

# Spray drying of a subcritical extract using *Marrubium vulgare* as a method of choice for obtaining high quality powder

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Received: date; Accepted: date; Published: date

**Abstract:** White horehound (*Marrubium vulgare* L.), is a grey-leaved perennial herb, belonging to Lamiaceae family, distributed in Eurasia and northern Africa zones. Despite the fact that *M. vulgare* is used since ancient times in treating diverse diseases, it is only in the last decade that scientists have been able to lay the foundation for potential pharmacological actions from the results observed through the prism of ethnopharmacological use of this species. The novelty of this study was that subcritical water extraction, acknowledged as a powerful extraction technology to recover phenolic compounds, was coupled with spray drying. The subcritical horehound extract, obtained at optimal process parameters, was used as a liquid feed in spray drying. Maltodextrin was used as a carrier in concentration of 10%. Therefore, two *M. vulgare* powders, carrier-free and 10% MD, were produced. Comprehensive powders characterization was conducted in order to evaluate their quality. Results confirmed that spray drying can be used as a method of choice for obtaining high quality horehound powders which kept the amorphous structure constant after 6 months.

**Keywords:** *Marrubium vulgare*, subcritical water extraction, spray drying, powder characterization

## 1. Introduction

The aerial parts and root of *Marrubium vulgare* L. have been traditionally used in the genus *Marrubium*, formed by nearly 30 species [1]. This genus is an abundant source of secondary metabolites, including nine different types of diterpenes and their derivatives, more than ten flavonoid constituents, and phenylpropanoids and their glycosides [2]. The most studied secondary metabolite marrubiin, a labdane diterpenoid, was isolated for the first time from *M. vulgare* leaves [3]. The reason why this chemotaxonomic marker was subject of many studies is due to owning several activities such as antioxidant, anti-inflammatory and vasorelaxant [4]. This perennial plant with morphological characteristics reminiscent of a mint [5], shows numerous diverse pharmacological effects. It was reported that a hydroalcoholic extract of aerial parts and root of horehound exerts significant antispasmodic activity which means that it can be used as an expectorant for acute/chronic bronchitis, colds and in cases of asthma [6]. According to another study, the hydroalcoholic extract also shows analgesic effects when administered per os or

48 intraperitoneally [7]. The series of *in vivo* experiments were performed in rats seeking for  
49 antidiabetic effect of horehound. The results were positive since there was more than 60% decrease  
50 of blood glucose level when aqueous extracts were administered [8]. The methanol extract of  
51 horehound herba revealed moderate activity when evaluated against five Gram positive bacteria:  
52 *Bacillus subtilis*, *Staphylococcus epidermidis* and *S. aureus*, *Pseudomonas vulgaris* and *Escherichia coli* [9].  
53 Furthermore, this plant demonstrated a strong effect against methicillin-resistant *Staphylococcus*  
54 *aureus*. [10]. Beside its medicinal use, extracts of horehound herba are also used as flavouring  
55 agents, especially by the brewing industry as a substitute for hops [5], in candies [11], and as an  
56 ingredient of cough pastilles [12].

57 According to a market research report, horehound was reported as the top-selling herbal  
58 dietary supplement ingredient in mainstream US retail outlets for the fifth consecutive year. In this  
59 channel, horehound supplement sales in 2017 increased for 12.3% from 2016 [13]. Records of the  
60 medicinal use of horehound confirm that this herb is still commonly used for its expectorant and  
61 cough-suppressant properties, especially in the form of cough drops and lozenges. According to  
62 European Medicines Agency guidelines, horehound is usually used in combination with 3 to 5  
63 herbal substances in Europe. In agreement with guidelines from the US FDA, dietary supplements  
64 appear in a number of forms inclusive of tablets, powders, capsules, softgels, gelcaps and liquids.  
65 Powders have many benefits over liquid extracts including higher stability, reduced bulk size,  
66 higher concentration of bioactives, simple manipulation and shipment, and finally easier  
67 standardization [14]. Therefore, spray drying imposes as technique of choice for obtaining a solid  
68 phase herbal powders from liquid feed in a single step.

69 The main purpose of this study was to estimate the efficiency of spray drying technology to  
70 microencapsulate phenolic compounds from horehound subcritical extract obtained at optimal  
71 conditions of process parameters. This extract, used as liquid feed, was obtained through subcritical  
72 water extraction which has been acknowledged as a powerful extraction technology to recover  
73 phenolic compounds from different matrices [15,16]. The obtained powders were further  
74 investigated with reference to their physical and chemical properties. To the best of our knowledge,  
75 the subcritical horehound extract has not been applied yet as a liquid feed for spray drying process.

## 76 2. Materials and Methods

### 77 2.1. Plant material

78 *M. vulgare* was bought from the local supplier of cultivated plants *Chamomilla* (Banatski  
79 Karlovac, Serbia), harvested in 2015. The aerial parts of *M. vulgare* were air-dried in thin layer,  
80 collected in the paper bags, and stored at a room temperature. Afterwards, the dried *M. vulgare*  
81 herba was grounded in a domestic blender and the particle size of grounded material was  
82 determined using vibration sieve sets (LISA, Ledaceria, Spain). The mean particle size of *M. vulgare*  
83 herba used in investigation was 0.28 mm.

### 84 2.2. Chemicals

85 Reagents used in methods, 1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), Folin-Ciocalteu  
86 and (±)-Catechin were purchased from Sigma (Sigma-Aldrich Chemie GmbH, Sternheim,  
87 Germany). The following reagents were also purchased from Sigma-Aldrich Chemie: iron (III)-  
88 chloride, potassium hexacyanoferrate (III), sodium hydrogen phosphate anhydrous, sodium  
89 dihydrogen phosphate and trichloroacetic acid. Gallic acid was purchased from Sigma (St. Luis,  
90 MO, USA). Maltodextrin of dextrose equivalent (DE) 16.5–19.5 (Sigma-Aldrich Chemie GmbH,  
91 Steinhelm, Germany) was used as a carrier material. All other chemicals and reagents were of  
92 analytical grade.

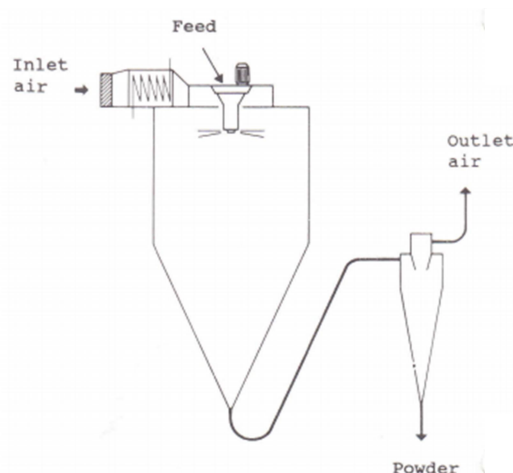
### 93 2.3. Liquid extract and liquid feed preparations

94 Subcritical water extraction (SWE) at optimal process conditions (temperature of 200 °C,  
95 extraction time of 20.29 min and absence of HCl) defined in our previous study, was used to obtain

96 liquid extract which was further used as a liquid feed. The scheme of subcritical water extraction  
 97 apparatus used is presented in previously published paper [17]. In certain amount of liquid feed no  
 98 carrier was added prior to the drying. Maltodextrin (MD) of dextrose equivalent (DE) 16.5–19.5 was  
 99 used as a carrier material. Procedure of preparation of liquid feed with 10% MD was described in  
 100 our previous study [14]. Therefore, two *M. vulgare* powders (MVPs) were obtained (0% MD and  
 101 10% MD).

#### 102 2.4. Spray drying process and its efficiency

103 The pilot scale spray dryer (APV Anhydro AS, Denmark) used for spray drying of prepared  
 104 liquid feed is presented in Fig 1. A laboratory peristaltic pump was used to transfer the liquid feed  
 105 into the drying chamber. For each run, 2L of liquid feed was dried. Liquid feeds were dried at inlet  
 106 temperature,  $T_i = 130 \pm 5$  °C, while outlet temperature,  $T_o$  was maintained at 75–80 °C. During the  
 107 production of the dry extract (powder), rotary disk, within atomizer, delivered speed from 20,000 to  
 108 21,000 rpm. The obtained powder was separated from heating medium in a cyclone and collected in  
 109 glass bottles, sealed and kept protected from air and humidity. The particle production efficiency  
 110 (i.e. powder recovery) is determined gravimetrically as ratio of mass of the powder obtained in the  
 111 collecting vessel after spray drying and mass of total solids measured in the liquid feed. Process  
 112 efficiency is expressed as the weight percentage.



126  
127 **Figure 1.** Schematic diagram of pilot scale spray dryer

128

#### 129 2.5. Analysis of MVPs stability properties

##### 130 2.5.1. Moisture content

131 Moisture contents of MVPs were determined according to standard procedure described in  
 132 official Pharmacopeia (Ph. Jug. IV). The gravimetric method, based on water removal by heating,  
 133 was carried out in an oven at 105 °C until achieving constant mass. Measurement of the moisture  
 134 content was performed promptly after the spray drying. All experiments were performed in three  
 135 replicates.

##### 136 2.5.2. Hygroscopicity

137 All powder samples (approx. 1 g) were placed in desiccator supplied with NaCl saturated  
 138 solution (70% RH) at 25 °C. The hygroscopicity was measured after 48 h. Hygroscopicity was  
 139 expressed as a gram of absorbed water per 100 g of dry extract powder. All experiments were  
 140 performed in three replicates [14].

## 141 2.6. Analysis of MVPs solubility and wettability properties

### 142 Water solubility (WSI) and water absorption (WAI) indexes

143 The WSI and WAI were determined according to a previously described method [18]. The  
144 certain amounts of powder (1.25 g) and water (15 mL) were strongly mixed in a 50 mL centrifuge  
145 tube. Afterwards, the mixture was incubated in a water bath at 30 °C for 30 min, and centrifuged for  
146 15 min at 3000 rpm. The supernatant was decanted in a pre-weighed Petri dish, while particles were  
147 concentrated as a solid pellet at the bottom of the centrifuge tube. Both supernatant and pellet were  
148 placed in an oven and dried at 105 °C overnight. The amount of solids in the dried supernatant was  
149 calculated as a percentage of the total dry solids in the 1.25 g of sample, and represents WSI. WSI,  
150 reconstitution property, is used as an indicator of degradation of powder constituents. WAI was  
151 calculated as the mass of solid pellets remaining after centrifugation divided by the mass of the  
152 original dry sample. WAI is a measure of the products ability to absorb water. WAI depends on the  
153 availability of hydrophilic groups and on the gel-forming capacity of macromolecules. The  
154 hydrophilic groups are responsible for binding of water molecules. The low WAI indicates better  
155 stability during the storage. All experiments were performed in three replicates.

## 156 2.7. Analysis of MVPs flow behavior properties

### 157 2.7.1. Bulk density

158 Bulk density was determined by measuring the volume of a known mass of powder sample in  
159 a graduated glass cylinder. 1 g of *M. vulgare* powder was placed into a 25 ml graduated cylinder.  
160 Afterwards, the bulk density was calculated from the difference between the mass of empty glass  
161 cylinder and the mass of glass cylinder with powder sample. Bulk density was expressed as mg of  
162 powder per ml.

### 163 2.7.2. Powder characterization

164 Powder flowability, a key property in filling and by calculating the Hausner ratio and the Carr  
165 Index (CI). The Hausner ratio is calculated from the ratio between the bulk and tapped densities of  
166 the powder. The Carr Index is another measure of flowability, also calculated from the two  
167 densities of the powder [19].

### 168 2.7.3. Particle size analysis

169 In order to measure the particle size distribution of the prepared powders, LEICA Image  
170 Processing and Analysis System (LEICA Q500MC, LEICA Cambridge Ltd., England) was used. The  
171 size was determined using 350 particles per product. The particles were described in detail by their  
172 length, breadth, surface area, perimeter and roundness. The Malvern apparatus (Malvern  
173 Mastersizer Scirocco 2000; Malvern Instruments Ltd., Worcestershire, UK) was used for laser  
174 diffraction required for determination of powders particle size distributions. The sample (approx. 1  
175 g) was loaded into the feeder tray. The dispersion air pressure was fixed at 2.0 bar to determine if  
176 particle attrition has occurred. Obscuration was kept between 10.0% and 15.0% throughout the  
177 whole measurement duration. The particle size distribution was characterized by the D (0.1), D (0.5)  
178 and D (0.9) values and the specific surface area (SSA).

### 179 2.7.4. Morphology-Scanning electron microscopy (SEM)

180 The morphology of the MVPs particles was examined by SEM (Hitachi S4700, Hitachi Scientific  
181 Ltd., Tokyo, Japan). In order to induce electric conductivity on the surface of the samples, a sputter  
182 coating apparatus (Bio-Rad SC 502, VG Microtech, Uckfield, UK) was applied. The air pressure was  
183 1.3–13.0 mPa.

## 184 2.8. Analysis of MVPs crystallographic and thermal properties

### 185 2.8.1. Differential scanning calorimetry (DSC)

186 The Mettler Toledo DSC 821e thermal analysis system with the STARe thermal analysis  
187 program V6.0 (Mettler Inc., Schwerzenbach, Switzerland) was used for DSC measurements. The  
188 sample (approx. 2–5 mg) was examined in the temperature range between 25 °C and 300 °C. The  
189 heating rate was 10 °C min<sup>-1</sup>. During the DSC investigation, argon was used as inert carrier gas, at a  
190 flow rate of 10 L/h.

### 191 2.8.2. X-ray powder diffraction analysis (XRDP)

192 The physical state of samples was evaluated by X-ray powder diffraction (XRPD). The  
193 BRUKER D8 advance X-ray powder diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) with  
194 Cu-K  $\lambda$ I radiation ( $\lambda=1.5406$  Å) and a VÅNTEC-1 detector (Bruker AXS GmbH, Karlsruhe,  
195 Germany) were used for analyses of diffraction patterns. Scanning of samples were performed at 40  
196 kV and 40 mA. The angular range was 3°–40° 2 $\theta$ , at increment time of 0.1 seconds and increment  
197 size of 0.007°. All operations, including K $\alpha$ 2 stripping, background removal and smoothing of the  
198 area under the diffractograms peaks, were performed using the DIFFRACplus EVA software.

## 199 2.9. Analysis of MVPs bioactive compounds

### 200 2.9.1. Total phenols content

201 The contents of total phenolic compounds (TP) in horehound herbal powders were determined  
202 by the Folin–Ciocalteu procedure [20]. Gallic acid was used as standard compound for preparation  
203 of calibration curve, and absorbance of the samples was measured at 750 nm (6300  
204 Spectrophotometer, Jenway, UK). Content of phenolic compounds in dry extracts was expressed as  
205 mg GAE per g of dry extract (mg GAE/g DE). All experiments were performed in three replicates,  
206 and results are expressed as mean values.

### 207 2.9.2. Total flavonoids content

208 The total flavonoids content (TF) was determined in MVPs using aluminum chloride  
209 colorimetric assay [21]. Catechin was used as a standard for creation of calibration curve, and  
210 absorbance was measured at 510 nm. Content of flavonoids in dry extracts was expressed as mg CE  
211 per g of dry extract (mg CE/g DE). All experiments were performed in triplicate, and results were  
212 expressed as mean values.

### 213 2.9.3. DPPH assay

214 The free radical scavenging activity of extracts produced from horehound herbal powder was  
215 determined using a simple and fast spectrophotometric method [22]. Briefly, the subcritical extracts  
216 were mixed with 90  $\mu$ M 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and methanol (95%) to provide  
217 different final concentrations of extract. After 1 hour at room temperature, the absorbance was  
218 measured at 517 nm, in triplicates by 6300 Spectrophotometer, Jenway, UK. Radical scavenging  
219 capacity (RSC (%)) was calculated according to Eq. (1). and expressed as IC<sub>50</sub> value, which  
220 represents the concentration of extract solution required for obtaining 50% of radical scavenging  
221 capacity.

$$\%RSC=100- ((A_{\text{sample}} \times 100))/A_{\text{blank}} \quad (1)$$

222 where A<sub>sample</sub> is the absorbance of sample solution and A<sub>blank</sub> is the absorbance of control.

223

### 224 2.9.4. FRAP assay

225 The reducing power of horehound herbal powder was determined by a previously described  
226 method [23]. Various concentrations of subcritical extracts were mixed with sodium phosphate

227 buffer (2.5 mL, 0.2M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide ( $K_3Fe(CN)_6$ ). The mixture  
228 was incubated at 50 °C for 20 min. After incubation, 10% trichloroacetic acid aqueous solution (2.5  
229 mL) was added to the mixture, and the mixture was centrifuged for 10 min at 3000 rpm. The  
230 obtained supernatant (2.5 mL) was mixed with bidestillated water (2.5 mL) and 0.1%  $FeCl_3$  solution  
231 (0.5 mL). Absorbance was measured at 700 nm. Antioxidant activity was expressed as EC50 value  
232 (mg/mL), which causes reduction of 50%  $Fe^{3+}$  ions in reaction mixture. All experiments were  
233 performed in triplicate.

234

### 235 2.9.5. HPLC analysis

236

237 Phenolic compounds in MVPs samples (MVP 0% MD and MVP 10% MD) were analysed using  
238 Agilent 1200 Series HPLC with DAD detector (Agilent Technologies, Palo Alto, CA, USA) equipped  
239 with Lichrospher® 100 RP 18e column (5  $\mu$ m, 250 x 4 mm). Mobile phase A was formic acid in  
240 water (0.17%), while mobile phase B was acetonitrile. The injection volume was 10  $\mu$ L, and flow rate  
241 0.8 mL/min with gradient program (0–53 min 0–100% B). Stop time of the analysis was 55 min.  
242 Compounds were determined by comparing the retention times and absorption spectra (200–400  
243 nm) of unknown peaks with the reference standards (ferulic acid, p-coumaric acid, caffeic acid,  
244 rutin, hyperoside, 5-hydroxy-2-methylfurfural). The powders were reconstituted in methanol (1:10),  
245 macerated for 24 hours and filtrated prior analysis. The investigated samples were analyzed in  
246 triplicate.

## 247 3. Results

### 248 3.1. Process efficiency

249 The optimal spray drying conditions must be satisfied in order to obtain an adequate process  
250 efficiency. The dominant factors in spray drying that need to be optimized and monitored  
251 throughout the process are feed temperature and air inlet/outlet temperatures [24]. There are  
252 several processing obstacles which indirectly affects the properties and yield of the final product.  
253 One of them is certainly wall deposition. The wall deposition is created when particles deposit on  
254 the surfaces of the inner walls of drying chamber This phenomenon deteriorates the yield of the  
255 powder and therefore increase the costs of manufacturing and maintenance [25]. Particles deposit  
256 on the wall by attaching to it due to their stickiness which occur above the glass transition  
257 temperature,  $T_g$  [26,27]. Apart from monitoring the air inlet temperature, so that on the surface of  
258 the product it does not reach more than 10–20 °C above  $T_g$ , feed flow rate needs to be constant.  
259 When the feed flow rate increases, larger droplets are created and the evaporation rate is lower [28].  
260 When atomizer is supplied with more feed, the particles retain shorter in drying chamber hence the  
261 drying time is reduced, contributing in wetter particles. Under these conditions, the particles are  
262 more cohesive which cause increase of deposition rate and decrease of yield [25].

263 Water and ethanol are the most acceptable "green" cosolvents for food-grade products [29].  
264 Despite being safe for human consumption, ethanol has the drawback of being highly flammable,  
265 which may limit its wider use in industry. On the contrary, water has the benefits of being  
266 nonflammable, flavorless and less restricted in terms of residual solvent. Consequently, the use of  
267 water as a common entrainer in a high-pressure extraction process is very attractive and convenient  
268 [30]. In our study we used subcritical water extract as liquid feed. The main idea behind  
269 introducing subcritical water extraction was to improve extraction yields of desired bioactives and  
270 overcome common drawbacks of standard solid-liquid extraction. SWE stands out as a promising  
271 technique regarding facilitated analyte diffusion, favoured mass-transfer kinetics, decreased  
272 viscosity and surface tension of water when temperature is increased. Temperature is the priority  
273 factor that affects efficiency and selectivity of SWE [31]. The water in a subcritical state is used as  
274 extraction solvent in SWE. Water is regarded as subcritical at temperatures between 100 °C and 374  
275 °C and at a pressure high enough to keep it in a liquid state [32]. The drying in pilot scale spray-  
276 dryers is considered efficient when recovery in the cyclone is higher than 50% [33]. The efficiency of



277 two investigated spray drying processes can be considered high since in both cases it was above  
 278 50% (0% MD:  $\eta=58.36\%$ ; 10% MD:  $\eta=77.07\%$ ). Furthermore, process efficiency was increased by  
 279 maltodextrin supplementation which can be related to the influence of MD concentration on the  
 280 formation of surface core prior to the formation of crust enclosing the drying droplets [34]. Finally,  
 281 regarding all criteria, in the first place absence of stickiness, absence of wall deposition  
 282 phenomenon and recovery greater than 50%, process conditions of MVPs production can be  
 283 considered as suitable.

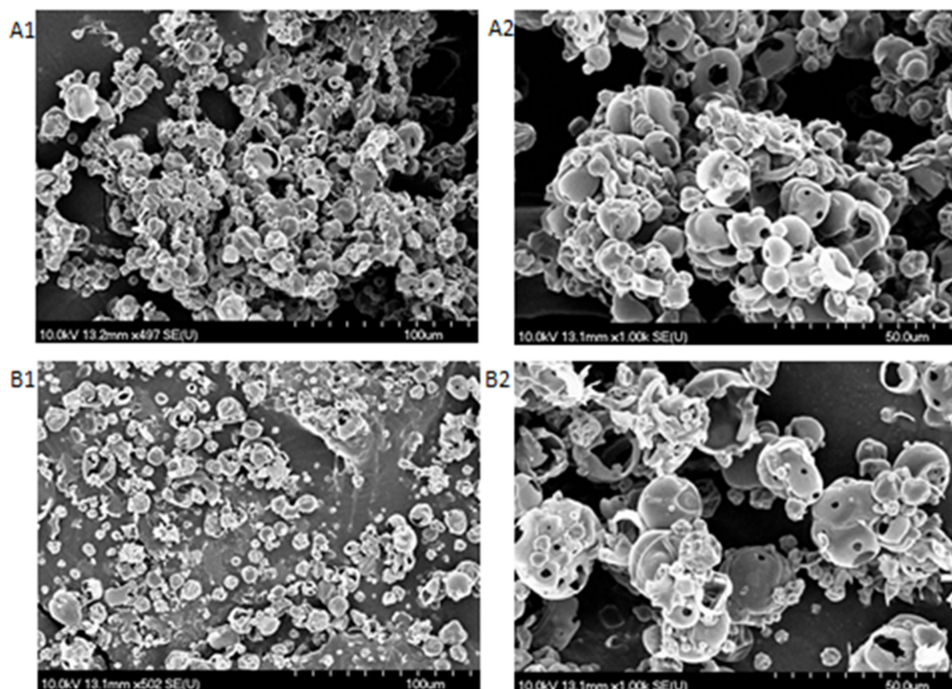
### 284 3.2. Evaluation of micrometric properties and structure of the MVPs

285 According to the literature, the diameter of spray-dried particles depends on the several factors  
 286 including atomization method used, concentration and viscosity of the encapsulated material and  
 287 finally drying conditions [35]. Some authors also emphasized that the particle size is significantly  
 288 affected by the type of carrier, with the largest sizes resulting from using starch or gum arabic as  
 289 carriers. There are studies that correlated larger particles with an increased encapsulation efficiency  
 290 [36]. In Table 1, average length, width, perimeter, area and roundness are presented.

291 **Table 1.** Particle size analyses of MVPs obtained by optical microscope.

Sample		Length	Width	Perimeter	Area	Roundness
		[ $\mu\text{m}$ ]	[ $\mu\text{m}$ ]	[ $\mu\text{m}$ ]	[ $\mu\text{m}^2$ ]	
MVP 0% MD	Average	4.43	3.57	15.49	14.40	1.33
	SD $\pm$	0.12	0.38	0.99	1.17	0.07
MVP 10% MD	Average	6.94	4.37	21.60	23.70	1.55
	SD $\pm$	2.65	1.60	6.99	12.92	0.44

292 In our study, the existence of maltodextrin caused an increase in the average particle size. In  
 293 sample MVP 0% MD, particles are smaller (Table 1) than in powder with carrier but aggregation  
 294 occurred due to presence of cohesiveness. In sample MVP 10% MD, particles are bigger and more  
 295 scattered which results in lower level of cohesiveness and their appearance as separated, more  
 296 individual particles (Fig. 2).  
 297  
 298  
 299



**Figure 2.** Micrographs of the MVPs particles using SEM with 500x magnification (A1, B1) and 1000x magnification (A2, B2) where A1, A2 represent MVP 0% MD and B1, B2 represent MVP 10% MD.

Fig. 2. introduces the morphology of the MVPs particles, changed after SD process, using SEM with 500x (A1, B1) and 1000x (A2, B2) magnifications. As stated in another study [14], before SD process the raw MD particles were large sized crystals with irregular needle shape. After SD process, small individual spherical particles with a smooth surface emerged. In our study, particles in both MVPs are nearly spherical with smooth surface. At 1000x magnification, small holes on the particles surface could be detected due to evaporation of solvent. To visually compare A1, A2 with B1, B2 in SEM pictures, it could be seen that without MD (A1, A2) aggregated postures of particles were produced, however using 10 % MD (B1, B2) the individuality of the particles was determinative. There is a strong adherence of smaller particles to the surface of higher magnitude particles (Fig. 2, B2) which confirmed the lack of crystalline and the presence of amorphous surfaces.

The decreasing trend of particle size when 5% and 10% MD were added was reported [14], while in our study opposite was noticed when 10% MD was added. Tonon et al. (2008) also found that a higher maltodextrin concentration in feed solution could lead to the production of larger particles in spray drying, which may be related to the increased feed viscosity with maltodextrin addition [37]. According to Phisut et al. (2012), the mean droplet size alters directly with the feed viscosity at constant atomizer speed. The higher the feed viscosity, the larger the droplets created during atomization. Therefore, the larger particles obtained by spray drying [36]. Table 2 lists the particle-size distribution of two samples, MVP 0% MD and MVP 10% MD.

**Table 2.** Particle size distribution of MVPs obtained by laser diffraction.

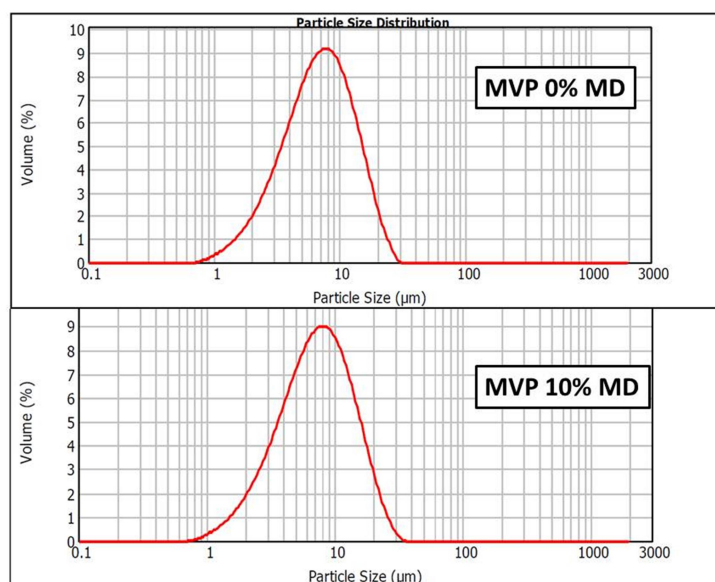
Sample	D 0.1 [ $\mu\text{m}$ ]	D 0.5 [ $\mu\text{m}$ ]	D 0.9 [ $\mu\text{m}$ ]	SSA
MVP 0% MD	2.700	6.920	14.840	1.150



MVP 10% MD	2.791	7.252	15.882	1.100
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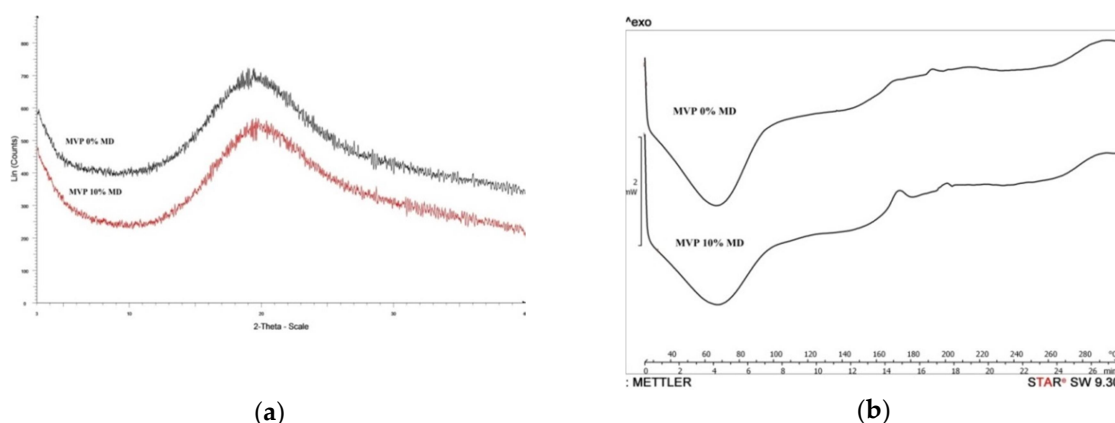
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Spray-drying of the subcritical extract resulted in microsized particles in both samples, with quite similar distribution (Fig. 3). Both distribution curves showed log normal shape. Fine decrease in specific surface area, when 10% MD was added, confirmed that particles are bigger in MVP 10% MD.



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Figure 3. Size distribution of the 0% MD MVP and 10% MD MVP.



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Figure 4. (a) XRPD patterns of the 0% MD MVP and 10% MD MVP; (b) DSC curves of the 0% MD MVP and 10% MD MVP.

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The results of XRPD analysis (Fig. 4 (a)) indicate the amorphous state of analysed MVPs without characteristic peak intensities. The amorphous state is convenient since it can provide very fast dissolution of herbal powders. The thermal behaviors of MVPs were similar. According to DSC curves (Fig. 4 (b)), water loss was detected below 100 °C, while no sharp endotherm peaks were detected which indicates an amorphous character without melting point of crystalline materials. According to literature reference, MD has a melting point at around 240 °C, ([https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB0379122.htm](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB0379122.htm)), which also disappeared after the spray drying procedure. The structural characterizations were repeated after

343 6 months and the character of the amorphous structure was unchanged, which confirmed its  
344 stability.

### 345 3.3. MVPs stability

346 The stability, particle size, morphology and rheological behaviour of powders are the main  
347 properties affected by moisture content [38]. The lowest moisture content that can be accomplished  
348 is favored in terms of adequate storage and manipulation. The most important shift occurs at the  
349 glass transition temperature (T<sub>g</sub>), which involves a second-order transition from a rubber-like  
350 liquid to a glassy solid state [39]. The main consequences of glass transition are the exponential  
351 decrease of molecular mobility and free volume, and an increase in viscosity at temperatures below  
352 T<sub>g</sub>, resulting in structural transformations that are time dependent [40]. Since water has very low  
353 T<sub>g</sub> (-135 °C), it is the major component responsible for significant T<sub>g</sub> depression in food material.  
354 Accordingly, water is considered to be a strong plasticizer in food systems [41] and that is why, if  
355 present in high amounts in produced dry powders, water could jeopardize powders quality by  
356 decrease of free flowing properties and increase of caking property.

357 Moisture contents in MVP 0% MD were 4.41% and 3.29% in MVP 10% MD. According to Ph.  
358 Eur. classification method regarding weight gain due to moisture sorption, obtained MVPs can be  
359 considered as moderately hygroscopic (2-15% w/w). The slight decrease in moisture content, with  
360 maltodextrin supplemented, was expected. The moisture contents of two obtained horehound  
361 powders were similar and lower than 5%, as in the case of *S. montana* powders. This low moisture  
362 content can provide sufficient shelf life of the dry extracts due to rare occurrence of microbiological  
363 contaminations [42]. Results in the same order of magnitude (3-5%) were previously observed [43]  
364 when moisture content of instant tea powder was evaluated. The moisture content of *A. millefolium*  
365 powders (6.10-7.68%) showed to be higher than in horehound powders [14]. Literature review  
366 supports the hypothesis that there is an effect of moisture content on the physico-mechanical  
367 properties of powders. In pharmaceutical industry, microcrystalline cellulose is a common tableting  
368 excipient. The moisture content of microcrystalline cellulose is about 3 to 4% which is in accordance  
369 with the United States Pharmacopeia monograph specifications which restrict moisture content not  
370 to be more than 5%. According to these data it is clear that MVPs are adequate not only for  
371 application in various food and dietary supplements but also in pharmaceutical industry.

372 Hygroscopicity is also a key property which represents the ability of powder to absorb the  
373 moisture from a high relative humidity environment [44]. Hygroscopicities of MVPs were similar,  
374 with no significant difference. After 48 h, hygroscopicity of investigated carrier-free powder was  
375 21.12% and 19.83% for 10% MD powder. Slight decrease in hygroscopicity was noticed with 10%  
376 MD supplementation, which is expected and consistent with moisture content, since MD increases  
377 the T<sub>g</sub> of liquid feed. They also observed that the lowest level of hygroscopicity was achieved when  
378 the highest maltodextrin concentrations were used [45]. Investigated powder properties are  
379 summarized in Table 3.

380 **Table 3.** Characterization of MVPs obtained from subcritical liquid feed.

Powder properties	MVP 0% MD	MVP 10% MD
Moisture content (%)	4.41	3.29
Hygroscopicity after 48 h (%)	21.12	19.83
WSI (%)	93.18	91.19
WAI (%)	1.80	1.97

### 381 3.4. Water solubility (WSI) and water absorption (WAI) indexes

382 The wettability is defined as the ability of a powder bulk to be penetrated by a liquid due to  
 383 capillary forces [46]. The process of dispersing a dry powder into a liquid can be classified into four  
 384 steps: wetting, submerging, dispersing and dissolving. The physical properties of a powder related  
 385 with these four steps are usually labeled under the term - instant properties [47]. The water  
 386 solubility index (WSI) is an unavoidable parameter in characterization of dry powders since it  
 387 demonstrates the powders ability to dissolve in water. Opposite to WSI, water absorption index  
 388 (WAI) shows powder ability to absorb water. High values of WSI and low values of WAI are  
 389 favourable. In investigated MVPs, WSI were similar and quite high (above 90%) (Table 3). This  
 390 outcome is expected since liquid feeds were prepared from subcritical extracts where water was  
 391 used as extractant. WSI slightly decreased as 10% MD was added. The concentration of MD affects  
 392 the size of the powdered particles and eventually decreases the solubility of the horehound  
 393 powder. The highest reported WSI for *S. montana* powder with 50% MD was 90.55%. In our study,  
 394 WAI had preferred low values (WAI= 0.0180 g/g of dry powder for carrier-free sample and WAI=  
 395 0.0197 g/g of dry powder for 10% MD sample) comparable with ones obtained for *S. montana*  
 396 powder with 50% MD [42]. In investigated *A. millefolium* carrier-free and 10% MD powders, WSI  
 397 were above 70% while WAI were below 20% [14].

### 398 3.5. MVPs flow behavior properties

399 One of the most important parameters that characterize powders is definitely bulk density. The  
 400 powders have to meet bulk density targets to provide consistent weight during packaging [48]. The  
 401 higher bulk density and lower moisture content in powder bulk are desired properties for  
 402 packaging and storage [49]. The bulk density of the amaranthus powder increased with a higher  
 403 maltodextrin concentration [50]. There is a correlation between bulk density and particle size.  
 404 Particles with smaller size reduced the void spaces among them and arranged themselves in closer  
 405 form. Consequently, the lower particle size led to a higher bulk density [51]. The bulk densities in  
 406 investigated MVPs were 83.33 mg/mL in carrier-free powder and 86.96 mg/mL in 10% MD powder.  
 407 The bulk density was slightly increased with carrier supplementation which is in contrast with  
 408 published results about decreasing of bulk density of pomegranate powders when MD  
 409 concentration increased [52]. These values are magnitude of order of *S. montana* powder obtained  
 410 by adding 10% MD (82.4 mg/mL) [42]. The bulk density measured in *A. millefolium* powder with  
 411 10% MD was twice lower (41.31 mg/mL) than bulk densities of MVPs [14]. The cohesive powders  
 412 favor creation of an open structure supported by the interparticle forces. Consequently, the  
 413 outcome is a relatively low bulk density of powders [53]. In our case, MVP 0% MD showed good,  
 414 free flow character while MVP 10% MD showed improved cohesive forces between the particles,  
 415 however we can state that its flowability is passable (Table 4).

416 **Table 4.** MVPs flowability expressed by Carr index and Hausner ratio.

Sample	Carr Index (%)	Hausner Ratio	Flow character
MVP 0% MD	15.01	1.18	Good/free flow
MVP 10% MD	23.23	1.30	Passable/cohesive

### 417 3.6. Polyphenol content in MVPs

418 Polyphenols comprise one of the most diverse groups of secondary plant metabolites, which  
 419 possess a wide palette of biological activities, among them antioxidant, anti-inflammatory,  
 420 antibacterial, and antiviral functions stand out as most relevant [54]. In addition, a large pool of  
 421 preclinical research and epidemiological data confirm that plant polyphenols can decelerate the

422 progression of some cancers, reduce the risks of cardiovascular disease, neurodegenerative  
 423 diseases, diabetes and osteoporosis [55,56,57]. Since remarkable bioactive potential has been  
 424 attributed to polyphenolic compounds, it is necessary to determine their content in dry extracts  
 425 which could be further implemented in various pharmaceutical formulations and dietary  
 426 supplements.

427 In comparison with TP values obtained in 10% MD powders of two herbs, *Satureja montana*  
 428 and *Achillea millefolium*, total phenols in MVPs (TP=85.20 mg GAE/g DE in 0% MD sample; TP=72.98  
 429 mg GAE/g DE in 10% MD sample) were lower. Consequently, total flavonoids (TF=31.37 mg CE/g  
 430 in 0% MD sample; TF=26.59 mg CE/g in 10% MD sample) were also lower in relation to TF in *S.*  
 431 *montana* powder with 10% MD (TF=118.69 mg CE/g) [42,14]. Total flavonoids in rosemary powder,  
 432 obtained by spray drying of ethanolic extract, were comparable with TF values of MVPs [58]. The  
 433 contents of total phenols and flavonoids decreased with the maltodextrin supplement due to  
 434 dilution of bioactive compounds encapsulated in powder with inert carrier. Some authors also  
 435 investigated recovery of encapsulated polyphenols in two *Salvia officinalis* powders (carrier-free and  
 436 20% MD). The powders were produced by spray drying of subcritical water extracts. They reported  
 437 slightly higher values for total phenols (TP= 106.26 mg GAE/g for 0% MD sample and TP= 91.35 mg  
 438 GAE/g for 20% MD sample) and total flavonoids (TF= 58.97 mg CE/g for 0% MD sample and TF=  
 439 56.98 mg CE/g for 20% MD sample). However, they also observed that in extracts obtained by SWE  
 440 using water as extractant, significantly lower selectivity towards polyphenols was demonstrated in  
 441 relation to aqueous ethanol applied as extractant in other modern extraction techniques [59].  
 442 Polyphenol contents and antioxidant activities for two MVPs are presented in Table 5.

443 **Table 5.** Polyphenol content (total phenols (TP) and total flavonoids (TF)) and antioxidant  
 444 activity of MVPs determined by DPPH and reducing power assays.

Sample	Total solids [mg/mL]	TP [mg GAE/g]	TF [mg CE/g]	IC <sub>50</sub> [mg/mL]	EC <sub>50</sub> [mg/mL]
MVP 0% MD	43.7	85.1975	31.3668	0.0204	0.0708
MVP 10% MD	52.8	72.9810	26.5851	0.0188	0.0756

445 In order to identify dominant phenolic compounds in MVPs, HPLC analyses were engaged  
 446 and the results are presented in Table 6. The major compounds are phenolic acids (ferulic acid, p-  
 447 coumaric acid and caffeic acid) and flavonoids rutin and hyperoside. It could be observed that in all  
 448 cases, recoveries of both phenolic acids and flavonoids were distinctly higher when maltodextrin  
 449 was added as carrier (Table 6). In case of rutin, addition of 10% MD resulted in more than 4-fold  
 450 increase of rutin content. This suggests that MD addition protects bioactives from thermal  
 451 degradation.

452 **Table 6.** Polyphenol content in MVPs obtained using HPLC-DAD

Sample	Ferulic acid	p-Coumaric acid	Caffeic acid	Rutin	Hyperoside
(µg/mL extract)					
0% MD MVP	48.77	26.42	14.27	134.46	17.43
10% MD MVP	70.69	49.61	20.96	584.55	33.28

### 453 3.7. Antioxidant activity

454 There is a discrepancy in the concentrations of polyphenols that appear effective *in vitro* and  
 455 the one that are measured *in vivo*, which are often of an order of magnitude lower. The potency of

456 nutraceuticals to prevent diseases depends on retaining the bioavailability of their active  
 457 ingredients [60]. Some authors investigated retention of antioxidant activity of the encapsulated  
 458 polyphenols of spray-dried grape seeds, apple skins and olive leaves extracts. They concluded that  
 459 there is a notable retention of antioxidant activity after encapsulation accomplished by spray  
 460 drying [61]. In order to test if microencapsulation by spray drying might be useful to protect  
 461 polyphenols of horehound, two in vitro assays, DPPH and reducing power, were employed.  
 462 Antioxidant activities of MVPs, expressed as IC<sub>50</sub> (IC<sub>50</sub>=20.4 µg/mL 0% MD sample; IC<sub>50</sub>=18.8  
 463 µg/mL for 10% MD sample) were lower than the antioxidant activities of herbal powders of *A.*  
 464 *millefolium* and *S. montana* obtained in our previous studies [42,14]. However, obtained  
 465 antioxidant activities for horehound powders were in line with IC<sub>50</sub> values (ranging from 17.6 to  
 466 24.4 µg/mL) of spray dried rosemary hydroalcoholic extract [58]. Reducing power of horehound  
 467 powders, expressed as EC<sub>50</sub> value, were 70.8 µg/mL in 0% MD sample and 75.6 µg/mL in 10% MD  
 468 sample.  
 469

#### 470 4. Conclusions

471 Spray drying is a well-recognized technique for transferring fruit juices into powders but not so  
 472 common when liquid feed is water/hydroalcoholic extract of plant material. The major challenge in  
 473 spray drying is creation of standardized herbal dried extract that has the required content of active  
 474 compounds. Since herbal extracts contain a numerous chemical constituents and are inconsistent in  
 475 composition, it is particularly difficult to conform them to a standard. However, this study shows  
 476 that spray drying of a subcritical horehound extract can be used as a method of choice for obtaining  
 477 high quality powders which kept the amorphous structure constant after 6 months storage time.  
 478 Furthermore, recoveries of both phenolic acids and flavonoids were distinctly higher when 10%  
 479 maltodextrin was added as carrier, which suggests that maltodextrin addition protects bioactives  
 480 from thermal degradation. This is particularly emphasized in the case of rutin content which was 4-  
 481 fold higher when carrier was included. Considering the antiasthmatic activity of rutin, this study  
 482 could initiate developing of a dry powder inhalation formulation based on *M. vulgare* to treat  
 483 respiratory disorders.

484 **Author Contributions:** A.G., S.V., J.V. S.J. and R.A. designed the experiments. J.V. S.J., A.G, R.A., P.S-R and  
 485 M.T. performed the experiments. A.G., S.V., J.V. S.J. and R.A. analyzed the data. All the authors discussed and  
 486 planned the paper. J.V. S.J., A.G, R.A., P.S-R and M.T. drafted the manuscript. M.B. funding acquisition.

487 **Acknowledgments:** The financial support was presented by Bilateral collaboration between Hungary and  
 488 Serbia, grant number: HU-SERB bilateral cooperation, TÉT\_16-1-2016-0167 and as part of the “Atrium of  
 489 Knowledge” project co-financed by the European Union from the European Regional Development Fund and  
 490 the Operational Programme Competitiveness and Cohesion 2014-2020.

491 **Conflicts of Interest:** The authors declare no conflict of interest.

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