







Article

# Spouted Bed Dried *Rosmarinus officinalis* Extract: A Novel Approach for Physicochemical Properties and Antioxidant Activity

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**Abstract:** In this study, a conical-cylindrical spouted bed dryer with Teflon<sup>®</sup> beads as spouting material was used for producing powdered rosemary (*Rosmarinus officinalis* L.) extract. The influence of the inlet drying gas temperature ( $T_{gi}$ ) and the percentage ratio between the feed rate of concentrated liquid extract by the maximum evaporation capacity of the spouted bed ( $W_s/W_{max}$ ) on selected physicochemical properties of the finished products were investigated. Antioxidant properties of the concentrated liquid extract and dried extracts were also evaluated by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH<sup>•</sup>) and lipid peroxidation induced by Fe<sup>2+</sup>/citrate (LPO) methods; and compared with the values obtained for a lyophilized extract (used as a control). Colloidal silicon dioxide (Tixosil<sup>®</sup> 333) and maltodextrin (DE 14) at a 2:1 ratio was added to the concentrated extract before drying (4.4% *w/w*) to improve the drying performance. The drying variables  $W_s/W_{max}$  and  $T_{gi}$  have statistically significant influence on total polyphenols and total flavonoid contents of the dried powders. The concentrated extract (on dry basis—being absolute solid content) showed superior antioxidant activity (AA) compared to both the spouted bed dried and the lyophilized extracts; exhibiting IC<sub>50</sub> values of  $0.96 \pm 0.02$ ,  $2.16 \pm 0.04$  and  $3.79 \pm 0.05 \mu\text{g mL}^{-1}$  (DPPH<sup>•</sup> method) and  $0.22 \pm 0.01$ ,  $1.31 \pm 0.01$  and  $2.54 \pm 0.02 \mu\text{g mL}^{-1}$  (LPO method), respectively. These results of AA are comparable to values obtained for quercetin, a flavonoid compound often used as a reference standard due to its potent antioxidant activity; with IC<sub>50</sub> of  $1.17 \mu\text{g mL}^{-1}$  (DPPH<sup>•</sup>) and  $0.22 \mu\text{g mL}^{-1}$  (LPO). However, the dried rosemary extracts are about 13.5 times more concentrated than the initial concentrated extract (dry weight), with a concentration of total flavonoids and polyphenols compounds ranging from 4.3 to 12.3 and from 1.2 to 4.7 times higher than the concentrated extract values (wet basis). The AA per dry product mass was thus significantly higher than the values measured for concentrated extractive solution, irrespective of some losses of AA apparently due to the drying process.

**Keywords:** herbal medicinal product; dried extract; spouted bed drying; antioxidant activity; *Rosmarinus officinalis*; powder properties

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## 1. Introduction

The use of herbal materials as medicines and food is as old as mankind. Plant-derived natural products are widely acclaimed to be cheap and safe, currently making them a preferred source of medicines and nutritional supplements [1–5]. These products are necessarily processed from their crude natural form to an acceptable finished product for reasons of convenience, standardization, stability enhancement, and improvement in physicochemical characteristics among other considerations. However, several factors affect the quality of herbal products, such as the climate, harvest period, and post-harvest treatments (e.g., drying and storage conditions) [6–11]. Therefore, a strict control of the steps involved in the production of a herbal product is needed to achieve consistent and appropriate level of bioactive substances, ensuring quality, efficacy and safety. The production control can be very difficult, since herbs and their preparations are a complex mixture of substances with varied physicochemical properties [8,9,12–16].

Herbal preparations are commonly marketed as a liquid, viscous preparations, or powders resulted from dried and comminuted plant materials (e.g., leaves, flowers, roots or the whole plant), or from the drying of an extractive solution. Compared to conventional liquid forms, the dried extracts show several advantages compared to liquid preparations such as lower costs of transport and storage, high concentration and stability of active substances. However, the processing conditions used during the manufacture of the dried extract affect the physicochemical properties of the product and might cause varying degrees of loss of active compounds [17].

Drying techniques, including spray drying (SD), freeze-drying, and fluidized beds, have been commonly used in the production of dry powders including herbal extracts [17–19], with spray drying being particularly used in herbal processing industries [20]. Various studies reported in literature focus on the search of alternative and innovative drying methods for herbal extracts drying, such as the spouted bed technology (SB). Spouted bed drying is considered a cheap, efficient, and reproducible method for drying herbal extracts, capable to produce high-quality powdered products suitable for pharmaceutical/nutraceutical applications [17,21,22]. A description of the fundamentals of the spouted beds and their application in drying can be found elsewhere [23,24].

Polyphenols are an important class of plant metabolites, which attract high interest linked to their postulated health protecting properties, in particular their antioxidative activity, attributed to the ability to scavenge free radicals and/or to prevent oxidation of low-density-lipoprotein [8,9,25–27]. However, adequate intakes and absorption rate of polyphenols would be recommended to ensure any beneficial outcome [28].

*Rosmarinus officinalis* L. (rosemary) is a common aromatic plant grown in many parts of the world, being native of the Mediterranean region. It can be used as ornamental plant, spice in cooking, preservative in food, and medicinal plant. The profile of rosemary bioactive compounds includes principally phenolic compounds such as caffeic acid, chlorogenic acid, carnosic acid, carnosol, rosmarinic acid, and ursolic acid found in the extract; and constituents identified in the essential oil such as  $\alpha$ -pinene, oleanolic acid, camphor, eucalyptol, rosmadial, rosmanol, rosmaquinones A and B, secohinokio, and derivatives of eugenol and luteolin [29,30].

Rosemary extracts are commercially available in Europe and USA for use as natural antioxidants in the food industry. They have received Generally Recognized as Safe (GRAS) status from the US Food and Drug Administration. The antioxidant activity of rosemary is linked to the high content of polyphenols, such as carnosic acid, carnosol, rosmanol, epirosmanol, and methyl carnosate, but over 90% of the antioxidant profile is attributed to carnosol and carnosic acid. The development of high quality standardized dried extracts of rosemary is a highly noteworthy study.

A comparison between SD and SB for drying of rosemary extract has been previously reported [31], in which the feasibility of the process at very strict processing conditions has been demonstrated. The aim of the present work was thus to determine the effects of the SB operating variables, namely, the inlet drying gas temperature ( $T_{gi}$ ) and the percentage ratio between the feed rate of concentrated liquid extract by the maximum evaporation capacity of the spouted bed ( $W_s/W_{max}$ ), on concentration of some antioxidant compounds (flavonoids and polyphenols) and on product moisture, using statistical methods. The antioxidant activities of the concentrated liquid extract and SB dried extract were also evaluated by both DPPH• and lipid peroxidation induced by  $Fe^{2+}$ /citrate (LPO) methods; and compared with the values obtained from a lyophilized extract (used as a reference). To the best of our knowledge this is the first time that antioxidant activity of SB dried rosemary extract was determined.

## 2. Materials and Methods

### 2.1. Herbal Material and Reagents

Dried leaves of *Rosmarinus officinalis* were purchased from Oficina de Ervas Pharmacy (Ribeirão Preto, São Paulo, Brazil). The dried vegetable material was milled in a knife mill (model MA 680, Marconi, Piracicaba, São Paulo, Brazil) until all particles passed through an 800  $\mu$ m sieve. The reagents, standard materials, and drying carriers utilized in this study include ethyl acetate, methanol, isobutanol, acetone, aluminum chloride, ethanol, chloridric acid, *o*-phosphoric acid, sodium hydroxide, potassium chloride, dimethyl sulfoxide (DMSO) and sucrose obtained from Labsynth (Vinhedo, Brazil), hexamethylenetetramine, sodium tungstate, phosphomolibdic acid obtained from Vetec, (Duque de Caxias, Brazil), colloidal silicon dioxide (Tixosil® 333, Rhodia, São Paulo, Brazil), maltodextrin (Mor Rex® 1914, Corn Products, São Paulo, Brazil), dehydrated quercetin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), ammonium ferrous sulfate hexahydrate, thiobarbituric acid (TBA) and MDA (malondialdehyde tetrabutylammonium salt, purity >98%) from Sigma-Aldrich (Steinheim, Germany).

### 2.2. Experimental Procedure

#### 2.2.1. Extraction of the Bioactive Compounds from Rosemary Leaves

The extractive solution was obtained by dynamic maceration in a bench top extraction system comprising a jacketed vessel coupled to a mechanical stirring unit. The vessel was connected to a thermostatic water bath (Marconi, MA-184), which circulate water at constant temperature; thereby maintaining desired extraction temperature. As described by Souza et al. [31], the extractive solution was obtained using a hydroalcoholic solvent (70% *v/v* ethanol) at the following conditions: extraction time of 1 h; extraction temperature of 50 °C; and plant to solvent mass ratio of 0.2. The crude extractive solution was vacuum filtered at 650 mm Hg through filter paper (grade 80G) and concentrated three times in a rotary evaporator at a temperature of 50 °C under a vacuum pressure of 650 mm Hg.

#### 2.2.2. Physicochemical Characterization of the Powdered Raw Herbal Material and Extractive Solution

The powdered herbal material was characterized by determination of the mean particle diameter, moisture content, total extractable matter and total flavonoids and polyphenols content. The concentrated extract was characterized for its density (pycnometry), solids concentration, and total flavonoids and polyphenols contents.

The moisture content ( $X_p$ ) of the powdered herbal material was carried out by gravimetric analysis (using the oven method). About 2 g of herbal material weighed into a dry Petri dish was dried to constant weight in an open tray oven operated at  $102 \pm 1$  °C.  $X_p$  was determined as the percentage ratio of the difference between the wet and dried herbal material by the dried mas of herbal material (dry basis).

The total flavonoids and polyphenols contents were determined by UV-Vis spectrophotometry, using a HP 8453 spectrophotometer running the software HP Chem-Station® (Agilent Life Sciences

and Chemical Analysis, Santa Clara, CA, USA). The procedure for flavonoid quantification involves the hydrolysis of the glycosides, the extraction of the flavonoids with ethyl acetate and the color development with the addition of a solution of aluminum chloride [32,33]. The reaction between aluminum chloride ( $\text{AlCl}_3$ ) and flavonoids results in a bathochromic shift of absorption wavelength of flavonoids. In this manner, concentration of flavones and flavonols in the extractive materials can be determined spectrophotometrically, measuring the absorbance at  $\lambda_{\text{max}} = 425 \text{ nm}$ , after 30 min of the addition the  $\text{AlCl}_3$  solution. Other phenolic compounds can equally complex with  $\text{AlCl}_3$ , but show  $\lambda_{\text{max}}$  at 434 nm. Quercetin, a well-recognized flavonol, was used as reference standard; and the results expressed as quercetin equivalent (QE). The quantification of the total polyphenols content was determined using the Folin-Denis method [34]. The procedure is based on the reduction of the phosphomolybdic-phosphotungstic acid by the polyphenols in a basic medium producing a dark blue color measured spectrophotometrically at 750 nm [31]. Gallic acid, a standard polyphenolic acid, was used as the reference substance, being the results expressed as gallic acid equivalent (GAE).

The solids content (Cs) of the concentrated extract was carried out by gravimetric analysis (using the oven method). About 2 g of liquid extract weighed into a dry Petri dish was evaporated to constant weight in an open tray oven operated at  $102 \pm 1 \text{ }^\circ\text{C}$  and the solid content determined by the ratio between the dry mass by the corresponding mass of the liquid extract. Results are expressed as average of three determinations ( $\pm$ standard deviation).

### 2.2.3. Spouted Bed Drying

#### Experimental Apparatus

Powdered extract of rosemary was obtained from the extractive solution following incorporation of adjuvants denominated as drying carrier. Previous experiments by our group evaluating different proportions of the drying carriers showed that the best ratio for spouting bed drying (under the operating conditions studied in this work) was 2:1 (40:20 Tixosil<sup>®</sup>:maltodextrin) [31]. The drying carrier was added to the concentrated extractive solution at a proportion of 4.4% *w/w*. The concentrated extract plus carrier were standardized to a solids content of 11.3%. The drying runs were carried out in a stainless steel, conical-cylindrical spouted bed, constituted by a conical base, with internal angle of  $40^\circ$  and inlet orifice diameter of 33 mm, connected to a cylindrical column with diameter of 150 mm and height of 400 mm. The upper part of the equipment is constituted by another cone and a powder collecting system (cyclone). The drying gas, heated by an electric heater (total power of 5000 W), was supplied into the spouting chamber through a 7.5 hp blower. Teflon<sup>®</sup> beads having a mean diameter of 5.45 mm, density of  $2160 \text{ kg m}^{-3}$ , surface area of  $5.27 \text{ cm}^2 \text{ g}^{-1}$  and shape factor of 0.96 were used as spouting particles. For spouted bed drying of pharmaceutical products, Teflon beads are an excellent choice, due to its inert nature, thermal stability, low coefficient of friction, insolubility and lack of toxicological effects [35]. The extract feed system consists of a 0.8 mm double fluid atomizer with internal mixing (installed at the top of the fountain), a peristaltic pump and an air compressor. A temperature control system, thermocouples, and rotameter were employed in the equipment instrumentation.

#### Drying Procedure

Drying operation started with the feed (through the inlet entrance orifice), of heated drying air to the spouted bed previously loaded with Teflon<sup>®</sup> beads (static bed height of 14 cm). At the desired drying temperature, the concentrated extract incorporated with the respective drying carrier was fed at the top of the fountain of the spouting bed through the double fluid atomizer, together with the atomizing air, at a preset flow rate. Measurements of the outlet gas temperature,  $T_{\text{go}}$ , were taken at regular intervals in order to detect the moment when the dryer reach the steady state ( $\pm 15 \text{ min}$ ). Samples of the dried extract were withdrawn and used for determination of its physicochemical properties and AA. The studied process parameters were the inlet drying gas temperature,  $T_{\text{gi}}$  (80 and

150 °C); and the percentage ratio of the mass feed flow rate of the concentrated extract to the evaporation capacity of the dryer,  $W_s/W_{max}$  (15, 45 and 75%) (Table 1). Study on the evaporation capacity for this dryer have been reported elsewhere [31]. The mass flowrate of the drying gas was maintained at  $0.0340 \text{ kg s}^{-1}$ , corresponding to 1.4 times the minimum spouting mass flowrate ( $Q_{ms}$ ). The atomizing air feed flow rate was fixed at  $20 \text{ L min}^{-1}$  at a pressure of 196.1 kPa.

**Table 1.** Spouted bed drying parameters used in manufacture of the dried extract.

$T_{gi}$ (°C)	$W_s/W_{max}$ (%)	$W_s$ (g $\text{min}^{-1}$ )
80	15	6.0
80	45	18.0
80	75	30.0
150	15	10.0
150	45	33.0
150	75	49.0

$T_{gi}$ , inlet drying gas temperature;  $W_s/W_{max}$ , the percentage ratio of the mass feed flow rate of the concentrated extract to the evaporation capacity of the dryer;  $W_s$ , mass feed flow rate of the concentrated extract (g  $\text{min}^{-1}$ ).

A portion of the crude concentrated extract was lyophilized and the product used as a reference sample, since dehydration occurred at a low temperature. The lyophilization was carried out in a Thermo Fisher Scientific freeze dryer model SNL 108 (Waltham, MA, USA) containing a Micromodulyo 1.5 L freeze-drying unit ( $305 \times 330 \times 432 \text{ mm}$ ), stainless steel condenser; 1/4 hp compressor and 0.30 kw power; ultra-vacuum pump VLP 195 FD-115 and freeze-drying vials with independent valves. No adjuvant was added to the concentrated extract prior to dehydration.

#### 2.2.4. Physicochemical Characterization of Dried Rosemary Extract

The physicochemical characterization of the spouted bed dried rosemary extract was carried-out by measurement of product moisture content, total flavonoids, total polyphenols contents, extract bulk density ( $\rho_b$ ); tapped density ( $\rho_{1250}$ ), and the flow and compressibility parameters; Hausner ratio ( $I_{Hausner} = \rho_{1250}/\rho_b$ ) and Carr index ( $I_{Carr} = (\rho_{1250} - \rho_b)/\rho_b$ ), and product morphology.

Moisture content, total flavonoids and polyphenols contents were determined by the methods described in Section 2.2.2.

The bulk density ( $\rho_b$ ) was obtained as the ratio of the mass of a powder sample to the volume occupied by the same sample when placed freely in a measuring cylinder without any compaction; while the tapped density ( $\rho_{1250}$ ) was determined by a similar ratio using the volume occupied by the powder after the cylinder was tapped 1250 times through a distance of 3 cm using a tapped density tester mod. TDT 22 (Caleva, Frankfurt, Germany). Determinations were carried out in triplicates [36].

The product morphology was assessed from photomicrographs of the spouted bed dried extracts acquired in a scanning electronic microscope (SEM) with magnification of 1000 $\times$ . Samples were placed on an aluminum foil placed on a carbon tube. An auto fine coater was used to coat the samples by sputtering with platinum and analyzed on a Quanta 200-ESEM system (FEI, Eindhoven, The Netherlands) using gaseous secondary electron detector holding the chamber at 27 °C and imaging at 20 kV and 600 Pa.

#### 2.2.5. Antioxidant Activity of the Rosemary Extracts

The DPPH $\bullet$  scavenging and the lipid peroxidation assays were the methods selected to estimate the antioxidant activity of the concentrated extract, lyophilized extract and the spouted bed dried rosemary extract.

##### DPPH $\bullet$ Radical Scavenging Assay

Preliminary tests were carried out to determine concentration ranges of the concentrated extract and the dehydrated test samples required for the DPPH $\bullet$  assay. Ten  $\mu\text{L}$  of the concentrated extract at

various dilutions (1:10; 1:20; 1:50; 1:100 and 1:200) and of the dried rosemary extract (0.375–6.25 mg mL<sup>-1</sup>) in ethanol were added to the reaction mixture containing 1 mL of 0.1 M acetate buffer pH 5.5, 1 mL of ethanol, and 0.5 mL of 250 µM of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) in ethanolic solution. The concentrations of the concentrated extract and dried rosemary extracts in the reaction medium ranged from 0.15 to 2.93 µg mL<sup>-1</sup> and from 0.94 to 15.6 µg mL<sup>-1</sup> (dry basis), respectively. The change in absorbance was measured after 10 min at room temperature. The hydrogen donating ability of the dried extracts of rosemary to DPPH• was determined from the change in absorbance at 517 nm using the spectrophotometer HP 8453 running the HP Chem-Station® software [37]. All measurements were made in triplicate. The same test was also performed for the flavonoid quercetin (positive control), being a reference antioxidant substance, which present a dose-dependent response [38–40].

### Lipid Peroxidation Method

The lipid peroxidation (microsomal lipid peroxidation induced by Fe<sup>+2</sup>/citrate) was assayed by malondialdehyde generation [41] in the presence of different concentrations (determined in a preliminary experiment) of rosemary extracts. Mitochondria was prepared by standard differential centrifugation techniques as described by Rodrigues et al. [41]. The protein content was determined by the biuret reaction [42]. 10 µL of the concentrated extract at various dilutions (1:10 to 1:200) and of the dried rosemary extract at different concentrations (12.5 to 300 µg mL<sup>-1</sup> in DMSO) were added to 1.0 mL of a reaction mixture (125 mmol L<sup>-1</sup> sucrose, 65 mmol L<sup>-1</sup> KCl and 10 mmol L<sup>-1</sup> Tris-HCl, pH 7.4—medium I). Mitochondria was added to yield a final concentration of 1 mg of protein plus 50 µM (NH<sub>4</sub>)<sub>2</sub>Fe(SO)<sub>4</sub> and 2 mM sodium citrate for 30 min at 37 °C. The solids concentrations of the extractive solution and of dried extract in the reaction medium ranged from 0.15 to 6.25 µg mL<sup>-1</sup> and from 0.38 to 6.25 µg mL<sup>-1</sup>, respectively. 1 mL of 1% thiobarbituric acid (TBA) (prepared in 50 mM NaOH), 0.1 mL of 10 M NaOH and 0.5 mL of 20% H<sub>3</sub>PO<sub>4</sub> were added to the reaction medium, followed by incubation for 20 min at 85 °C. Malondialdehyde-thiobarbituric acid (MDA-TBA) complex was extracted with 2 mL of isobutanol. The samples were then centrifuged at 1660× g for 10 min. The measurement was performed on the supernatant at 535 nm using a UV-Vis spectrophotometer. Two controls were used for this test, a positive control (without the samples) and a negative control (without iron). The blank was prepared in the same way as the reaction mixture, but without the mitochondria. All measurements were made in triplicate.

### 2.3. Experimental Design

The drying runs were conducted according to two-factors and three-levels (2 × 3) experimental design, aiming to determine the effects of the processing drying variables (factors)  $W_s/W_{max}$  and  $T_{gi}$  on total flavonoids and polyphenols contents, and on product moisture ( $X_p$ ). The experimental data was submitted to an analysis of variance (ANOVA) test to detect the factors effects with statistical significance on the experimental responses ( $\alpha = 0.05$ ). Response surfaces were plotted using the Statistica 13.5 software (TIBCOTM, Palo Alto, CA, USA), to show the impact of the studied factors on the desired outcomes.

## 3. Results and Discussion

### 3.1. Physicochemical Characterization of the Powdered Herbal Material and of Extractive Solution

The vegetable material was milled to increase the surface area for greater solvent contact, thereby enhancing the extraction efficiency. The milling process resulted in powdered leaves with mean particle diameter of 0.3 mm (sieve), considered as moderately fine. The starting vegetable material showed moisture content of  $10.4 \pm 0.2$  (% w/w), extractable matter of  $22.2 \pm 0.01$  (% w/w), and total flavonoids and polyphenols contents of  $3.32 \pm 0.02$  (mg.QE g<sup>-1</sup>—dry basis), and  $31.2 \pm 0.31$  (mg.GAE g<sup>-1</sup>—dry basis).

The extraction of the bioactive compounds from the powdered leaves of *Rosmarinus officinalis* was carried in an extraction system at a controlled temperature of 50 °C, adequate for extraction but

not to cause substantial degradation of the extracted bioactive compounds. Hydroalcoholic solvent was used for the extraction process to facilitate the extraction of the polar and non-polar substances with ethanol preferred due to its relatively low toxicity profile. The extractive solution obtained containing about 2.8% (*w/w*) of solids, was concentrated approximately 2.6 times by rotary evaporation of the solvent, thus increasing the solids content prior to spouted bed drying. The concentrated extract had a density of  $0.98 \pm 0.01$  ( $\text{g cm}^{-3}$ ), a solids content of  $7.32 \pm 0.06$  (% *w/w*), total flavonoid contents of  $11.87 \pm 0.02$  ( $\text{mg.QE g}^{-1}$ —dry basis) and a total polyphenols concentration of  $146.07 \pm 0.23$  ( $\text{mg.GAE g}^{-1}$ —dry basis).

### 3.2. Spouted Bed Drying and Physicochemical Characterization of the Dried Rosemary Extracts

Although spouted bed driers have been previously employed to process vegetable drug materials and phytopharmaceuticals [31], the initial attempts at drying the concentrated rosemary extracts were challenging. High amounts of the atomized feed material adhered to the surfaces of the inert material and the dryer wall, resulting in dryer collapse within a short processing time. In an attempt to solve this problem, drying carriers were added to the concentrated extract before the drying procedure [31]. A mixture of colloidal silicon dioxide (Tixosil 333®) and maltodextrin DE 14 at proportion of 40:20 (2:1) relative to the total solids content improved considerably the spouted bed dryer performance; with respect to atomization of the feed, adherence of extract on the inert material and dryer wall.

Table 2 shows the experimental results of total flavonoids and total polyphenols contents of the spouted bed dried extracts obtained at various processing conditions, together with the moisture content ( $X_p$ ).

**Table 2.** Total flavonoids and total polyphenols contents of the spouted bed dried extracts.

$T_{gi}$ ( $^{\circ}\text{C}$ )	$W_s/W_{max}$ (%)	$T_F$ ( $\text{mg.QE g}^{-1}$ ) *	$T_P$ ( $\text{mg.GAE g}^{-1}$ ) *	$X_p$ (% <i>w/w</i> )
80	15	$6.4 \pm 0.05$	$13.1 \pm 0.98$	$13.7 \pm 1.12$
80	45	$5.6 \pm 0.03$	$25.0 \pm 1.87$	$8.7 \pm 0.87$
80	75	$4.7 \pm 0.04$	$36.6 \pm 2.11$	$13.9 \pm 0.95$
150	15	$5.0 \pm 0.04$	$32.4 \pm 1.13$	$2.0 \pm 0.05$
150	45	$3.8 \pm 0.05$	$48.6 \pm 2.05$	$7.9 \pm 0.55$
150	75	$3.7 \pm 0.01$	$49.9 \pm 1.29$	$11.1 \pm 1.03$

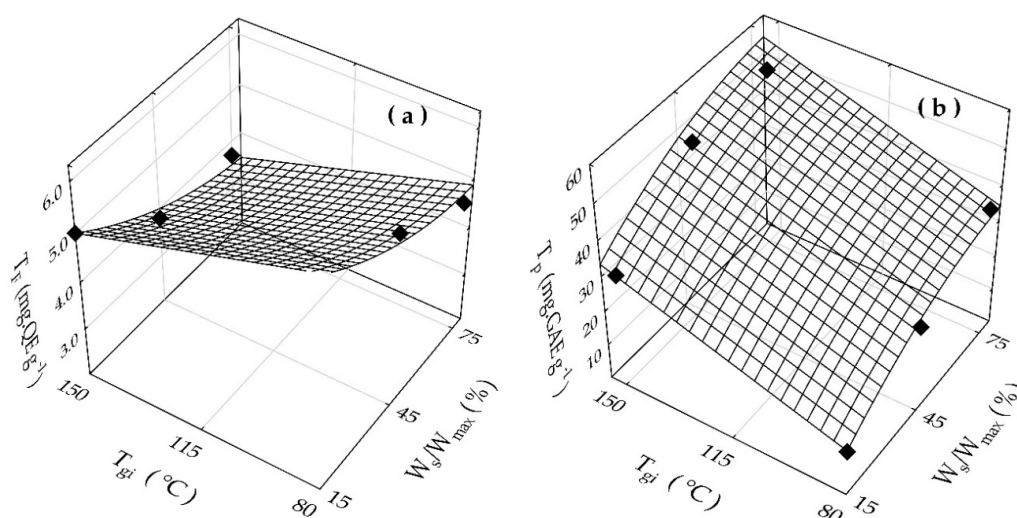
$T_{gi}$ , inlet drying gas temperature;  $W_s/W_{max}$ , the ratio of the mass feed flow rate of the concentrated extract to the evaporation capacity of the dryer; \* dry basis.

The experimental data of  $T_F$ ,  $T_P$ , and  $X_p$  were subjected to analysis of variance (ANOVA) to detect any significant effects of the processing variables on the responses. The sum of the interaction effects between the variables were used as an estimate of the experimental error. ANOVA results reveal that the linear effects of the factors  $T_{gi}$  and  $W_s/W_{max}$  exerts statistically significant influence on both  $T_F$  ( $p$ -values of 0.026 and 0.033) and  $T_P$  ( $p$ -values of 0.026 and 0.033), with a  $F_{calc}$  at least 1.52 times higher the critical value for an  $\alpha = 0.05$  ( $F_{1,2-0.05} = 18.513$ ). On the other hand, the ANOVA results for  $X_p$  did not show statistical significance of the investigated factors, even using an  $\alpha = 0.10$ , with the maximum  $F_{calc}$  value (obtained for the linear effect of  $T_{gi}$ ), at least 3.7 times lower than the critical tabled value ( $F_{1,2-0.10} = 8.526$ ). Figure 1a,b present the response surface plots showing the effects of drying variables  $W_s/W_{max}$  and  $T_{gi}$  on total flavonoids and total polyphenols contents in the dried rosemary extracts, respectively. It can be seen from Figure 1a that increases in  $W_s/W_{max}$  and  $T_{gi}$  exert negative effects on  $T_F$ . The decrease observed for  $T_F$  might be linked to the thermal degradation of flavonoids, for example due to the occurrence of oxidative reactions or decomposition of thermolabile compounds, increasing concentration of other phenolic substances. The extent of degradation varies from compound to compound and is dependent on the dehydration method [43]. Flavones (e.g., luteolin) and flavonols (e.g., quercetin) concentration have been shown to reduce by up to 50% within 15 min of exposure to heat above 130  $^{\circ}\text{C}$  [44]. This behaviour could explain the corresponding increase of  $T_P$  as values of study variables increase (Figure 1b). For example, thermal degradation of quercetin resulted in

accumulation of protocatechuic acid, a phenolic compound [45]. Such compounds might be detected by the quantification reaction of the Folin-Denis method [34], which measures total phenolics substances. The phenolic compounds comprise a large number of organic molecules with heterogeneous structure, including the flavonoids. These substances present high reactivity to  $O_2$  and have an important role as antioxidants [46].

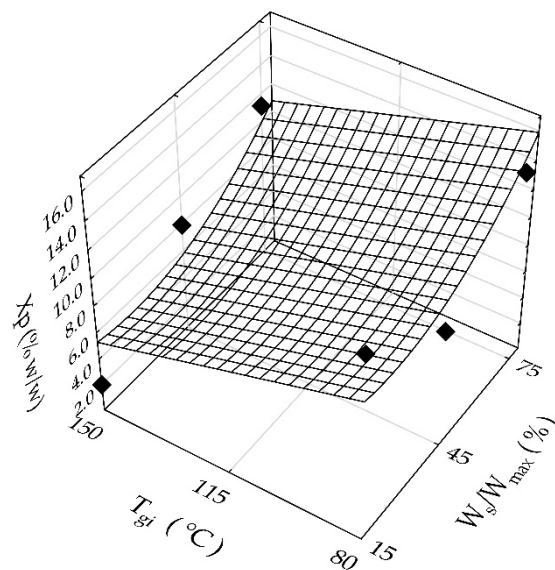
The moisture content of biological dried materials and the water activity are important quality parameters, since they are linked to product stability. The physicochemical and microbiological stability tended to increase conversely with the water content (linked to water activity) of the dried products. The decrease in product moisture content implies a reduction on water activity; although there is a minimum value given by the monolayer moisture content. Below this value, the water is strongly bonded to material structure; reducing the rate of degradation reactions and hindering microbial growth. The monolayer moisture content is usually considered the lowest value to which food and agricultural material can be dried to ensure product stability [47,48]. Although ANOVA did not evidence any statistically significant effects of the drying variables on  $X_p$  ( $F_{calc} < F_{1,2-0.10}$ ), the data presented in Table 2, give evidences that the increase of the inlet gas temperature and/or reduction in the feed flow rate of the concentrated extract tends to reduce the product moisture content; as can be seen qualitatively on Figure 2. For dried extracts of medicinal plants, the Brazilian Pharmacopoeia recommends maximum moisture content of 4%. From Table 2, it can be seen that only the dried extracts produced at  $T_{gi} = 150\text{ }^\circ\text{C}$  and  $W_s/W_{max} = 15\%$  falls in the recommended range. The powder production at this drying condition provided the higher powder production rate (yield  $\approx 65\%$ ), perhaps due to the decrease of the adhesion tendency of the powder product to equipment wall; a positive effect of its low moisture content. Since the biological activities of rosemary extract is attributable to several phenolic constituents rather than a singular compound, it is appropriate that simultaneous retention of these bioactive principles is put in perspective in order to set the best processing conditions.

The characterization of the flow properties of the spouted bed dried extracts were carried out through the determination of the loosely packed bulk density,  $\rho_b$ , tapped bulk density,  $\rho_t$ , the real density,  $\rho_r$ , Carr's index, and the Hausner ratio. These properties have been shown to have direct relationship with the behavior of the product during storage, manipulation and industrial processing. The spouted bed dried rosemary extracts has an Hausner ratio  $\approx 1.81$  and compressibility index  $\approx 45\%$ ; mean loosely packed bulk density around  $430 \pm 10\text{ kg m}^{-3}$  and tapped bulk density  $\approx 780 \pm 20$ , being the true density, measured with a helium pycnometer, equal to  $1493.7 \pm 2.9\text{ kg m}^{-3}$  [31]. These results are indicative of powders with poor flow and compression properties.



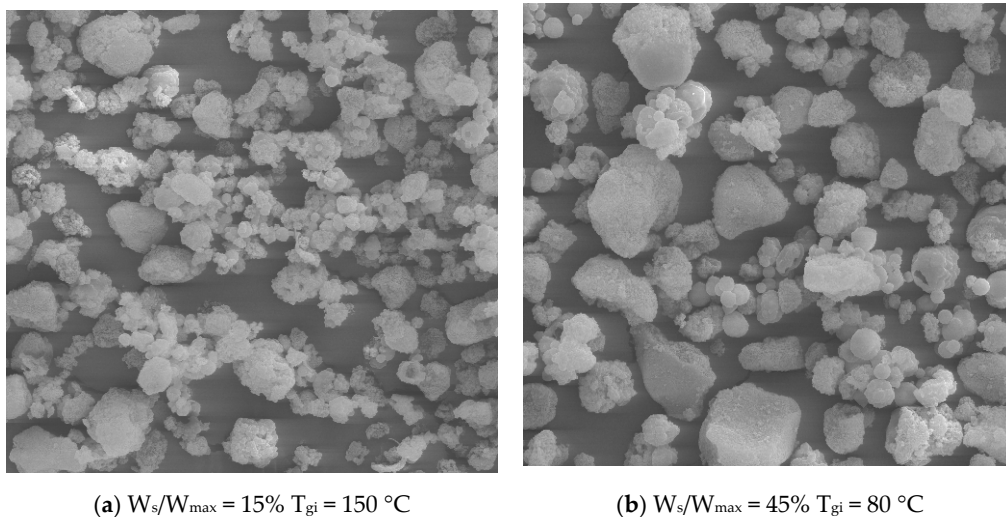
**Figure 1.** Response surface plots showing the effects of drying variables  $W_s/W_{max}$  and  $T_{gi}$  on total flavonoids (a), and total polyphenols content in the dried rosemary extract (b).





**Figure 2.** Qualitative surface plot showing the effects of drying variables  $W_s/W_{max}$  and  $T_{gi}$  on product moisture content ( $X_p$ ) of the dried rosemary extract.

Figure 3 shows photomicrographs obtained by SEM at two distinct processing conditions; showing the presence of irregular to rounded powder particles, with polydisperse characteristics. Figure 3b corresponds to the product obtained at high  $W_s/W_{max}$  (45%) and low  $T_{gi}$  (80 °C), which shows  $X_p$  almost 4.4 times superior to the value measured for the product of Figure 3a (8.7 vs. 2.0%  $w/w$ —see Table 2). The bigger particles in Figure 3b might be caused by the increase in atomized droplets size and on  $X_p$  by the high feed flowrate of rosemary extract to the spouted bed [49], and lower drying temperature.



**Figure 3.** Typical SEM photomicrographs (magnification of 1000 $\times$ ) of spouted bed dried rosemary extracts produced at distinct operating conditions: (a) at  $W_s/W_{max}$  (15%) and  $T_{gi}$  (150 °C), (b) at  $W_s/W_{max}$  (45%) and  $T_{gi}$  (80 °C) (see Table 2).

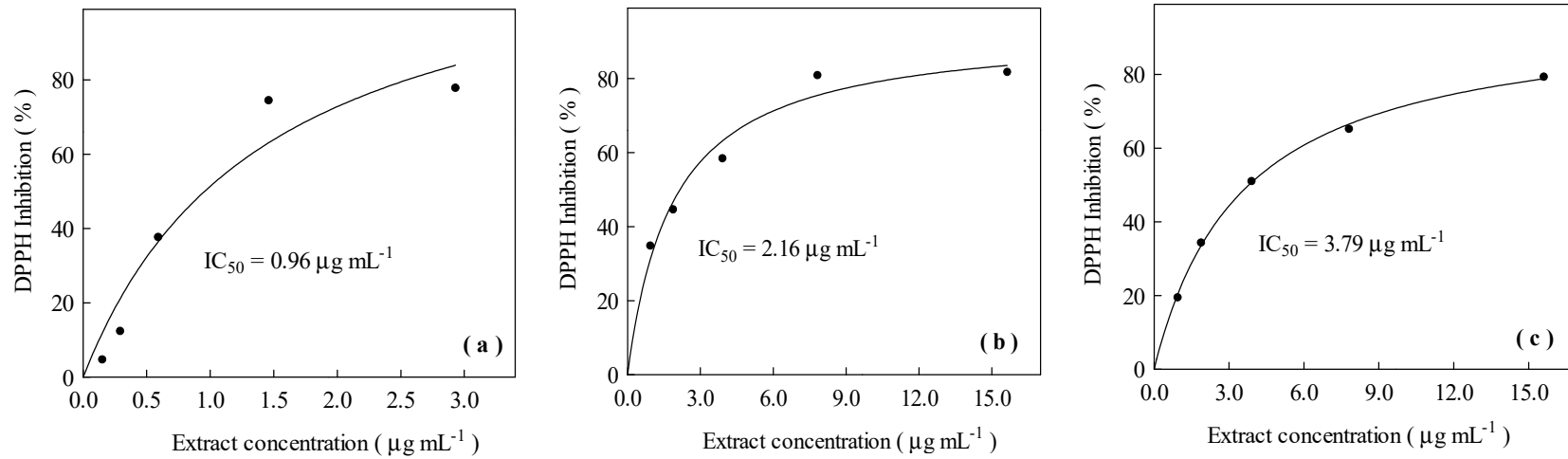
### 3.3. Antioxidant Activity of the Concentrated and Dried Rosemary Extracts

Deleterious free-radical reactions have been linked to a variety of degenerative pathological conditions including cancer, autoimmune disorders, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [50–53]. This effect has been described as a consequence of the

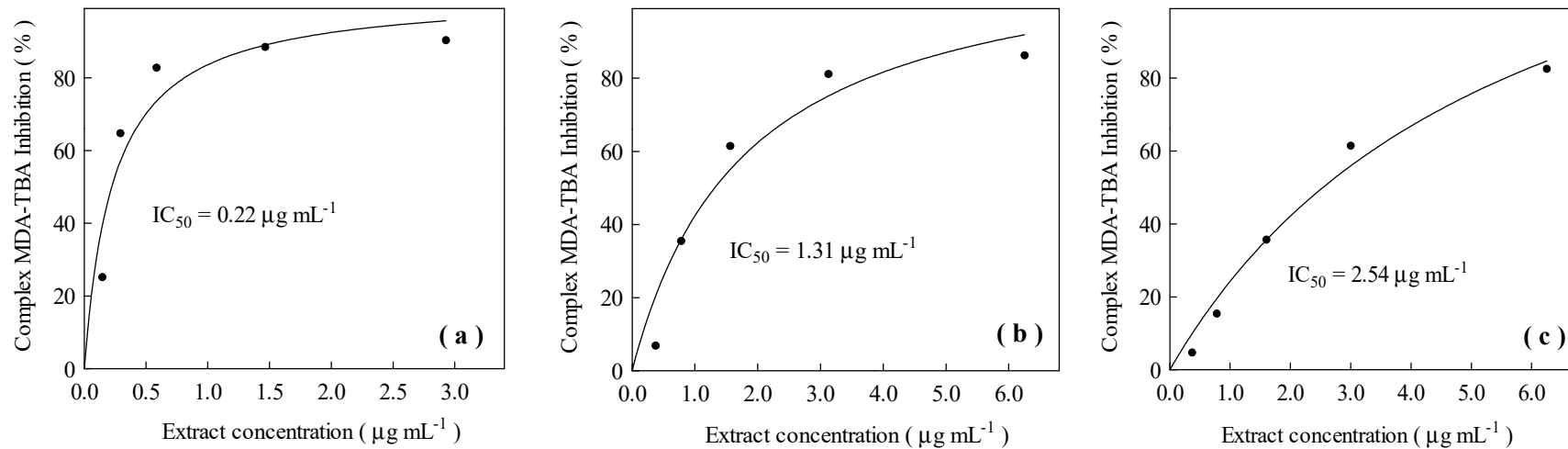
involvement of reactive oxygen species in initiating autoxidation of cellular lipid membranes, which are mainly composed of polyunsaturated fatty acids, leading to cellular necrosis [54,55]. There is no single method that alone can evaluate the antioxidant activity accurately and quantitatively, since oxidative processes involve multiple active species, reaction characteristics, and mechanisms [56]. The literature recommends the simultaneous use of several methods based on different mechanisms of the inhibition of oxidation to examine the antioxidant capacity of a given sample. In this study, the evaluation of the antioxidant activity was carried out for the concentrated extract, SB dried extract obtained at  $W_s/W_{max}$  of 15% and  $T_{ig}$  of 150 °C (which presents good  $T_F$  and  $T_P$  values, and has moisture content below the maximum value recommended by the Brazilian pharmacopoeia), and for a sample of lyophilized extract (used as a control). The antioxidant activity was determined by two distinct methodologies, namely: DPPH• scavenging (DPPH•) and the inhibition of the lipid peroxidation (LPO) methods. Figure 4a–c shows the results of DPPH• inhibition produced by the concentrated extractive solution, spouted bed dried, and lyophilized extracts, respectively (dry basis). It can be seen from the Figures that DPPH• inhibition exhibits a dose dependent behavior regardless of the sample analysed. The concentrated extract exhibited an  $IC_{50} = 0.96 \pm 0.02 \mu\text{g mL}^{-1}$  (dry-basis), with a maximum DPPH• inhibition of approximately  $78 \pm 1.6\%$  at concentration of  $2.93 \pm \mu\text{g mL}^{-1}$ , being the steady state obtained thereafter (Figure 4a). Figure 4b,c show a maximum DPPH• inhibitions of  $79 \pm 1.1$  and  $81 \pm 1.3\%$ , respectively for the spouted bed dried and the lyophilized extracts. These results were reached at concentrations of  $15.6 \pm 0.2 \mu\text{g mL}^{-1}$  of the dried extract sample; with  $IC_{50}$  values of  $2.16 \pm 0.04$  and  $3.79 \pm 0.03 \mu\text{g mL}^{-1}$ , respectively.

The AA of the spouted bed dried extract determined by this method was lower than the one measured for the concentrated extract (in dry basis), but higher than the value determined for the lyophilized extract, used as a control. Nevertheless, considering that the spouted bed dried extract has in its composition 60% of drying carriers, the concentration of active constituents of the extract is about 62.5% diluted in relation to those present in the concentrated extract. Performing this correction, it can be seen that the loss of AA due to drying is less than 30%. Tests (control) performed with synthetic quercetin, a flavonoid with potent antioxidant properties, resulted in an  $IC_{50}$  of  $1.17 \mu\text{g mL}^{-1}$  [57]. The rosemary extracts caused instantaneous decrease in the absorbance of DPPH•, in a manner similar to that presented by quercetin, which supports its AA.

The effects of the rosemary extracts on lipid peroxidation induced by  $Fe^{+2}$ /citrate were accessed by the production of MDA-TBA complex (absorbance reading at 535 nm). Figure 5a–c show, respectively, the experimental results of the inhibition of lipid peroxidation obtained for the concentrated extract, spouted bed dried, and lyophilized rosemary extracts, respectively (dry basis). It can be seen that the inhibition of the LPO activity produced by the rosemary extracts samples were dose dependent, the same behavior observed with the DPPH• method. The maximum inhibition falls between 82% and 86%. The  $IC_{50}$  values obtained for the concentrated extract (a), spouted bed dried rosemary extract (b), and for the lyophilized sample (c) were, respectively,  $0.22 \pm 0.01$ ,  $1.31 \pm 0.02$  ( $0.82 \pm 0.01$  with dilution correction), and  $2.54 \pm 0.02 \mu\text{g mL}^{-1}$  in the reaction medium; while the flavonoid quercetin showed an  $IC_{50}$  of  $0.22 \mu\text{g mL}^{-1}$  [58]. The significant AA exhibited by the rosemary extracts samples by DPPH• and LPO assays are in agreement with studies reported in the literature [59].  $IC_{50}$  values reported for concentrated extract and SB extracts of *Bauhinia forficata* L. were 15.2 and 12.2  $\mu\text{g/mL}$  (DPPH• assay) [60], and 22.5 and 25.9  $\mu\text{g/mL}$  for the LPO method; values significantly higher than the values here reported. The maximum inhibition of DPPH exhibited by the actual spouted bed dried rosemary extract was also superior to those reported for SB dried *Bauhinia forficata* extract ( $79\% \times 69\%$ ), and ( $86.3\% \times 80.3\%$ ). Notwithstanding, these values were reached at significantly lower concentrations for the rosemary SB dried extract compared to *Bauhinia forficata*. Altogether, these results evidenced a higher AA for the SB dried rosemary extract compared to *B. forficata* dried extract [60].



**Figure 4.** Experimental results of the DPPH• inhibition produced by the concentrated extract (a), spouted bed dried extract (b), and lyophilized extract (c).



**Figure 5.** Experimental results of the inhibition of the lipid peroxidation induced by Fe<sup>+2</sup>/citrate promoted by the concentrated extract (a), spouted bed dried extract (b), and lyophilized extract (c).

The results of AA of the rosemary extracts assessed by DPPH<sup>••</sup> and LPO assays evidenced a slight to moderate decrease in the AA of the rosemary extracts caused by the SB drying operation, apparently due to degradation of bioactive compounds on exposure to the processing conditions used in this study; mainly heated air. It is known that polyphenolic compounds are thermolabile and their efficacy may be compromised on exposure to heat [61]. However, the SB dried rosemary extracts here produced are near 13.5 times more concentrated than the concentrated extract (in dry weight); and have high concentration of bioactive compounds by extract mass (in wet basis). A simple calculation with the experimental results of  $T_F$  and  $T_p$  presented in Table 2, shows that the concentration of total flavonoids and polyphenols compounds presented in the spouted bed dried extract ranges from 4.3 to 12.3 and from 1.2 to 4.7 times the values in the concentrated extract, respectively. Therefore, even considering the losses of bioactive compounds and of AA caused by the drying, the SB dried extracts have advantages compared to the concentrated extract, regarding the concentration bioactive compounds, lower storage volume, and perhaps higher stability.

#### 4. Conclusions

The results here reported indicate that the production of dried extract of *Rosmarinus officinalis* by the spouted bed technology is feasible. The processing conditions should be strictly controlled in order to ensure the product quality with an acceptable dryer performance. The use of experimental design could facilitate the identification of the significant variables and allow the optimization of the processing variables. The drying variables  $W_s/W_{max}$  and  $T_{gi}$  exert statistically significant effects on the content of bioactive compounds in the dried rosemary extract. The antioxidative properties of rosemary extracts were evaluated by both DPPH<sup>\*</sup> and lipid peroxidation methods. The rosemary extracts caused instantaneous decrease in the absorbance of DPPH<sup>\*</sup> (in a manner similar to that presented by quercetin) and were able to inhibit the lipid peroxidation induced by Fe<sup>+2</sup>/citrate, indicating their high antioxidant profile. The drying operation caused a slight to moderate decrease in the antioxidant activity of the spouted dried rosemary extracts; but the spouted dried rosemary extracts have higher concentration of bioactive compounds by extract mass (in wet basis) compared to concentrated extract; ranging from 4.3 to 12.3 and from 1.2 to 4.7 times, respectively for  $T_F$  and  $T_p$ . The spouted bed dried extracts showed powerful antioxidant activity and have potential to be used as a natural antioxidant or phytoactive ingredient in food, cosmetic and pharmaceutical applications. Further work still needs to be carried out in order increase the production yield and to improve product properties to respond to the specifications from the pharmaceutical regulatory bodies.

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