Optimization of spray drying conditions for production of *Achillea millefolium* extract powder

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Abstract

In this study, a spray drying process of yarrow (*Achillea millefolium* L.) liquid extracts was optimized by using the response surface methodology. The study aimed to determine the influence of temperature (120-195 °C), liquid flow rate (3-16.5 cm^3 min^{-1}) and dry matter content in the liquid extract (0.3-1.5 %) on the yield of the drying process, the total polyphenols content and the antioxidant activity of the dry powder. Under the tested conditions the yield varied dramatically and ranged from 8 to 75 %, while the effects on the polyphenol content and antioxidant activity were lower. The optimized conditions for the maximum antioxidant activity and maximal yield of the dried extract were as follows: temperature of 130 °C, liquid flow rate of 7.5 cm^3 min^{-1} and dry matter content of 1.2 %. Under the optimal conditions, the yield was 66 %, while there was a slight decrease in the polyphenol content in the dried extract as compared to that in the liquid extract (145 mg of gallic acid equivalents [GAE] per g of the total dry matter *vs*. 152 mg GAE g^{-1}, respectively). Consequently, antioxidant activity of the dry powder was only slightly reduced as compared to that of the liquid extract (DPPH neutralization was 58 *vs*. 64 %, respectively). The dried yarrow powder preserved its antimicrobial activity against pathogenic bacteria *Staphylococcus aureus* (MIC value of 10 mg cm^{-3}) and *Pseudomonas aeruginosa* (MIC value of 20 mg cm^{-3}).

Keywords: yarrow; polyphenols; antioxidant activity; antimicrobial activity.

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1. INTRODUCTION

Medicinal plants are widely used in medicine, cosmetics and human nutrition. The *World Health Organization* (WHO) estimates that 65 to 80 % of the world population uses medicinal plants as a primary form of health care [1]. The positive effect of medicinal herbs is explained by the presence of bioactive compounds, secondary metabolites, which can exhibit various biological activities, such as antimicrobial, antioxidant, anti-inflammatory, cytotoxic, diuretic, spasmolytic and many other effects [2]. Plant organisms contain pharmacologically active compounds, while the efficacy of the produced plant preparations depends on the preparation method, which can dramatically influence the quality of the final product. It is well established that different extraction methods, different solvents and drying conditions are investigated for maintaining the quality of herbal products [3-5].

Achillea millefolium L. (yarrow) is among the most widely used medicinal plants belonging to the family of *Asteraceae*. Yarrow is commonly found in Europe, North Africa, North America, and Asia [6]. The plant is used in the folk medicine to treat respiratory tract infections, diabetes, kidney disorders, to improve digestion and as an appetite-enhancing drug [6,7]. Yarrow has proven antimicrobial, antitumor, antioxidant and anti-inflammatory properties due to the presence of bioactive compounds that primarily belong to the group of polyphenols [8-10], which are mostly phenolic acids and flavonoids [8,11].

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In order to prepare herbal products, medicinal herbs are subjected to various processes including drying, mechanical processing (grinding, sieving and separation), distillation and extraction in various solvents. Extraction of the pharmacologically and biologically active ingredients from the plant material yields liquid extracts, which upon drying produce semi-solid or solid-consistency preparations [5,12]. Dried plant extract powder is the most common and widely used form of medicinal plant products because the solid form offers higher physicochemical and microbiological stability than the corresponding liquid form, while transport and storage become easier [13-16]. Several techniques including spray drying, freeze-drying, spouted bed and fluidized bed drying can be used to obtain powder extracts [17,18]. Spray drying is a commonly used technique that may be employed to produce powders of fine particles with higher concentrations and stability of active substances [19,20]. This technology offers high production rates and lower operating costs in comparison to alternatives, and it is a single step drying process [21,22]. Spray drying consists of atomization of feed, droplet-air contact, drying, and separation of the dried product from the gas phase [23]. The feed solution is dispersed into droplets that are rapidly dried due to the high surface area and intimate contact with the drying gas. The obtained dried powder is protected from overheating by rapid removal from the drying zone and the final product can be removed from the gas stream by cyclones and/or filters [19].

In order to improve the spray drying process and avoid problems such as low yields, sticking and high moisture content in the obtained particles, operating variables (inlet air temperature, the flow rate of the drying gas and liquid feeds, and atomizing conditions) should be optimized [21,24,25]. In addition, during the drying process degradation of bioactive compounds should be prevented by selecting appropriate conditions.

The aim of this study was to optimize spray drying conditions to produce *A. millefolium* L. dry extract with preserved bioactive components belonging to the group of polyphenols. Influence of spray drying parameters (temperature, liquid flow rate and dry matter content in the dried liquid extract) on the yield, polyphenol content and antioxidant activity of the dry powder were modeled by using the response surface methodology (RSM). Upon optimization, antimicrobial activity of the dry powder produced under optimal condition was tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

2. MATERIALS AND METHODS

2. 1. Materials

For this study, waste yarrow was used as a raw material, which was obtained from the Institute of Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia. The experimental material was a dust waste discarded after production of tea, for which yarrow was grind and fractionated and the plant material with particle sizes lower than 0.3 mm was discarded as dust. The yarrow dust was stored in the dark, at room temperature in paper bags. Ethanol (96 %) supplied by Zorka Pharma, Serbia was used as a solvent for the extraction process. Folin-Ciocalteu reagent, methanol, gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Aldrich, while sodium carbonate was purchased from Lach-Ner, Czech Republic. Resazurin was purchased from Acros Organics, Geel, Belgium. All chemicals were the highest commercial grades and were used as received without any further purification.

2. 2. Microwave-assisted extraction (MAE) of waste yarrow

Liquid yarrow extracts were prepared by microwave-assisted extraction (MAE) at conditions previously optimized to maximize the yield of total polyphenols and biological activity in a scaled-up process [11]. In short, the waste yarrow was mixed with 70 % ethanol at a liquid/solid ratio of 40 $cm^3\ g^{-1}$ in total volume of 420 cm^3. Extraction was performed in a microwave oven (Samsung Electronics Euro QA Lab, United Kingdom) for 30 s at the microwave power of 600 W. The obtained liquid extract was separated from solids by filtration by using a vacuum pump (V-700, Buchilabortechnik AG, Fanil, Switzerland) and concentrated in a rotary evaporator (Buchilabortechnik AG, Fanil, Switzerland) at a vacuum pressure of 150 mbar and temperature of 60 °C. After the evaporation process, dry matter content in the extracts was determined by using a moisture analyser (Kern MLS-A, Balingen, Germany).

2. 3. Experimental design of spray drying

In order to determine the optimal conditions for spray drying resulting in the maximum yield, total polyphenol content and antioxidant activity, RSM and central composite design (CCD) were used. RSM is the most commonly used mathematical and statistical method for modeling and analyzing a process (when the response of interest is affected by various variables and the objective of the method is to optimize the response) [26]. According to the experimental design, the effect of process factors was analyzed within following range: inlet air temperatures (95-195 °C), liquid flow rates (3 – 21 cm^3 min^{-1}) and dry matter content in the liquid extracts (0.3 - 1.5 %). A statistical package, Design-Expert (Version 8, Stat-Ease, Inc., Minneapolis, United States) was used for experimental design and the experimental data analysis. Yarrow liquid extracts (200 cm^3) were fed into a drying chamber by a peristaltic pump connected to a two-fluid atomizer linked to an air compressor (Büchi Mini Spray Dryer B-290, Switzerland). The compressor forces air into the outer tube of the nozzle which causes the liquid to emerge as a fine, atomized spray. The feed system of drying gas constituted of a blower and an air filter. Dried product was collected in a cyclone. After performing 23 predicted experiments of spray drying, 8 series of experiments yielded products of poor quality (insufficiently dried material was obtained). These experimental points were eliminated from the calculations in the model, and the historical data design with 15 experimental runs was selected for modeling and analysis of the relationships between the inlet parameters and the responses. As a result, the process variables were reduced to the following ranges: temperature, 120-195 °C (*A*); liquid flow rate, 3-16.5 cm^3 min^{-1} (*B*) and dry matter content, 0.3-1.5 % (*C*). The influence of these variables on three system responses (yield (Y_1), total polyphenol content (Y_2) and antioxidant activity of the dried extract powders (Y_3)) was evaluated (Table 1). The experimental data were fitted to a cubic polynomial model, and by applying the analysis of variance (ANOVA) adequacy and statistical significance of the proposed mathematical models were assessed.

Table 1. Design matrix and corresponding responses (A is the temperature, B is the liquid flow rate, C is the dry matter content, Y_1 is the yield, Y_2 is the total polyphenol content, Y_3 is the antioxidant activity of the dried extract powders)

Run	Independent variable			Responses		
	A / °C	*B* / cm^3 min^{-1}	*C* / %	Y_1 / %	Y_2 / mgGAE g^{-1}	Y_3 / %
1	120	7.5	0.6	62	145	41.5
2	120	7.5	1.2	65	137	53
3	145	3	0.9	41	148	47
4	145	3	0.9	27	141	54
5	145	12	0.3	20	140	61
6	145	12	0.3	25	137	59
7	145	12	0.9	38	155	57
8	145	12	0.9	40	157	58
9	145	12	0.9	37	149	59
10	145	12	1.5	8	131	47
11	145	12	1.5	11	137	49
12	170	7.5	0.6	39	145	64
13	170	7.5	1.2	75	135	41
14	170	16.5	0.6	45	141	42
15	195	12	0.9	65	154	62

By using backward elimination, terms in the models that had a non-significant effect on a response ($p > 0.1$) were removed from the equations and the experimental data were refitted. The models were validated by performing additional experiment using the optimal conditions predicted by the RSM. The fitted polynomial equations were presented as surface plots in order to visualize the relationship between the independent variables and the response variables.

2. 4. Yield

The drying yield was calculated as the ratio of the powder dry weight and the dry weight of the liquid extract and expressed as %.

2. 5. Determination of the total polyphenol content

The total polyphenol content was quantified colorimetrically by using a modified Folin-Ciocalteu method and gallic acid (GAE) as a reference standard [27]. For determination of phenolic content, 0.1 cm^3 of the liquid yarrow extract or dissolved dry extract at the final concentration of 10 mg cm^{-3} was combined with 0.5 cm^3 of the Folin-Ciocalteu reagent and 6 cm^3 of distilled water. The mixture was shaken for 60 s and subsequently, 2 cm^3 of 15 % Na_2CO_3 was added, and the mixture was additionally shaken for 30 s. Finally, distilled water was added to reach the final volume of 10 cm^3. After 2 h of incubation in the dark at room temperature, the absorbance was read at 750 nm by using a UV/visible spectrophotometer (Ultrospec 3300 pro, Amersham Bioscience, Sweden). The blank was prepared by using 70 % ethanol instead of a sample. Results were reported as mass of GAE per mass of the total dry matter. Three series of experiments were performed, and the results are presented as mean values.

2. 6. Determination of antioxidant activity

Antioxidant activity of the liquid and dried extracts was measured by using the DPPH radical-scavenging assay [28]. DPPH solution (0.2 mM) was prepared by dissolving the adequate amount of DPPH in methanol. To measure the DPPH neutralization capacity the total of 0.05 cm^3 of the liquid yarrow extract or dissolved dry extract in 70 % ethanol, at the final concentration of 1 mg cm^{-3}, was mixed with 3.95 cm^{-3} of methanol and 1 cm^{-3} of 0.2 mM DPPH methanol solution. The obtained solutions were vigorously shaken and kept in the dark at room temperature for 30 min. The absorbance was read against a blank (methanol) at 517 nm. A control sample was prepared by using 70 % ethanol solution instead of an extract. Results were expressed as inhibition percentages of free DPPH radicals relative to the control sample and calculated according to the equation:

$$\text{Inhibition} = \frac{A_c - A_s}{A_c} 100 \quad (1)$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. Three series of experiments were performed, and the results are presented as mean values.

2. 7. Determination of the minimum inhibitory concentration

The microdilution method was used to determine the minimum inhibitory concentration (MIC) of *A. millefolium* L. dry extract obtained under optimal drying conditions with resazurin as an indicator of cell growth. The dry yarrow extract was dissolved at the final concentration of 40 mg cm^{-3} in 20 % ethanol. Resazurin solution (6.75 g dm^{-3}) was prepared by dissolving the appropriate amount in distilled water, vortexed and filter sterilized (0.45 μm filter). The Gram-positive bacterium *Staphylococcus aureus* ATCC 25923 and Gram-negative bacterium *Pseudomonas aeruginosa* PAO1 were grown in Trypton soy broth (TSB, Torlak, Serbia). TSB was prepared by dissolving 30 g of the commercially available TSB powder and 6 g of yeast extract in 1 dm^3 of distilled water. The medium was boiled for 15 min and then sterilized in an autoclave for 30 min at 120 °C.

The nutrient medium (TSB 0.1 cm^3) was poured into all wells of a 96 well microtiter plate, followed by addition of 0.1 cm^3 of yarrow extracts in the first column (therefore the concentration of the extract in the first column was 20 mg of total dry matter per cm^3). A series of double dilutions was achieved by transferring 0.1 cm^3 of the mixture of medium and yarrow extracts to the next column so that the final volume in each well was 0.1 cm^3. Eight series of dilutions were made so that the yarrow concentration varied in the range from 0.16 to 20 mg of the total dry matter per cm^3. Next, 0.01 cm^3 of the resazurin solution and 0.01 cm^3 of bacterial suspension were added to each well so that the final concentration was 5×10^6 CFU cm^{-3}. Controls were prepared in parallel with the samples. The positive control contained 0.1 cm^3 of TSB medium, 0.01 cm^3 of resazurin and 0.01 cm^3 of bacterial suspension, and the negative control contained 0.05 cm^3 of TSB medium, 0.05 cm^3 of yarrow extract and 0.01 cm^3 of resazurin. Microtiter plates were incubated for 24 h at 37 °C. After the incubation, the minimum inhibitory concentration was determined visually based on the colour. Any change in colour from purple to pink or colourless was considered as positive (indicative of microbial growth). The lowest concentration at which colour change not was observed was adopted as the MIC value [29]. The tests for both strains were performed twice, and the results are presented as mean values.

3. RESULTS AND DISCUSSION

3. 1. Model determination

Multiple regression analysis was applied to the experimental data resulting in correlations between the spray drying parameters (temperature, liquid flow rate and dry matter content) and the yield, polyphenol content and antioxidant activity of the powders. By application of the analysis of variance (ANOVA) the adequacy and statistical significance of all models was assessed. Two measured responses, *i.e*. the yield and the antioxidant activity of the dry powders fitted the third order polynomial equation. The total polyphenol content in dry powders was not significantly influenced by the drying conditions, and only a mean value for this response was obtained.

Values of the coefficient of determination for the yield and antioxidant activity between the response function and the experimentally obtained results were high amounting to 0.98 and 0.90, respectively. "Adequate Precision" measures the signal to noise ratio, and a ratio greater than 4 is desirable. The ratio of 16.4 and 12.2 for the yield and antioxidant activity, respectively, indicated an adequate signal. The lack of fit of both models was not significant, and after removing the effect of non-significant factors by using backward reduction, the relationship between the two responses and tested variables could be explained by the following equations:

$$Y_1 = 22.75 - 24.50A - 38.42B - 28.83C - 128.25AB - 67.00AC - 113.17BC + 66.75A^2 - 22.33C^2 - 183.50ABC \quad (2)$$

$$Y_3 = 56.54 + 4.38A + 6.37B + 18.72AC + 30.20BC + 90.64ABC \quad (3)$$

where Y_1 (the yield) and Y_3 (the antioxidant activity) are the responses, and the independent variables are *A* (temperature), *B* (liquid flow rate) and *C* (dry matter content).

3. 2. Influence of drying parameters on the dried extract yield

High yield is one of the objectives of spray drying processes. Under the tested drying conditions, the yield varied from 8 to 75 % (Table 1). The highest product yield was 75 % at an inlet air temperature of 170 °C, liquid flow rate of 7.5 $cm^3\ min^{-1}$ and dry matter content of 1.2 % (run 13). According to the analysis of the experimental data three linear terms (*A*, *B* and *C*), three interaction parameters (*AB*, *AC* and *BC*), two quadratic terms (A^2 and C^2) and one cubic interaction parameter (*ABC*) had significant effects on the dried extract yield. Positive sign of the quadratic term of temperature (Eq (2) A^2) indicated presence of a function minimum, while the negative sign of the quadratic term in relation to dry matter content (C^2) indicated existence of a function maximum. Interactions between the temperature and the liquid flow rate, between the temperature and the dry matter content, and between the liquid flow rate and the dry matter content had an antagonistic effect on the dried extract yield. In order to visualize influence of the interacting variables (*AB*, *AC* and *BC*) on the dried extract yield, response surface plots were created (Fig. 1).

The dried extract yield increased when the dry matter content was in the range 0.9-1.2 % and achieved the highest value at 1.2 % of dry matter content (Table 1, run 13). It is well documented in the literature that when feed concentration increases, the amount of moisture is decreasing together with the energy needed to evaporate water [30]. With the higher feed concentration, more complete drying prevents sticking of the product resulting in better separation [31,32]. Furthermore, more concentrated solutions provide larger particles with better cyclone separation [33,34]. However, further increase in the feed concentration (in this study up to 1.5 % of the dry matter content) can produce even larger particles, which are drying more slowly than the smaller ones, obtained at lower solid contents in the final product. This phenomenon increases deposition in the drying chamber and hence reduces the yield [35].

The maximum dried extract yield was obtained at the liquid flow rate of 7.5 $cm^3\ min^{-1}$ (Table 1, run 13). Existence of the function maximum could be explained by the liquid residence time. In specific, as the liquid flow rate is decreased, the liquid residence time is increasing providing droplets the chance for drying. Correspondingly, as the flow rate is increased, the residence time is decreased resulting in incomplete drying and poorer yield. The same trend as observed in our study was reported by others [36,37]. Both research groups favored lower flow rates that allowed more complete water evaporation and decreased the probability of dispersion and condensation on the chamber walls, which consequently resulted in better process yields. Moreover, in another study it was concluded that the pump rotation speed could induce a negative effect on the yield since higher flow rates can cause clogging of the atomization nozzle,

resulting in lower drying mass yields [38]. Negative influence of higher flow rates on the yield, could be also explained by reduction of the outlet air temperature, and consequent lower thermal energy supply that was probably insufficient for complete drying. In this case, sticking occurred in the drying chamber, lowering the yield and increasing the moisture content, in line with previous reports [39].

Temperature had a different influence on the drying yield within the tested temperature range (up to 170 °C). Figure 1 shows that high yields are obtained in the drying process under two scenarios: 1) at low temperature, lower liquid flow rate and high dry matter content, or 2) at high temperature, low liquid flow rate and high dry matter content. In general, at a temperature of 170 °C the yield reached the maximum, while a slightly lower yield was observed at a temperature of 120 °C with the same dry matter content in the liquid extract. In our study, at lower temperatures and very high liquid flow rates, the particles were sticking together, which is in line with the observation of others [39,40]. When higher inlet air temperatures are used, heat and mass transfers are enhanced so that the probability of moist particles to stick to the drying chamber wall is decreasing [41-45].

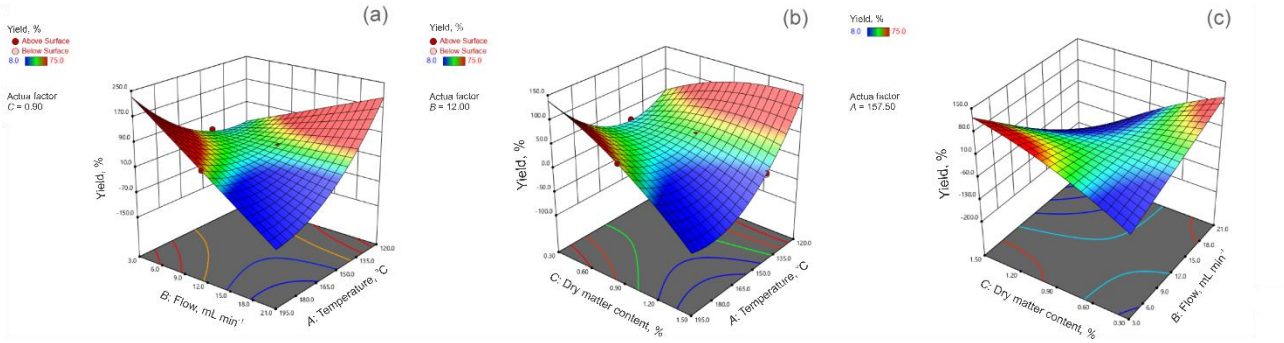

Figure 1. Surface plots of interactive effects on the dried extract yield of drying parameters: a) temperature and liquid flow rate (AB), b) temperature and the dry matter content (AC) and c) liquid flow rate and the dry matter content (BC)

3. 3. Influence of drying parameters on the total polyphenol content

Total polyphenol contents in the dried extracts obtained under the tested spray drying conditions were in the range of 131-157 mg GAE g^{-1} dry matter, which was comparable to the total polyphenol content in the starting liquid extract (feed) amounting to 152 mg GAE g^{-1} dry matter. Although it is typical to observe a slight decrease in the polyphenol content in the powder extracts as a result of the exposure to high temperatures [46,47], selection of relatively mild drying conditions in this study apparently prevented significant polyphenol degradation.

3. 4. Influence of drying parameters on antioxidant activity

Antioxidant activity of dry extracts was significantly influenced by two linear (*A*, *B*), two interaction parameters (*AC*, *BC*), and one cubic interaction parameter (*ABC*). The Eq. (3) shows that both linear parameters (temperature and liquid flow rate) had positive effects on antioxidant capacity, indicating that the higher antioxidant activity of the dried extract was achieved with the increase in these factors. Interactions between the temperature and the dry matter content, between the liquid flow rate and the dry matter content, and between all tested parameters (*ABC*) had synergetic and positive effects on the antioxidant activity. In order to visualize the influence of the interacting variables on the antioxidant activity, response surface plot was created (Fig. 2).

Although there was significant influence of the drying parameters on the antioxidant activity of the dried extracts, it should be stressed that compared to the antioxidant activity of the liquid extract (64 % DPPH scavenging activity), only a slight change in the antioxidant activity after the drying process was observed.

Antioxidant activities of the obtained powders correlate with the total polyphenol content. Souza et al. reported that high temperatures generate higher water evaporation; therefore, drying is more rapid, and the loss of labile compounds is lower [48]. There are also examples of increased total polyphenol content after heat treatment of various food products: green pepper, green beans, spinach [49], or onion powder [50]. This increase is attributed to the liberation of phenolic compounds by the cleavage of esterified and glycosylated bonds [50]. While in this study negligible

effects of the drying conditions on the total polyphenol content were observed, the observed relative decrease in the DPPH scavenging activity could be attributed to some subtle changes in the chemical nature of the polyphenols. Similar results of slight decrease of antioxidant activity upon drying were noted by others [51,52].

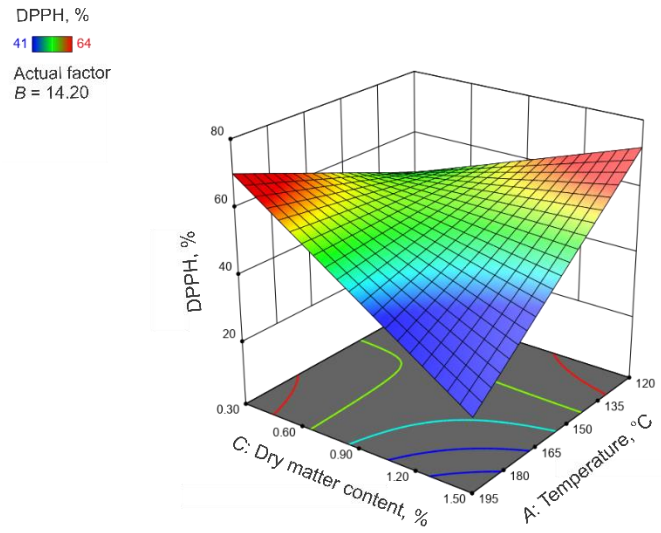

Figure 2. Surface plot of interactive effects of temperature and the dry matter content (AC) on the total antioxidant activity

3. 5. Validation of the models

The objective of this study was to optimize spray drying conditions for obtaining the maximal yield of yarrow powder extracts with the maximal antioxidant activity. The optimal conditions were determined by using the desirability function approach yielding: 130 °C; 7.5 cm^3 min^{-1} the liquid flow rate and 1.2 % of dry matter content in the feed. Predicted values for the two outcomes were the following: the antioxidant activity of 57 %, and maximal yield of 61 %. Measured values for dry extracts obtained under the optimal conditions fitted within the 95 % Pis for all parameters and were close to the most probable predicated values: the antioxidant activity (58 %) and dried extract yield (66 %). The DPPH radical scavenging activity of the dry extract obtained under the optimal conditions was 58 %, which marked a slight decrease in the radical scavenging activity as compared to that of the liquid extract that valued 64 %. Compared to the polyphenol content in the liquid extract (152 mg GAE g^{-1}), there was a slight decrease in the polyphenols content after drying of extract (145 mg GAE g^{-1}). This result indicated a more significant reduction in antioxidant capacity then that in the total polyphenol content. About 91 % of antioxidant capacity was retained after the drying process, while 95 % of total polyphenols were preserved in the dried extract.

3. 6. Antimicrobial activity of liquid and dried extracts

Antimicrobial activity of medicinal plants results from the combined effect of compounds, mostly polyphenols. In this study we evaluated the antimicrobial activity of the dry powder obtained under optimal conditions against two pathogens. The results showed that the antimicrobial activity of yarrow extract against *S. aureus* and *P. aeruginosa* was preserved after the drying process. Sensitivity of *P. aeruginosa* was similar to the liquid and dry extracts (MIC value of 20 mg cm^{-3}), while, in line with the slight decrease in the antioxidant activity, the dry powder was slightly less potent when suppressing the growth of *S. aureus* (MIC value of the liquid extract was 5 mg cm^{-3}, and 10 mg cm^{-3} for the dry extract).

It is well established that yarrow extracts exhibit antibacterial activity against Gram-positive bacterial species, while the results against Gram-negative species are less evident, which is in line with our findings [53]. The resilience of Gram-negative bacteria can be ascribed to the structure of their cell wall, which contains lipopolysaccharide, which is relatively impermeable to polyphenols [54,55]. The observation that Gram-negative bacteria are less sensitive to the *A. millefolium* antibacterial activity than Gram-positive was also shown by the research of Tajiket al. [56]. *A. millefolium* L. in addition to flavonoids that are typical for the genus *Achillea*, contains sesquiterpene lactones as active compounds [57]. It is believed

that the α-methylene lactone moiety of the molecule is responsible for the activity, because the activity disappears when the double bond is reduced, as shown in the case of α-methyl lactones from *Achillea atrata* [58].

4. CONCLUSION

The results reported in this study indicate the significant impact of spray drying conditions on properties of the obtained *Achillea millefolium* L. dry extract. RSM was successfully applied to model the effects of temperature, liquid flow rate and the dry matter content in the feed on the yield and antioxidant activity of the dry yarrow extract. The obtained mathematical models were applied to optimize drying conditions, which yielded production of a dry extract in which 91 % of the antioxidant potential was preserved. In addition to these properties, powder obtained under the optimal drying conditions, showed a preserved antimicrobial activity against *S. aureus* and *P. aeruginosa*. Therefore, it can be concluded that drying conditions predominantly influence the yield of the product with a moderate effect on the biological activity of the dry extract, under tested conditions. The obtained spray dried yarrow powder was green/brown in color with evident bioactive properties suitable for various medical or pharmaceutical applications. This research is useful as it identifies the most appropriate operating conditions for spray drying of liquid yarrow extracts, and it can be used as a reference for further research.

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REFERENCES

[1] Palhares RM, Gonçalves Drummond M, dos Santos Alves Figueiredo Brasil B, Pereira Cosenza G, das Graças Lins Brandao M, Oliveira G. Medicinal plants recommended by the world health organization: DNA barcode identification associated with chemical analyses guarantees their quality. *PloS one*. 2015;10:e0127866. https://doi.org/10.1371/journal.pone.0127866

[2] Popovic V, Tatic M, Sikora V, Ikanovic J, Drazic G, Djukic V, Mihailovic B, Filipovic V, Dozet G, Jovanovic Lj, Stevanovic P. Variability of yield and chemical composition in soybean genotypes grown under different agro-ecological conditions of Serbia. *Rom Agric Res*. 2016;33:29-39.

[3] Pham HNT, Nguyen VT, Vuong QV, Bowyer MC, Scarlett CJ. Effect of extraction solvents and drying methods on the physicochemical and antioxidant properties of *Helicteres hirsuta* Lour. leaves. *Technol*. 2015;3:285-301. https://doi.org/10.3390/technologies3040285

[4] Azmin SNHM, Manan ZA, Alwi SRW, Chua LS, Mustaffa AA, Yunus NA. Herbal processing and extraction technologies. *Sep Purif Rev*. 2016;45:305-320. https://doi.org/10.1080/15422119.2016.1145395

[5] Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products:a comprehensive review. *Chin Med-UK*. 2018;13:2-26. https://doi.org/10.1186/s13020-018-0177-x

[6] Lakshmi T, Geetha RV, Roy A, Kumar SA. Yarrow (*Achillea Millefolium Linn*.) a herbal medicinal plant with broad therapeutic use-A review. *Int J Pharm Sci Rev Res*. 2011;9:136-141.

[7] Cavalcanti AM, Baggio CH, Freitas CS, Rieck L, de Sousa RS, Da Silva-Santos JE, Mesia-Vela S, Marques MC. Safety and antiulcer efficacy studies of *Achillea millefolium* L. after chronic treatment in Wistar rats. *J Ethnopharmacol*. 2006;107:277-284. https://doi.org/10.1016/j.jep.2006.03.011

[8] Benedek B, Kopp B. *Achillea millefolium* L. s.l. revisited:Recent findings confirm the traditional use. *Wien Med Wochenschr*. 2007;157:312-314. https://doi.org/10.1007/s10354-007-0431-9

[9] Saeidnia S, Gohari AR, Mokhber-Dezfuli N, Kiuchi F. A review on phytochemistry and medicinal properties of the genus *Achillea*. *DARU*. 2011;19:173-86.

[10] Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. *Front Microbiol*. 2019;10:911. https://doi.org/10.3389/fmicb.2019.00911

[11] Milutinovic M, Radovanovic N, Corovic M, Siler-Marinkovic S, Rajilic-Stojanovic M, Dimitrijevic-Brankovic S. Optimisation of microwave-assisted extraction parameters for antioxidants from waste *Achillea millefolium* dust. *Ind Crop Prod*. 2015;77:333-341. https://doi.org/ 10.1016/j.indcrop.2015.09.007

[12] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J TraditComplem*. 2011;8:1-10. https://doi.org/10.4314/ajtcam.v8i1.60483

[13] Oliveira WP, Bott RB, Souza CRF. Manufacture of standardized dried extracts from medicinal Brazilian plants. *Dry Technol*. 2006;24:523-533. https://doi.org/10.1080/07373930600612073

[14] Bakowska-Barczak AM, Kolodziejczyk PP. Black currant polyphenols:their storage stability and microencapsulation. *Ind Crop Prod*. 2011;34:1301-1309. https://doi.org/10.1016/j.indcrop.2010.10.002
[15] Rocha GA, Favaro-Trindade CS, Grosso CRF. Microencapsulation of lycopene by spray drying:Characterization, stability and application of microcapsules. *Food Bioprod Process*. 2012;90:1-6. https://doi.org/10.1016/j.fbp.2011.01.001
[16] Yatsu FKJ, Borghetti GS, Bassani VL. Technological characterization and stability of *Ilex paraguariensis* St. Hil. Aquifoliaceae (Mate) spray-dried powder. *J Med Food*. 2011;14:413-419. https://doi.org/10.1089/jmf.2010.0044
[17] Souza CRF, Oliveira WP. Powder properties and system behaviour during spray drying of *Bauhinia forficata* link extract. *Dry Technol*. 2006;24:735-749. https://doi.org/10.1080/07373930600685905
[18] Cortes-Rojas DF, Oliveira WP. Physicochemical properties of phytopharmaceutical preparations as affected by drying methods and carriers. *Dry Technol*. 2012;30:921-934. https://doi.org/10.1080/07373937.2012.666608
[19] Amaro MI, Tajber L, Corrigan OI, Healy AM. Optimisation of spray drying process conditions for sugar nanoporousmicroparticles (NPMPs) intended for inhalation. *Int J Pharm*. 2011;421:99-109. https://doi.org/10.1016/j.ijpharm.2011.09.021
[20] Nedovic V, Kalusevic A, Manojlovic V, Petrovic T, Bugarski B. Encapsulation Systems in the Food Industry. In:Advances in Food Process Engineering Research and Applications, Boston, MA:Springer Nature America, Inc;2013:229-253. https://doi.org/10.1007/978-1-4614-7906-2_13
[21] Chegini GR, Ghobadian B. Spray dryer parameters for fruit juice drying. *World J Agric Sci*. 2007;3:230-236.
[22] Patel RP, Patel MP, Suthar AM. Spray drying technology:an overview. *Indian J Sci Technol*. 2009;2:44-47. https://doi.org/10.17485/ijst/2009/v2i10.3
[23] Andrea T, Marcela F, Lucia C, Esther F, Elena M, Simona M. Microencapsulation of lipase and savinase enzymes by spray drying using Arabic gum as wall material. *JEAS*. 2016;6:161-173. https://doi.org/10.1007/978-1-4614-7906-2_1310.4236/jeas.2016.64012
[24] Ozmen L, Langrish TAG. An experimental investigation of the wall deposition of milk powder in a pilot-scale spray dryer. *Dry Technol*. 2003;21:1253-1272. https://doi.org/10.1081/DRT-120023179
[25] Kudra T. Sticky region in drying:Definition and identification. *Dry Technol*. 2003;21:1457-1469. https://doi.org/10.1081/DRT-120024678
[26] Montgomery DC. Response Surface Methods and Designs. In:Design and analysis of experiments, Eighth Edition John Wiley and Sons, Inc, New Jersey;2005:478.
[27] Singleton V, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965;16:144-158.
[28] Lee SK, Mbwambo Z, Chung H, Luyengi L, Gamez E, Mehta R, Kinghorn A, Pezzuto J. Evaluation of the antioxidant potential of natural products. *Comb Chem High Throughput Screen.*1998;1:35.
[29] Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*. 2007;42:321-324. https://doi.org/10.1016/j.ymeth.2007.01.006
[30] Tontul I, Topuz A. Spray-drying of fruit and vegetable juices:Effect of drying conditions on the product yield and physical properties. *Trends Food Sci Tech*. 2017;63:91-102. https://doi.org/10.1016/j.tifs.2017.03.009
[31] Billon A, Bataille B, Cassanas G, Jacob M. Development of spray-dried acetaminophen microparticles using experimental designs. *Int J Pharm.* 2000;203:159-168. https://doi.org/10.1016/s0378-5173(00)00448-8
[32] Nguyen DQ, Nguyen TH, Mounir S, Allaf K. Effect of feed concentration and inlet air temperature on the properties of soymilk powder obtained by spray drying. *Dry Technol*. 2017;36(7):817-829. https://doi.org/10.1080/07373937.2017.1357040
[33] Prinn KB, Costantino HR, Tracy M. Statistical modeling of protein spray drying at the lab scale. *AAPS Pharm Sci Tech*. 2002;3:E4. https://doi.org/10.1208/pt030104
[34] Poozesh S, Jafari SM, Akafuah NK. Interrogation of a new inline multi-bin cyclone for sorting of produced powders of a lab-scale spray dryer. *Powder Technol*. 2020;373:590-598. https://doi.org/10.1016/j.powtec.2020.07.012
[35] Maurya M, Murphyb K, Kumarb S, Shib L, Leea G. Effects of process variables on the powder yield of spray-dried trehalose on a laboratory spray-dryer. *Eur J Pharm Biopharm*. 2005;59:565-573. https://doi.org/10.1016/j.ejpb.2004.10.002
[36] Gallo L, Llabot Juan M, Allemandi D, Bucala V, Pina J. Influence of spray-drying operating conditions on *Rhamnus purshiana* (Cascara sagrada) extract powder physical properties. *Powder Technol*. 2011;208:205-214. https://doi.org/10.1016/j.powtec.2010.12.021
[37] Telang AM, Thorat BN. Optimization of process parameters for spray drying of fermented soy milk. *Dry Technol*. 2010;28:1445-1456. https://doi.org/10.1080/07373937.2010.482694
[38] Toneli J, Park K, Negreiros A, Murr F. Spray-drying process optimization of chicory root inulin. *Dry Technol*. 2010;28:369-379. https://doi.org/10.1080/07373931003645017

[39] Mihajlovic T, Ibric S, Mladenovic A. Application of design of experiments and multilayer perceptron neural network in optimization of the spray-drying process. *Dry Technol*. 2011;29:1638-1647. https://doi.org/10.1080/07373937.2011.592960
[40] Tee LH, Chuah L, Pin KY, Abdull Rashih A, Yusof YA. Optimization of spray drying process parameters of *Piper betle*L. (Sirih) leaves extract coated with maltodextrin. *J Chem Pharm Res*. 2012;4:1833-1841.
[41] Goula AM, Adamopoulos KG. Spray drying of tomato pulp in dehumidified air:I. The effect on product recovery. *J Food Eng*. 2005;66:25-34. https://doi.org/10.1016/j.jfoodeng.2004.02.029
[42] Tonon RV, Brabet C, Hubinger MD. Influence of process conditions on the physicochemical properties of acai *(Euterpe oleraceae* Mart) powder produced by spray drying. *J Food Eng*. 2008;88:411-418. https://doi.org/10.1016/j.jfoodeng.2008.02.029
[43] Ramos FdeM, Oliveira CCMde, Soares ASP, Silveira Junior V. Assessment of differences between products obtained in conventional and vacuum spray dryer. *Food Sci Technol*. 2016;36 (4):724-729. https://doi.org/10.1590/1678-457X.09216
[44] Jafari SM, Ghalenoei MG, Dehnad D. Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder. *Powder Technol*. 2017;311:59-65. https://doi.org/10.1016/j.powtec.2017.01.070
[45] Pinon-Balderrama CI, Leyva-Porras C, Teran-Figueroa Y, Espinosa-Solis V, Alvarez-Salas C, Saavedra-Leos MZ. Encapsulation of Active Ingredients in Food Industry by Spray-Drying and Nano Spray-Drying Technologies. *Processes.* 2020;8 (8):889. https://doi.org/10.3390/pr8080889
[46] Eldridge JA, Repko D, Mumper RJ. Retention of polyphenolic species in spray-dried blackberry extract using mannitol as a thermoprotectant. *J Med Food*. 2014;17:1064-1069. https://doi.org/10.1089/jmf.2013.0177
[47] Bastias-Montes JM, Choque-Chavez MC, Alarcon-Enos J, Quevedo-Leon R, Munoz-Farina O, Vidal-San-Martin C. Effect of spray drying at 150, 160, and 170 °C on the physical and chemical properties of maqui extract (Aristotelia chilensis (Molina) Stuntz). *Chil J Agric Res*. 2019;79 (1):144-152. https://doi.org/10.4067/S0718-58392019000100144
[48] Souza V, Thomazini M, Balieiro J, Trindade C. Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification byproducts of the Bordo grape (*Vitis labrusca*). *Food Bioprod Process*. 2015;93:39-50. https://doi.org/10.1016/j.fbp.2013.11.001
[49] Turkmen N, Sari F, Velioglu YS. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chem*. 2005;93:713-718. https://doi.org/10.1016/j.foodchem.2004.12.038
[50] Sharma K, Ko EY, Assefa AD, Ha S, Nile SH, Lee ET, Park SW. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *J Food Drug Anal*. 2015;23:243-252. https://doi.org/10.1016/j.jfda.2014.10.005
[51] Fang Z, Bhandari B. Effect of spray drying and storage on the stability of bayberry polyphenols. *Food Chem*. 2011;129:1139-1147. https://doi.org/10.1016/j.foodchem.2011.05.093
[52] Edrisi M, Langrish TAG. Spray drying bioactive orange-peel extracts produced by Soxhlet extraction:Use of WPI, antioxidant activity and moisture sorption isotherms. *LWT - Food Sci. Technol*. 2016;72:1-8. https://doi.org/10.1016/j.lwt.2016.04.033
[53] Frey FM. and Meyers R. Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State. *BMC Complement Altern Med.* 2010;10:64. https://doi.org/10.1186/1472-6882-10-64
[54] Nostro A, Germano MP, D´Angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl Microbiol*. 2000;30:379-384. https://doi.org/1046/j.1472-765x.2000.00731.x
[55] Li Z-H, Cai M, Liu Y-S, Sun P-L, Luo S-L. Antibacterial activity and mechanisms of essential oil from *Citrus medica* L. *var. sarcodactylis*. *Molecules*. 2019;24:1577. https://doi.org/10.3390/molecules24081577
[56] Tajik H, Jalali FSS, Sobhani A, Shahbazi Y, Zadeh MS. *In vitro* assessment of antimicrobial efficacy of alcoholic extract of *Achillea millefolium* in comparison with penicillin derivates. *J Anim Vet Adv*. 2008;7:508-511.
[57] Lyss G, Glasl S, Jurenitsch J, Pahl HL, Merfort I. A sesquiterpene and sesquiterpene lactones from the *Achillea millefolium* group possess antiinflammatory properties but do not inhibit the transcription factor NF-γB. *Pharm Pharmacol Lett*. 2000;10:13-15.
[58] Aljancic I, Vajs V, Menkovic N, Karadzic I, Juranic N, Milosavljevic S, Macura S. Flavones and sesquiterpene lactones from *Achillea atrata* subsp. *multifida*:antimicrobial activity. *J Nat Prod*. 1999;62:909-911. https://doi.org/10.1021/np980536m

SAŽETAK

Optimizacija uslova sušenja raspršivanjem za proizvodnju praha ekstrakta *Achillea millefolium*

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(Naučni rad)

U radu je optimizovan postupak sušenja raspršivanjem tečnog ekstrakta hajdučke trave (*Achillea millefolium L.*) primenom metode odzivnih površina. Ispitan je uticaj temperature (120-195 °C), protoka tečnosti (3-16,5 cm^3 min^{-1}) i sadržaja suve materije u tečnom ekstraktu (0,3-1,5 %) na prinos sušenja, sadržaj polifenola i antioksidativnu aktivnost suvog ekstrakta. Pod ispitivanim uslovima prinos je drastično varirao (od 8 do 75%), dok je uticaj sušenja na sadržaj polifenola i antioksidativnu aktivnost dobijenog praha bio manji. Optimizovani uslovi za maksimalnu antioksidativnu aktivnost i maksimalan prinos suvog ekstrakta bili su sledeći:130 °C pri protoku od 7,5 cm^3 min^{-1} tečnog ekstrakta sa 1,2 % suve materije. Pri optimalnim uslovima prinos sušenja je iznosio 66 %, dok je zabeležen blagi pad sadržaja polifenola u suvom ekstraktu u poređenju sa tečnim ekstraktom (145 mg ekvivalenta galne kiseline [GAE] po g suve materije naspram 152 mg GAE g^{-1}, redom). Antioksidativna aktivnost suvog praha neznatno je smanjena u poređenju sa tečnim ekstraktom (neutralizacija DPPH (di(fenil)-(2,4,6-trinitrofenil)iminoazanijum) je bila 58 % naspram 64 %). U osušenom prahu hajdučke trave očuvana je antimikrobna aktivnost prema patogenim bakterijama *Staphylococcus aureus* (vrednost minimalne inhibitorne koncentracije (MIK) je bila 10 mg cm^{-3}) i *Pseudomonas aeruginosa* (MIK vrednost je bila 20 mg cm^{-3}).

Ključne reči: hajdučka trava; polifenoli; antioksidativna aktivnost;antimikrobna aktivnost