freitas, 2010). The dryer type and operating conditions used in the drying process of a liquid extract play important roles in determining the properties and cost of a product (Souza, Schiavetto, Thomazini, & Oliveira, 2008). Hence, factors related to the drying process make the development of the phytopharmaceutical binomial formulation/process a complex task. Among the widely used drying techniques, spray drying is the most commonly used in both the food and phytopharmaceutical industries (Georgetti, Casagrande, Souza, Oliveira, & Fonseca, 2008). Spray drying presents several advantages over other drying technology, such as operational flexibility, applicability for heat sensitive materials and affordability (Wendel & Celik, 1987).

SDRE properties used as quality indicators in this investigation were the contents of total polyphenols, total flavonoids, total tannins and rosmarinic acid. Additional information on process adequacy is supplied by *"in vitro"* antioxidant activity, which is closely related to the suitability of powder for further therapeutic use. The results of complete powder characterisation are presented in Table 1, which also displays the Box–Behnken design matrices and the coded levels of the factors studied.

From a phytopharmaceutical technology point of view, a major challenge is to produce a standardised extract that has the desired content of bioavailable active compounds. In the obtained products, the levels of TP_C, TF_C, TT_C and RA_C ranged from 12.9% to 17.4%, 4.35% to 8.60%, 5.72% to 7.83% and 2.32% to 7.50% (w/w), respectively. These values have degradation ratios ranging from 42.5% to 57.3%, 5.80% to 53.4%, 10.8% to 34.9% and 29.8% to 78.3%, respectively. It is interesting to note that the different sets of drying conditions used in this study affected the polyphenolic

Table 1
Results of spray-dried products characterisation.

Product	X_1	X_2	X_3	TP_C	TF_C	TT_C	RA_C	*AOA*
1	−1	−1	0	16.5	6.83	6.77	5.37	18.8
2	+1	−1	0	15.4	5.98	6.81	5.88	21.9
3	−1	+1	0	14.1	5.15	7.75	3.74	24.4
4	+1	+1	0	15.4	5.89	7.39	5.74	18.6
5	−1	0	−1	17.4	7.64	5.72	6.9	19.9
6	+1	0	−1	17.1	7.01	6.54	7.5	21.0
7	−1	0	+1	12.9	4.9	7.83	2.32	19.5
8	+1	0	+1	15.4	5.2	5.79	5.87	20.5
9	0	−1	−1	15.8	8.6	5.81	6.67	18.3
10	0	+1	−1	16.9	7.19	6.45	6.45	19.5
11	0	−1	+1	14.6	4.35	5.94	4.97	19.3
12	0	+1	+1	14.5	5.37	7.53	4.53	18.3
13	0	0	0	13.2	5.91	6.26	5.5	19.3
14	0	0	0	14.7	6.38	6.25	5.85	19.6
15	0	0	0	13.7	6.53	6.63	5.52	17.6

X_i: Coded factors in the experimental design; −1, 0, +1: coded levels in the experimental design; TP_C: total polyphenol contents (% w/w); TF_C: total flavonoid contents (% w/w); TT_C: Total tannin contents (% w/w); RA_C: rosmarinic acid contents (% w/w); *AOA*: antioxidant activity (IC_{50}, $\mu g \cdot mL^{-1}$).

compounds differently, with the highest ranges observed in RA_C and TF_C. In earlier investigations comparing spray and spouted bed drying of rosemary extracts, Souza et al. (2008) observed similar TP_C and TF_C degradation profiles. According to these authors, the degradation of the polyphenols may have been caused by oxidative condensation phenomena and decomposition of thermolabile compounds induced by in-process factors such as heating.

In addition to physicochemical quality control, the evaluation of several functional properties is essential for a full characterisation and validation of pharmaceutical powder technology processes. Among them, antioxidant activity plays an important role in the development of rosemary's pharmaceutical dosage forms (Ibarra et al., 2010). The SDRE presented IC_{50} values ranging from 17.6 to 24.4 $\mu g \cdot mL^{-1}$, which indicates that some activity is lost during the spray drying process (1.68% to 41.3%). Better recovery was found for SDRE submitted to spray drying of HRE at intermediate levels of extract feed rate, drying air inlet temperature and spray nozzle airflow rate (exp. 15). It is accepted that potent DPPH· free radical scavenging by polyphenols is due to their ideal, although heterogeneous, chemical structures, since they are comprised of hydroxyl groups varying in number and position (Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005). SDRE at a final concentration of 125 $\mu g \cdot mL^{-1}$ in the medium were able to inhibit approximately 90% of radical-scavenging activity (data not shown). The resulting *AOA* values are plausible, since 125 $\mu g \cdot mL^{-1}$ methanolic rosemary extracts from other areas possessing diverse amounts of total polyphenols and rosmarinic acid have been evaluated by DPPH· free radical scavenging and the inhibition observed varied from 90.6% to 94.7% (Yesil-Celiktas, Girgin et al., 2007). These results, together with the fact that the process can be modified to allow higher TP_C, TF_C, TT_C, RA_C and *AOA* recovery, suggest that although SDRE lost some polyphenols, they still present excellent antioxidant activity, indicating potential for use in nutraceutical therapy and food preservatives.

The SDRE had diverse properties when different sets of conditions were applied in the drying process (Table 1). Thus, correct selection of the processing conditions is important to guarantee the physicochemical and functional quality of the spray-dried rosemary products. Interestingly, with a high extract feed rate, high drying air inlet temperature and intermediate spray nozzle air flow rate (exp. 4) TP_C, TF_C, TT_C, RA_C and *AOA* ranged from intermediate to high levels, reaching 15.39%, 5.89%, 7.39%, 5.74% and 18.56 $\mu g \cdot mL^{-1}$, respectively. Accordingly, spray drying processes may be an attractive and promising alternative for the development of new pharmaceutical dosage forms of rosemary.

3.2.2. Product quality interactions

The complex results of the individual powder characterisations (Table 1) require further investigation regarding their significance, and the interactions of the quality indicators and the studied factors. In order to precisely determine the interactions of the process factors with the quality indicators, ANOVA and correlation analyses were performed. The tables with complete ANOVAs for each powder property are omitted, but a summary of the main effects and their significance values are listed in Table 2, where the levels of significance are displayed as percentages. Table 2 also displays comments on the interactions shown to be highly significant and arrows indicate the sign of the effect (positive or negative). In addition, the response surface analysis allows the fitting of polynomial equations of the dependent variables as a function of the significant factors for predicting quality indicators. The response surfaces of the parameters studied, as functions of the factors that were shown to be significant, are shown in Figs 1–4.

The ANOVA showed that only the S_A exerted an influence on the TP_C at a significance level of 5%. None of E_F, E_F^2, *IT*, IT^2, S_A^2 nor the interactive terms were significant. Moreover, increasing the S_A had

Table 2
Summary of factor effects and significances (p) on powder properties.

Factor	TP_C	TF_C	TT_C	RA_C	*AOA*
E_F	0.393	0.773	0.0604	↑0.0068[b]	0.831
E_F^2	0.125	0.267	↑0.016[c]	0.454	↑0.0283[c]
IT	0.605	0.104	↑0.0019[b]	0.166	0.4567
IT^2	0.2505	0.775	↑0.0227[c]	0.468	0.576
S_A	↓0.0119[c]	↓0.0002[a]	↑0.01[b]	↓0.0012[b]	0.773
S_A^2	0.099	0.449	↓0.0378[c]	0.409	0.576
$E_F \times IT$	0.222	0.094	0.416	0.219	↓0.0098[b]
$E_F \times S_A$	0.1779	0.2814	↓0.0014[b]	↑0.0388[c]	0.962
$IT \times S_A$	0.553	↑0.0253[c]	0.0889	0.844	0.380

Significant at: [a]0.1%, [b]1% and [c]5%; symbols: ↑ increase and ↓decrease; E_F: extract feed rate ($mL \cdot min^{-1}$); *IT*: drying air inlet temperature (°C); S_A: spray nozzle airflow rate ($L \cdot min^{-1}$); TP_C: total polyphenol contents (% w/w); TF_C: total flavonoid contents (% w/w); TT_C: total tannin contents (% w/w); RA_C: rosmarinic acid contents (% w/w); *AOA*: antioxidant activity (IC_{50}, $\mu g \cdot mL^{-1}$).

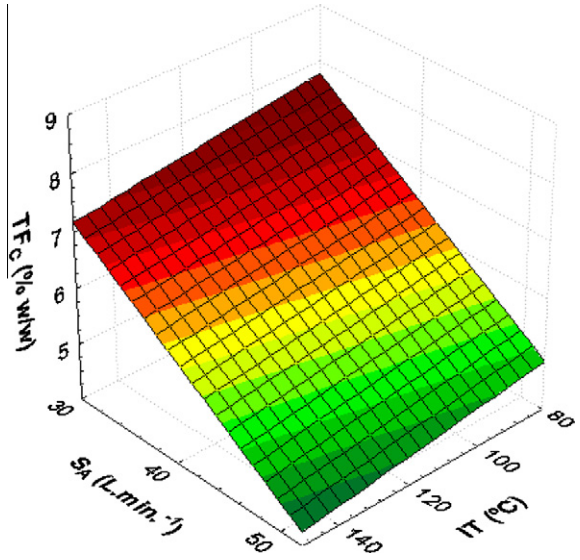

Fig. 1. Surface plot of total flavonoid content as a function of drying air inlet temperature and spray nozzle airflow rate.

a negative influence on total polyphenol content. The fitted equation, with correlation coefficient $r = 0.923$, is given by:

$$TP_C = 13.87 - 1.224\left(\frac{S_A - 40}{10}\right) \quad (4)$$

The surface response of TF_C as a function of *IT* and S_A is shown in Fig. 1. The spray nozzle airflow rate had a strong negative effect on TF_C, at a significance level of 0.1%. However, the interaction of *IT* with the S_A had a positive influence at 5%. The fitted equation, with correlation coefficient $r = 0.979$, is given by:

$$TF_C = 6.273 - 1.327\left(\frac{S_A - 40}{10}\right) + 0.607\left(\frac{IT - 110}{30}\right)\left(\frac{S_A - 40}{10}\right) \quad (5)$$

Fig. 2a–c presents the surface responses of TT_C as a function of E_F, *IT* and S_A. The surfaces show that E_F, *IT* and S_A all exerted a non-linear effect on TT_C. This effect was confirmed by the ANOVA, which demonstrated a significance level of 1% to both *IT* and S_A, and 0.1% for the squared terms (E_F^2, IT^2 and S_A^2). In addition, the trends of the curves for low or high E_F and S_A are inconsistent, which means that there is an interaction between these factors

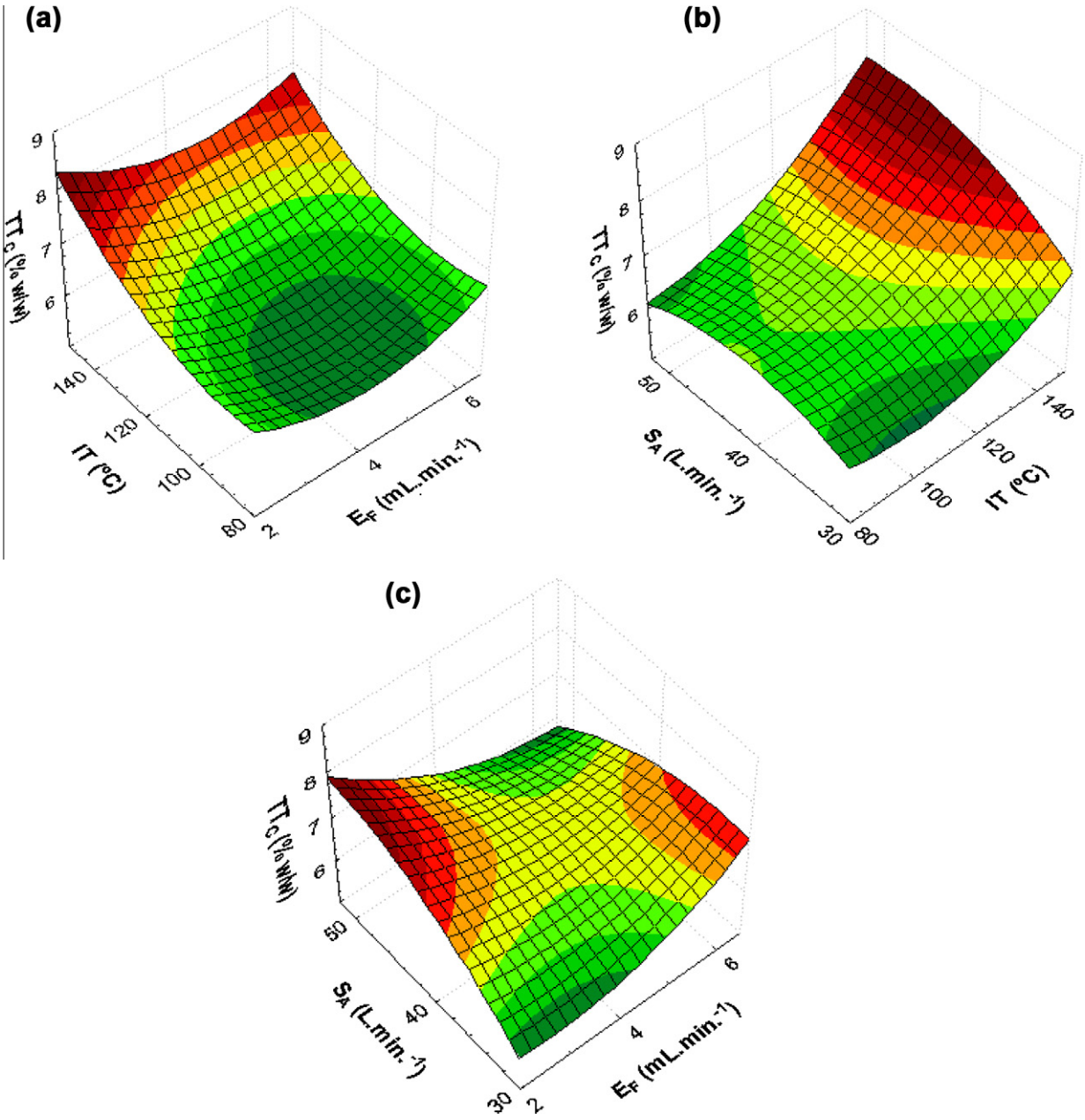

Fig. 2. Surface plot of total tannin content as a function of extract feed rate and drying air inlet temperature (a); drying air inlet temperature and spray nozzle airflow rate (b); extract feed rate and spray nozzle airflow rate (c).

(Fig. 2c). Using the ANOVA, this interactive effect occurs at a significance level of 1%, as shown in Table 2. The fitted equation, with correlation coefficient $r = 0.982$, is given by:

$$TT_C = 6.38 + 0.419\left(\frac{E_F^2 - 4}{2}\right) + 0.473\left(\frac{IT - 110}{30}\right) + 0.381\left(\frac{IT^2 - 110}{30}\right) + 0.321\left(\frac{S_A - 40}{10}\right) - 0.329\left(\frac{S_A^2 - 40}{10}\right) - 0.715\left(\frac{E_F - 4}{2}\right)\left(\frac{S_A - 40}{10}\right) \quad (6)$$

The effect of spray-drying factors on the rosmarinic acid contents of the products, RA_C, can be seen in Fig. 3. The E_F and S_A strongly affected RA_C, both at significant levels of 1%. Furthermore, RA_C depended on the interaction between E_F and S_A at 5%. The fitted equation, with correlation coefficient $r = 0.982$, is given by:

$$RA_C = 5.623 + 0.832\left(\frac{E_F - 4}{2}\right) - 1.229\left(\frac{S_A - 40}{10}\right) - 0.737\left(\frac{E_F - 4}{2}\right)\left(\frac{S_A - 40}{10}\right) \quad (7)$$

Fig. 4 shows a surface plot of antioxidant activity, *AOA*, as a function of the extract feed rate and drying air inlet temperature. The surface shows that the extract feed rate exerted a positive nonlinear effect on *AOA*. The nonlinear effect of E_F was confirmed by the ANOVA, which demonstrated a significance level of 5% for the squared term (E_F^2). However, the interaction between the *IT* and E_F had a strong negative effect on *AOA* at a significance level of 1%. The fitted equation, with correlation coefficient $r = 0.922$, is given by:

$$AOA = 18.83 + 1.75\left(\frac{E_F^2 - 4}{2}\right) - 2.227\left(\frac{E_F - 4}{2}\right)\left(\frac{IT - 110}{30}\right) \quad (8)$$

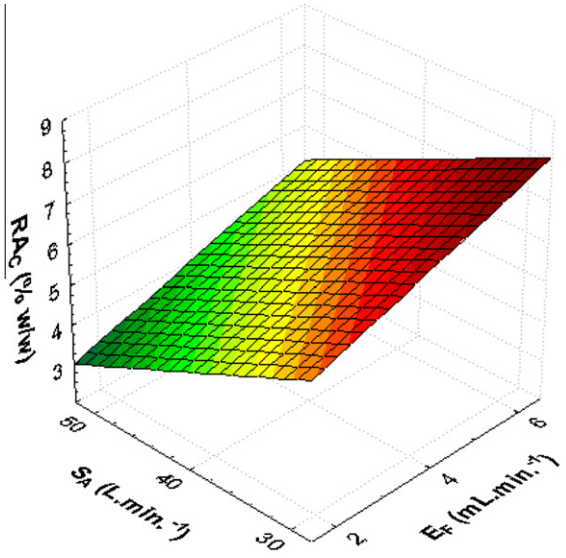

Fig. 3. Surface plot of rosmarinic acid content as a function of extract feed rate and spray nozzle airflow rate.

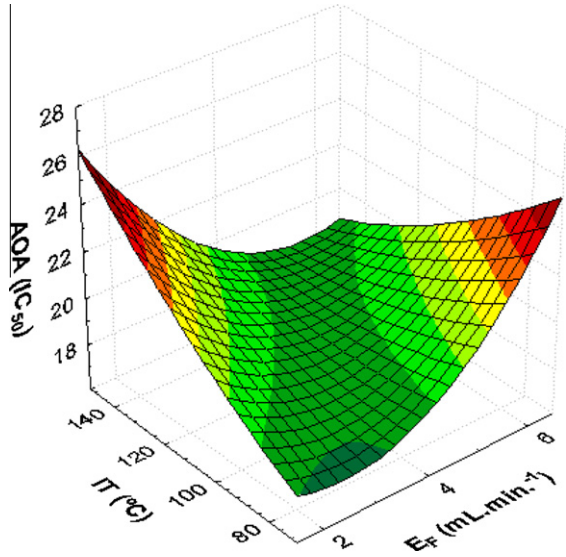

Fig. 4. Surface plot of antioxidant activity as a function of extract feed rate and drying air inlet temperature.

How the factors studied and quality indexes are connected remains unclear, since Table 2 does not show the interactions. To facilitate interpretation of the relationships between the factors studied and quality indices the correlation matrix of the process factors and the quality indices was prepared (data not shown). The correlation coefficients between the *AOA* and TP_C, TF_C, TT_C and RA_C on the SDRE were, respectively, 0.03, −0.27, 0.23 and −0.14. It is clear from the correlation coefficients that *AOA* does not correlate with any of the chemical markers contents. These results, together with the fact that the recovery of chemical markers was significantly lower than the recovery of the antioxidant activity, may indicate that the antioxidant activity is only partially related to the compounds observed here, and there may be other chemicals involved in its activity. In fact, antioxidants present in rosemary extracts are not restricted to polyphenols (Ibarra et al., 2010). Moreover, it is important to consider the occurrence of synergism between the chemical compounds in the whole extract, which makes the *AOA* dependent on both the chemical structure and interactions between the antioxidant substances, besides its concentration (Georgetti et al., 2008).

An r^2 of 0.77 was observed for the correlation between the RA_C and the total polyphenol contents, suggesting that approximately 77% of the polyphenols in the extracts are rosmarinic acid. The rosmarinic acid content may be related to the high selectivity of the solvent used in the extraction procedure.

4. Conclusion

This work confirms the feasibility of spray drying for the preparation of standardised dried rosemary extracts. However, the selection of the correct set of drying conditions is required to guarantee the physicochemical and functional quality of the products. Results indicate that the best conditions for obtaining dry extracts of *R. officinalis* with adequate physicochemical and antioxidant properties involves an extract feed rate of 6 mL · min^{-1}, a drying air inlet temperature of 140 °C and a spray nozzle air flow rate of 50 L · min^{-1}.

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