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Comparative evaluation of phenolics' profile and recovery in spray dried powders obtained from rosemary and oregano extracts in relation to their antibacterial activity *in vitro*

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Abstract

Spray dried powders were obtained from Rosmarinus officinalis (rosemary) and Origanum vulgare (oregano) leaves after cold and hot-temperature extraction followed by immobilization on maltodextrin (10 and 15%) at different inlet temperatures (120 and 145°C). The operating parameters and drying yields were optimized and correlated with the powder rheological properties (bulk and tapped density, Hausner ratio, Carr index, wettability). The total phenolic content (TPC) and the total flavonoid content (TFC) were determined to evaluate which type of extraction gives powders with an increased phenolic and flavonoid composition. The antibacterial activity in vitro (on agar plates, by disc diffusion method) was screened for each powder, against four different Gram positive and Gram negative bacteria. Generally, all powders obtained from hot extracts were richer in flavonoids than phenolic acids. The oregano powders showed stronger inhibition of Gram positive bacteria and greater sensitivity than Gram negative bacteria. B. cereus presented the greatest sensitivity from all the tested strains, oregano powders being generally, more efficient. Meanwhile, S. typhimurium showed greater sensitivity to cold rosemary powders. Both rosemary and oregano spray dried, phenolic-rich powders may function as potential biodesinfectants or phyto-pharmaceutical ingredients for herbal medicines.

Keywords: rosemary, oregano, phenolics, spray drying powders, antibacterial screening

1. Introduction

Rosmarinus officinalis (rosemary) and *Origanum vulgare* (Greek oregano) are cultivated worldwide for their aromatic taste, for their utility as flavoring spices, having antioxidant and preservative properties. Meanwhile, rosemary and oregano are considered medicinal plants due to their complex composition in bioactive compounds, known to act as antioxidants, antimicrobial, hepatoprotective, antidiabetic and anticancer agents, to mention only a part of properties which were scientifically proven. AL-SEREITI & al. [1] described the pharmacology of rosemary (*Rosmarinus officinalis Linn*.) and its therapeutic potential; recently, the European Medicines Agency: Community herbal monograph on *Rosmarinus officinalis L., aetheroleum* [2] described the characteristics of *Rosmarinus officinalis*, while VALLVERDU-QUERALT & al. [3], STAMENIC & al. [4] and MARTINS & al. [5]

reviewed largely the composition and applications of *Origanum vulgare*. The specific flavors of oregano and rosemary are due to volatile oils having antibacterial potential (VAUZOUR & al. [6], YÁÑEZ & al. [7], BASSOLÉ & al. [8]), but also their aroma is given by a complex composition in phenolics (mainly flavonoids), diterpenes (BICCHI & al. [9]), coumarins, stilbenoids, and lignans (CEYLAN & al. [10]). Former studies confirmed the antibacterial effect of flavonoids (CUSHNIE & al. [11]) and phenolic acids (CUEVA & al. [12]) originating from aromatic herbs. Flavonoids in *Rosmarinus officinalis* leaves were characterized by OKAMURA & al. [13], HO & al. [14] and recently by BORRÁS-LINARES & al. [15]. Flavonoid distribution during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis* and the biosynthetic pathways were studied by DEL BANO & al. [16] and BAI & al. [17]. The phytochemical profile of *Rosmarinus officinalis*, the correlations with their antioxidant and anti-proliferative activity were reported recently by KONTOGIANNI & al. [18], while the phenolic antioxidants from clonal oregano (*Origanum vulgare*) with specific antimicrobial activity against *Helicobacter pylori* by CHUN & al. [19].

Different methods to extract bioactive phenolics and diterpenes were applied for rosemary and oregano, from conventional extracts in hydroalcoholic solutions, to water infusion, using ultrasounds, microwaves or supercritical liquids. The development of a microwave-assisted extraction for the analysis of phenolic compounds from *Rosmarinus officinalis* was recently reported by ŠVARC-GAJIC & al. [20]. OKOH & al. [21] made a comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydro-distillation and solvent free microwave extraction methods. ZHANG & al. [22] using HPLC, followed the stability and degradation of isolated carnosic acid, carnosol, rosmarinic acid by comparison with the rosemary extract (*Rosmarinus officinalis* L.). Green processes for the extraction of bioactive molecules from rosemary were recently applied; the chemical and functional characterization being made by ultra-performance liquid chromatography-tandem mass spectrometry and *in vitro* assays (HERRERO & al. [23]).

The investigation of novel antimicrobial agents is of great scientific interest, considering the growing resistance of microorganisms (CEYLAN & al. [10]). Recent advances in understanding the antibacterial properties of flavonoids and phenolic acids against commensal, probiotic and pathogenic bacteria were reported (CUSHNIE & al. [11], CUEVA & al. [12]); anti-biofilm formation and anti-adhesive (to Hep-2 cells) effects of rosemary water extract against some food-related pathogens were developed and further studied by ELHARIRY & al. [24]. The antimicrobial activity of aromatic plants such as rosemary and oregano was mainly attributed to their essential oils having bacteriostatic and antifungal effects. Numerous *in vitro, in vivo,* and clinical studies are confirming that many essential oils exert antimicrobial activity against different strains of Gram-positive and Gram negative bacteria and fungi (CEYLAN & al. [10], BOZIN & al. [25], DEL CAMPO & al. [26]).

Antimicrobial activity of carnosol and ursolic acid, two antioxidant constituents of *Rosmarinus officinalis* L. was tested by COLLINS & al. [27]. The antioxidant and antibacterial properties of oregano extracts obtained by fractional supercritical fluid extraction (SFE) with carbon dioxide were recently investigated and compared with the properties of essential oil obtained by hydrodistillation STAMENIC & al. [4]. Such extract showed strong antibacterial activity against staphylococci, but did not affect *Escherichia coli* of normal intestinal flora, while the essential oil showed stronger antibacterial activity against

E. coli, *Salmonella* and *Klebsiella pneumoniae*, comparing to the supercritical extracts STAMENIC & al. [4]. If the bioactivity of oregano methanolic extracts and essential oils is well known, reports about aqueous extracts are scarce, since few were published about the composition of infusion, decoction and hydroalcoholic extracts MARTINS & al. [5], avoiding the toxic effects of essential oils.

In the last two decades, the application of microencapsulation as advanced spray drying technology is increasing, for immobilizing bioactive molecules from aromatic herbs or fruits. Spray drying represents one of the most efficient encapsulating techniques based on extract's drying on the surface of stable matrices, which provides a protective barrier against degradation (by oxidation, humidity) and a better diffusion control (and controlled release) assuring stable concentrations for encapsulated bioactives (PONCELET & al. [28], RÉ & al. [29]). The main encapsulation matrices, used in spray drying are proteins (lactose), gums (alginate, acacia gum) or carbohydrates like cellulose, maltodextrins or modified starch (FUCHS & al. [30], DESCAMPS & al. [31], FANG & al. [32]). The drying operating conditions are determinant parameters for the final product quality and represent a challenging task. Some literature data reports that different procedures are used to encapsulate volatile oils from rosemary and oregano compared to alcoholic extracts, by spray drying (BARANAUSKIENE & al. [33], COUTO & al. [34]).

Recently, a technique to encapsulate rosemary essential oil by spray drying using whey protein-inulin blends as carriers was reported by DE BARROS & al. [35], but meanwhile, data on encapsulating aqueous extracts is scarce. Also, few studies provide information about process optimization, powder characterization (specific properties), antibacterial activity or relationship between specific properties (phenolic profile, total phenolic and flavonoid content) and antibacterial activity for other aromatic herbs, such as *Syzygium cumini* and *Psidium guajava* (PEIXOTO & al. [36], FERNANDES & al. [37]). Using arabic gum as carrier, rosemary oil was microencapsulated by spray-drying the powder recovery ranged from 17.25%–33.96% when the optimized conditions were represented by 171°C-inlet air temperature (DE BARROS FERNANDES & al. [38]). BARANAUSKIENE & al. [33] reported also the preparation of oregano powder and its properties after flavor encapsulation into milk protein-based matrices. MESTRY & al. [39] reported recently the optimized parameters for spray drying of fermented mixed juice of carrot and watermelon. GOULA and ADAMOPOULOS [40] reported as well a new technique for spray-dried encapsulation of lycopene, while the physicochemical properties of phytopharmaceutical preparations and the influence of drying methods and carriers on powders was presented by CORTÉS-ROJAS & al. [41].

In this study we aimed to characterize comparatively rosemary and oregano water cold and hot extracts and spray dried powders obtained by immobilization on maltodextrin, optimizing different drying parameters. The spray dried powders were characterized for their rheologic properties, the recovery yield of phenolics. Finally, the antibacterial properties of powders were determined and correlated with the powders' composition and drying technology.

2. Materials and Methods

2.1 Reagents and bacterial strains

All solvents and reagents (HCl, Folin-Ciocâlteu reagent, Na_2CO_3, gallic acid, $NaNO_2$, $AlCl_3$, NaOH, quercetin, formic acid, acetonitrile, sodium formate) used were of high purity

(Merck, Darmstadt, Germany). Maltodextrin used as carrier agent for spray drying had a 10 Dextrose Equivalent value (Glucidex provided by Supremia, Romania). The tested microorganisms (provided by the microbiology laboratory of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca) were Gram positive (*Bacillus cereus,* ATCC 11778; MediMark, France and *Lysteria monocytogenes* (ATCC 35152; Liofilchem, Italy) and Gram negative (*Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028, from Microbiologics, USA). The selective growth media and the supplements for the antibacterial assay were: MYP (CM 0929, Oxoid, UK), egg yolk emulsion X073 (Lab M Limited, Lancashire, UK), Oxford (CM 0856, Oxoid, Hampshire, UK), selective supplement for *Listeria* (107006, Merck Milipore, Germany), TBX (CM 0945, Oxoid, Hampshire, UK) and XLD (IVD CM 0469 Oxoid, Hampshire, UK).

2.2 Plant samples, extracts and spray drying procedure

Dried leaves of oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) were grounded and extracted in distilled water containing 1% HCl. The concentration of plant in the extract was 15%. The extraction was done in duplicate, either at room temperature (cold extraction) or in hot solvent at 80°C (hot extraction) for 24 hours. The fresh extracts were divided in different aliquots which were mixed with maltodextrin (MD) in a weight ratio of 90:10 or 85:15. All mixes of extracts with MD were submitted to spray drying, using different operational parameters. The spray drying process was conducted in a Niro Mobile Minor atomizer (GEA, Søborg, Denmark).

The technological parameters were set as follows: inlet temperatures (T_i) of 120±5ºC or 145±5°C, and outlet temperatures maintained above 30°C; the feed flow rate ranged from 90.3 ml/min to 200 ml/min. The sample codification for spray dried powders of rosemary (R) and oregano (O), obtained from hot (HE) or cold (CE) extracts is presented in table 1, as well the abbreviations corresponding to powders obtained by spray drying, considering the inlet temperature and maltodextrin percentage. For chemical analysis, the raw extracts were stored at -20°C and the powders at 4°C, in dark and in the absence of humidity. In order to measure the rheological properties and test the antibacterial activity, the fresh obtained powders were used. To test the antibacterial activity, the powders were rehydrated in distilled water at different dilution rates, e.g. 30% (A) to 40% (B), 50% (C) and 60% (D).

Table 1. Sample codification for spray dried powders of rosemary (R) and oregano (O), obtained from hot (HE) or cold (CE) extracts, depending on the operating parameters (inlet temperature and maltodextrin percentage).

T_{IN} / % MD	Rosemary HE	Rosemary CE	Oregano HE	Oregano CE
120°C / 10%	RHE 120-10	RCE 120-10	OHE 120-10	OCE 120-10
120° C / 15%	RHE 120-15	RCE 120-15	OHE 120-15	OCE 120-15
145°C / 10%	RHE 145-10	RCE 145-10	OHE 145-10	OCE 145-10
145°C / 15%	RHE 145-15	RCE 145-15	OHE 145-15	OCE 145-15

*T_{in}-inlet temperature; %MD-percentage of maltodextrin;

2.3 Spray drying operating parameters and rheological powder properties

The operating parameters referred to inlet (T_{IN}) and outlet temperature (T_{OUT}), the yield (%), the flow rate (ml/min) and moisture content (MC %). Some parameters (establishment of inlet and outlet temperature, the flow rate) were optimized before, by

preliminary assays. The powder MC values were determined gravimetrically by oven-drying method, at 105ºC until constant weight (Association Official Analytical Chemists, AOAC 934.01). The wettability (W) of the powders was determined by the GOULA & al. method [42] with slight modifications. Shortly, a quantity of 2g powder were sprinkled over a surface of 100 ml water at 25ºC, under stirring at 900 rpm. The time taken by the particles to sediment, sink or be submersed and disappear from water's surface represented the wettability. Powders bulk density (B_D) was adapted after the GOULA & al. method [42] as follows: a quantity of 2 g powder was loaded gently in a 10 ml graduated cylinder, measuring the ratio between the powder mass (g) and the volume occupied (cm^3) in the cylinder. The tapped density (T_D) was determined by the same protocol as for B_D, but after the powder being tapped 1 min by a vortex. The Hausner ratio (H_R) was calculated applying the formula proposed by KENNEDY and PANESAR [43] $H_R = T_D/B_D$ and the Carr index (CI) was calculated by the formula of LAY-TZE [44] $CI= T_D - B_D / T_D *100$.

2.4 Determination of Total Phenolic Content of Extracts and Spray Dried Powders

The total phenolic content (TPC) of raw extracts and powders after rehydration in distilled water (10% powder in water) were determined by spectrophotometry, using the method Folin-Ciocâlteu method with a slight modification [45]. Briefly, 0.025 ml extract or rehydrated sample was mixed with 2.375 ml distilled water and 0.150 ml Folin-Ciocâlteu reagent and 0.450 ml solution Na_2CO_3 7.5% were added. The samples were kept in the dark for 2 hours. The absorbance was recorded at 750 nm (using Biotek multiplate reader). A standard solution of pure gallic acid (1 mg/ml) was used to make a calibration curve (y=0.9443x+0.0608, R=0.99). The results were expressed in milligrams of gallic acid equivalents (GAE) per gram plant or per gram powder.

2.5 Determination of Total Flavonoid Content of Extracts and Spray Dried Powders

The total flavonoid content (TFC) of raw extracts and spray dried powders after rehydration was determined as follows: briefly, to 1 ml of extract or rehydrated sample there were added 0.3 ml $NaNO_2$ solution 0.5g/L, 0.3 ml $AlCl_3$ solution 1g/L, 2 ml NaOH solution 1M. The absorbance was recorded at 510 nm. Pure quercetin was used as standard for the calibration curve (y=0.8095x+0.0475, R=0.99). The results were expressed as milligrams of quercetin equivalents (QE) per gram extract or powder.

2.6 Antibacterial assay by disk diffusion method

Before testing, all bacterial strains were reactivated in nutrient broth (2 ml bacterial suspension in 45 ml nutrient broth). The correlation between the absorbance of the bacterial suspension in broth and the number of colony forming units (CFU/ml) were established using their absorption at 660 nm. The cell counting was performed in parallel using a Funke Gerber colony counter (Funke, Berlin, Germany) with colored illumination. The antibacterial activity was determined using the disk diffusion method. A volume of 100 µl of each bacterial suspension (10^4 CFU/ml) was spread on Petri dishes containing selective growth medium. After 30 minutes, sterile paper disks (6 mm disks provided by Nordic, Romania) were placed on the surface of the inoculated medium, well spaced out. Volumes of 25 µl from each rehydrated powder suspensions in water (A=30%, B=40%, C=50% or D=60%) as described above, were added on the disk and placed on the Petri plate inoculated with bacteria. Gentamicin (40 mg/ml) was used as reference standard (positive control), as recommended

by the National Committee for Clinical Laboratory Standards (NCCLS) for inhibiting all the tested strains. In this case, the disks were impregnated with 2.5 µg gentamicin for *B. cereus*, 2.3 µg for *L. monocytogenes*, 0.29 µg for *E. coli* and 0.625 µg for *S. typhimurium*. Sterile water was used as negative control. All the plates were incubated for 24 h at 30°C (*B. cereus*) and 37°C respectively (for the other bacteria). The antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) around the disk in the plate. Two diameters were measured using a calliper, the mean value being considered as DIZ value.

2.7 Statistical analyses

All samples and analyses were done in triplicate and expressed as average value (x) ± standard deviation (SD). The statistical data analysis was performed using the XLSTAT statistical software (MS Excel, Addinsoft, New York, USA). The difference between different parameters and factors was evaluated using ANOVA ($p<0.05$). Fisher test ($p<0.05$) was used to identify the significance of differences among different groups and to compare the mean values. Two-way ANOVA within samples was performed to compare the analytical data.

3. Results and Discussions

3.1 Spray drying operating parameters and process yields

The spray drying operating parameters for each powder sample, namely the inlet (T_{IN}) and outlet temperature (T_{OUT}), the yield (%), flow rate (ml/min) and moisture content (MC %) are presented in Table 2.

Table 2. Spray drying operating parameters for each powder sample: inlet (T_{IN}) and outlet temperature (T_{OUT}) yield (%), flow rate (ml/min) and moisture content (MC%)

Sample	T_{OUT}	Yield (%)	Flow rate	MC%	Sample	T_{OUT}	Yield (%)	Flow rate	MC%
OCE 145-10	≥30	74.69	100	0.61±0.02	RCE 145-10	<29	56.4	127	1.31±0.13
OCE 145-15	<25	20	200	0.18±0.01	RCE 145-15	≥29	79.13	130	1.07±0.15
OCE 120-10	≥35	95.83	100	0.63±0.02	RCE 120-10	≥30	79.62	120	0.61±0.03
OCE 120-15	≥29	40.38	111	0.56±0.02	RCE 120-15	≥30	83.17	100	1.02±0.04
OHE 145-10	≥35	72.66	101	0.37±0.01	RHE 145-10	≥38	63.94	111	0.23±0.01
OHE 145-15	≥37	75.41	101	0.49±0.01	RHE 145-15	≥40	97.03	111	0.58±0.13
OHE 120-10	≥36.5	79.41	90.7	0.30±0.01	RHE 120-15	≥35	98.64	111	0.71±0.08
OHE 120-15	≥37	73.33	90.3	0.48±0.02	RHE 120-10	≥30	66.66	93.5	0.82±0.00

*** Values for moisture content represent the mean of three replicate measurements ± SD, flow rate (ml/min)

We can observe that the two inlet temperatures of 145°C or 120°C were coupled with outlet temperatures ranging from 20°C to 38° C. The process yields proved to be influenced by the inlet temperature, feed flow rate and maltodextrin concentration. For rosemary extracts

the yields were superior, but not significant, comparing with oregano. Increasing the inlet temperature and optimizing the feed flow rate, the process yield seems to be increased due to enlargement of mass transfer. The increasing maltodextrin percentage (from 10 to 15%), determined a decrease of yield in oregano powders, similar to data reported by DESCAMPES & al. [31], but increases of yields for rosemary powders, up to 98.64%. The texture and color was similar, in spite of slight darker nuances of powders obtained with 145°C inlet temperatures. The highest yields were obtained for RHE 145-15 and 120-15 as well for OCE 120-10. Flow rates around 100 ml/min proved to influence positively the yields. Generally the OHE powders had yields of around 75% while RHE powders, superior yields (up to 98%). TONON & al. [46] reported to obtain acai powders with a moisture content ranging from 0.64% to 2.89% at 10%-30% maltodextrin, which proved good protective role for thermolabile compounds. A study made by FERRARI & al. [47] showed a moisture content of 3.25% for blackberry powders including 7% maltodextrin.

3.2 Rheological properties of powders

Table 3 and table 4 present comparatively the rheological properties of all spray dried powders obtained from rosemary and oregano extracts, namely the bulk density(B_D), tapped density (T_D), Hausner ratio (H_R), Carr index (CI) and wettability (W). Bulk density and tapped density proved not to be influenced by inlet temperature values (120°C *vs* 145°C), having similar values for both oregano and rosemary. According to FAZAELLI & al. [48] these values were negatively correlated with solubility. In these experiments, the powders obtained by hot extraction (RHE, OHE), had lower B_D values (0.31-0.32 g/cm^3) and higher H_R (mean values of 1.55 and 1.53) comparing with the powders obtained from cold extracts (RCE, OCE), which and implicitly had an increased solubility. The tapped density did not show significant modifications, ranging from 0.44 to 0.51 g/cm^3. The H_R values in these experiments ranged from 1.15 to 1.58. According to H_R values reported in the literature by CORTÉS-ROJAS & al. [41], H_R values of around 1.2 correspond to large particles having low friction in between, while smaller and cohesive particles have H_R values of around 1.6, correlated with an increased tendency of friction and increased resistance to flow. In the experiments, the H_R values indicate to have a homogeneous particle density and dimension.

The CI values are indirect indicators of powder resistance to flow. In these experiments the CI values oscillated from 13.56 to 36.73, corresponding to a “normal towards low” flowabillity, as indicated by LAY TZE & al. [44] who classified the flow properties of powders from 5-15% good flow and >25% low flowabillity. Both H_R and CI are reversely correlated with particle size, moisture and Van der Waals and electrostatic forces (LAY TZE & al. [44]). Comparing the mean values for RCE vs RHE and OCE vs OHE, we can observe higher mean values for CI in powders from hot extracts and slightly higher values for rosemary than oregano. The wettability was also influenced by the extraction procedure and maltodextrin concentration, in agreement with literature data (FERNANDES & al. [37], GOULA & al. [40]). In our experiments, the powders obtained through hot extraction (OHE and RHE) had significant higher wettability values (669.75 and 744.5 sec) comparing with the wettability of powders from cold extracts (OCE, RCE). We can conclude that solubility of RHE and OHE powders is significantly inferior to powders from cold extracts, being explained by an increased extraction of lipophilic, water insoluble compounds.

Table 3. Comparative rheological properties of rosemary spray dried powders: bulk density (B_D), tapped density (T_D), Hausner ratio (H_R) and Carr index (CI). For abbreviations, see Table 1.

Sample-T_{IN}-%MD	B_D (g/cm^3)	T_D (g/cm^3)	H_R	CI	Wettability (sec)
RCE 145-10	0.34±0.00	0.51±0.00	1.50	33.33	268
RCE 145-15	0.34±0.00	0.51±0.00	1.50	33.33	292
RCE 120-10	0.34±0.00	0.50±0.00	1.47	32.00	310
RCE 120-15	0.34±0.00	0.51±0.00	1.50	33.33	279
Mean values RCE powders			**1.49**	**32.99**	**287.25**
RHE 120-15	0.32±0.00	0.49±0.00	1.53	34.69	672
RHE 145-10	0.32±0.00	0.50±0.00	1.56	36.00	658
RHE 145-15	0.31±0.00	0.49±0.00	1.58	36.73	696
Mean values RHE powders			**1.55**	**35.67**	**669.75**

* Values are average of three replicated measurements±standard deviation

Table 4. Comparative rheological properties of oregano spray dried powders: bulk density (B_D), tapped density (T_D), Hausner ratio (H_R) and Carr index (CI). For abbreviations, see Table 1.

Sample-T_{IN}-%MD	B_D (g/cm^3)	T_D (g/cm^3)	H_R	CI	Wettability (sec)
OCE 145-10	0.38±0.00	0.48±0.00	1.26	20.83	412
OCE 145-15	0.38±0.00	0.44±0.04	1.15	13.63	362
OCE 120-10	0.32±0.00	0.46±0.00	1.43	30.43	282
OCE 120-15	0.35±0.01	0.49±0.01	1.40	28.57	386
Mean values OCE powders			**1.31**	**23.36**	**360.5**
OHE 120-10	0.32±0.00	0.50±0.00	1.56	36.00	711
OHE 120-15	0.32±0.00	0.48±0.00	1.50	33.33	688
OHE 145-10	0.31±0.00	0.48±0.00	1.54	35.41	745
OHE 145-15	0.32±0.00	0.49±0.00	1.53	34.69	834
Mean values OHE powders			**1.53**	**34.85**	**744.5**

* Values are average of three replicated measurements±standard deviation

3. 3 Comparative Spectrophotometry Data of Total Phenolic Content and Total Flavonoids Versus Total Phenolics determined by LC-MS from Rosemary and Oregano Extracts and Powders

Tables 5 and 6 present comparatively the total phenolic content (TPC) expressed in gallic acid equivalents (GAE), total flavonoid content (TFC) expressed in quercetin equivalents (QE) and total phenolics determined by LC-MS, expressed in rutin equivalents (RE) for all rosemary and oregano extracts and powders. These methods are based on different analytical approaches and difficult to compare, but, positive correlations were expected.

Table 5. Mean values ± SD obtained for total phenol content (TPC), the total flavonoid content (TFC) and the total phenolics determined by LC-MS, for oregano raw extracts and powders. For abbreviations see Table 1.

Sample	TPC (mg GAE/g powder)	TFC (mg QE/g powder)	LC-MS(mg rutin/g powder)
OCE	15.35±0.17^{a}	8.22±0.38^{b,c}	14.54±0.13
OCE 120-10	14.307±1.25^{d,e}	1.117±0.02^{j}	17.61±0.33
OCE 120-15	9.267±0.39^{g}	1.462±0.02^{i,j}	14.89±0.26
OCE 145-10	12.264±0.17^{f}	2.222±0.14^{g,h,i}	-
OCE 145-15	13.671±0.12^{e}	2.278±0.12^{g,h}	-
OHE	16.56±0.30^{a}	8.46±0.17^{b}	29.02±0.63
OHE 120-10	21.477±0.23^{b,c}	5.422±0.25^{e}	3.37±0.03
OHE 120-15	20.869±0.35^{c}	4.232±0.10^{f}	7.60±0.06
OHE 145-10	22.016±0.21^{a,b}	5.973±0.08^{e}	7.78±0.05
OHE 145-15	22.777±0.29^{a}	4.352±0.05^{f}	9.49±0.13

*Values are average of three replicated measurements±standard deviation; different letters represent significant statistical differences (Fisher's test, 95% confidence interval); total phenolic content (TPC)expressed as mg equivalents of gallic acid/g powder; OHE, OCE, RHE, RCE are expressed as mg gallic acid equivalents (GAE)/g plant, total flavonoid content (TFC) expressed as mg quercetin equivalents/g powder.By LC-MS method, the total phenolics were expressed as mg rutin eq. /g plant or powder

Considering the TPC values, for oregano extracts and powders it was obvious that hot extraction released more phenolics from the plant matrix (from 16.56 mg GAE/g oregano plant to 20.86-22.77 mg GAE/g oregano powder and from 15.31 mg GAE/g rosemary plant to 20.92-21.89 mg GAE/g rosemary powder), comparing with inferior values for the cold extracts and derived powders. Oregano powders obtained by hot extraction (OHE) were 1.6 times richer in TPC than those obtained by cold extraction (OCE). Oregano powders (OCE) were two times richer in total phenolics than rosemary powder RCE, but these differences were not observed for OHE *vs* RHE. Therefore, hot extraction increased the concentration of phenolics by 50% in oregano and by 30% in rosemary extracts and also in powders.

Table 6. Mean values ± SD obtained for total phenol content (TPC), the total flavonoid content (TFC) and the total phenolics determined by LC-MS, for rosemary raw extracts and powders. For abbreviations see Table 1.

Sample	TPC (mg GAE/g powder)	TFC (mg QE/g powder)	LC-MS(mg rutin/g powder)
RCE	10.83±0.16^{d}	7.03±0.10^{d}	8.52±0.13
RCE 120-10	9.508±0.14^{g}	4.075±0.04^{f}	4.94±0.03
RCE 120-15	4.448±1.14^{j}	1.533±0.01^{h,i,j}	8.33±0.12
RCE 145-10	7.677±0.37^{h}	2.465±0.06^{g}	6.67±0.10
RCE 145-15	6.568±0.21^{i}	2.435±0.07^{g}	6.80±0.10
RHE	15.31±0.43^{c}	5.71±1.03^{e}	16.46±0.20
RHE 120-10	20.927±0.17^{c}	7.692±0.12^{a}	38.23±0.75
RHE 120-15	21.515±0.15^{b,c}	7.575±0.35^{c,d}	32.13±0.63
RHE 145-10	21.891±0.05^{a,b,c}	8.539±0.69^{b}	32.65±0.58
RHE 145-15	21.833±0.11^{a,b,c}	8.005±0.48^{b,c}	29.62±0.45

*Values are average of three replicated measurements±standard deviation; different letters represent significant statistical differences (Fisher's test, 95% confidence interval); total phenolic content (TPC)expressed as mg equivalents of gallic acid/g powder; OHE, OCE, RHE, RCE are expressed as mg gallic acid equivalents (GAE)/g plant, total flavonoid content (TFC) expressed as mg quercetin equivalents/g powder.By LC-MS method, the total phenolics were expressed as mg rutin eq. /g plant or powder

Considering TFC values, representing 10-25% of the total phenolics, there were noticed similar concentrations in OCE and OHE (8.22-8.46 mg QE/g plant) and lower concentration in RCE and RHE (7.03-5.71mg QE/g plant). In powders, the concentrations

were superior but similar in both OCE, OHE, RCE and RHE; significand differences were observed between total phenolics (TPC) and flavonoid content (TFC) of oregano and rosemary extracts and powders. Similar results were obtained by CHUN & al. [19] for TPC in oregano hot water extract . Oregano powders revealed higher values for TPC than rosemary powders, only when cold extracts were spray dried. Contrary, rosemary powders obtained from hot extracts, but not from cold extracts, were richer in TFC (flavonoids) than oregano. Comparing the influence of 10% vs 15% MD in powders, the concentration of TPC and TFC was higher using 10% MD than 15% MD, in agreement with other authors (VIDOVIĆ & al. [49]) who suggested that the increase of MD concentration may decrease the TPC and TFC content.

3.4 Screening of Antibacterial Activity

The antibacterial effect of various flavonoids is well documented, e.g. apigenin, epigallocatechin gallate, quercetin, kaempferol, luteolin and derivatives (11). Considering the profile of rosemary and oregano extracts, their antibacterial effect was screened. Fig. 1 represents the antibacterial effect of the different rosemary (RCE and RHE) hydrated powders obtained at 120°C with 10% MD) and oregano powders (OCE *vs* OHE) by comparison with gentamicin. A, B, C, D variables represent the different concentrations (30, 40, 50 and 60%, respectively) of hydrated powders added to media.

The intensity of antibacterial effect was measured by the inhibition diameter zone (DIZ) ranging from 0 to 3 mm. The antibacterial activity was identified starting at 30% rehydrated powder in the media. While RHE 120-10 presented a weak antibacterial activity (DIZ max=1mm) against *B. cereus*, RCE 120-10 showed an antibacterial activity (DIZ ~2mm) against *E. coli* and *S. typhimurium* (DIZ=1.25 mm). Powders obtained from OCE had antibacterial activity against *B. cereus*, *E. coli* and *S. typhimurium* (OCE 145) starting from 30% (A) for *B. cereus* and *E. coli*, while for *Salmonella* only at 40% (B). An activity anti-*Listeria* was observed only for OCE, at 50% and 60% concentrations (C and D). A large inhibitory spectrum was observed for oregano powders against *B. cereus*. Oregano powders (OHE) showed a slight antibacterial activity (DIZ aprox. 2mm) only against *B. cereus*. Meanwhile, RHE 145 and OHE 120 did not present any antibacterial activity. The same sensitivity of *Bacillus cereus* against hot water extracts of Thai medicinal plants was observed by TERPSON [50]. PROESTOS & al. [51] reported an inhibition of *Listeria monocytogenes*, *B. cereus* and *E. coli*, ranging from 1 to 10 mm by oregano methanolic extracts, but no antibacterial activity against *S. typhimurium*. Meanwhile, MARTINS & al. [5] observed inhibition of *E. coli* by oregano decoct.

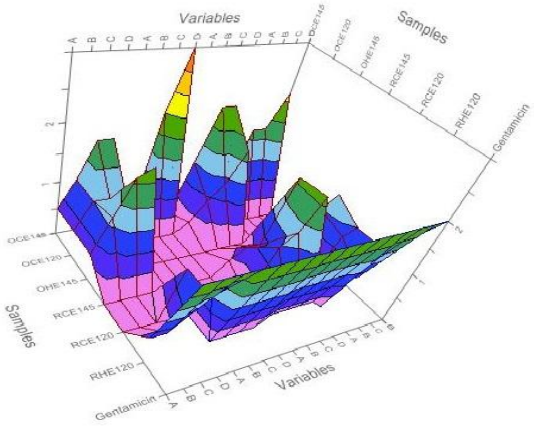

Fig 1. Representation of the antibacterial effect of the different rosemary (RCE and RHE – derived hydrated powders) and oregano powders by comparison with gentamicin. ***A,B,C,D variables represent the different concentrations (from 30 to 60%) of hydrated powder added to media. The intensity of antibacterial effect was measured by the inhibition diameter DIZ (range of values from 0 to 3mm).

We have to consider that the antibacterial activity is strain dependent and may be influenced by the affinity of the compounds to the bacterial membrane PISTELLI & al. [52]. In our case, the oregano powders had higher DIZ values for Gram positive bacteria like *B. cereus* and exhibited greater sensitivity than Gram negative bacteria. These specific effects can be explained by the hydrophobic surface of Gram negative bacteria which may exclude hydrophilic molecules like phenolic acids or flavonoids (CUEVA & al. [12]), as well the hypothesis of DO PRADO & al. [53], which showed that Gram negative bacteria can grow in the presence of tannins and monomeric catechins. The sensitivity of Gram positive bacteria to phenolic acids was stated by CUEVA & al. [12]. Fig. 2 represents the PCA biplot analysis of rosemary and oregano powders connected to DIZ. The two principal components explained 88.97% of the total variance. The first component (F1) explained 57.23% of the variance, while the second component (F2) explained 31.74% of the variance. It was observed a clear discrimination between powders based on the type of extraction used. *B. cereus* presented the greatest sensitivity for OCE powders, while for rosemary the sensitivity was observed only for powders obtained from RCE *S. typhimurium* presented sensitivity to RHE 120, while *E. coli* was sensitive to OCE and RCE.

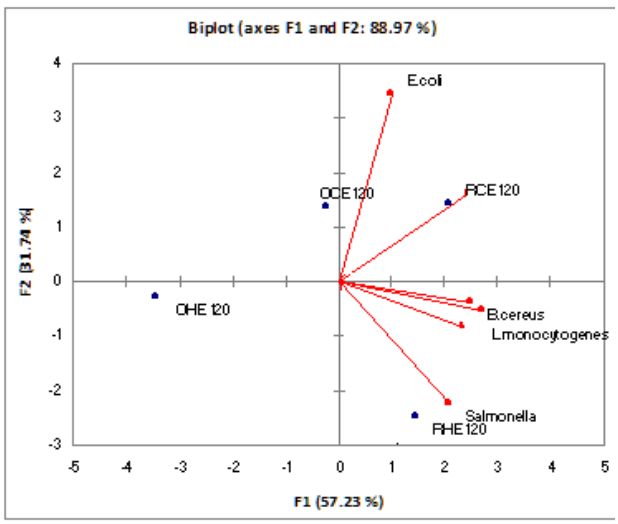

Fig 2. PCA biplot analysis to correlate the efficiency of antibacterial effects of rosemary RCE 120-10, RHE 120-10 and oregano OCE 120-10 and OHE 120-10 powders, dependent on the diameter of inhibition zone (DIZ)

4. Conclusions

The rheological properties of the powders were determined by different parameters, e.g. bulk and tapped density which proved not to be influenced by inlet temperature values (120°C *vs* 145°C), but by maintaining the outlet temperature above 30°C we observed an improvement in the recovery yield. The powders obtained by hot extractions had lower bulk density and higher Hausner ratios (mean values of 1.55 and 1.53) comparing with the powders obtained from cold-extracts, which had increased solubility. The Carr index values, as indicators of powder resistance to flow, ranged from 13.56 to 36.93, corresponding to a "normal towards low" powder flowabillity. The wettability was also influenced by the extraction procedure and maltodextrin concentration. In our experiments, the powders obtained through hot extraction (OHE and RHE) had significant higher wettability values (669.75 and 744.5 sec) comparing with the wettability of powders from cold extracts (OCE, RCE). Therefore, the low degree of solubility for RHE and OHE can be explained by an increased extraction of lipophilic, water insoluble compounds. Oregano powders obtained by hot extraction were 1.6 times richer in total phenolics than those obtained by cold extraction, and superior to rosemary powder.

Therefore, hot extraction increased the concentration of phenolics by 50% in oregano and by 30% in rosemary extracts and also in powders. Rosemary powders obtained from hot extracts, but not from cold extracts, were richer in TFC (flavonoids) than oregano. Comparing the influence of maltodextrin in powders, the concentration of phenolics and flavonoids was higher in 10% than 15% maltodextrin. We noticed that powders obtained from hot extracts were richer in flavonoids than phenolic acids. The oregano powders had higher inhibitory effects on Gram positive bacteria and exhibited greater sensitivity than Gram negative bacteria. *B. cereus* presented the greatest sensitivity from all the tested strains. *E. coli* and *B. cereus* had sensitivity to oregano and rosemary powders obtained from cold extracts, oregano being more efficient. Meanwhile, *S. typhimurium* showed greater sensitivity to cold rosemary powders than cold oregano powders. Further studies will develop the quantitative correlations between the qualitative and quantitative composition of powders and the specific inhibition of different Gram positive or Gram negative bacteria.

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