Enhancing the antimicrobial and antifungal activities of a coloring extract agent rich in betacyanins obtained from *Gomphrena globosa* L. flowers†

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Although less explored than beetroot (*Beta vulgaris* L.), the flowers of *Gomphrena globosa* L. are a very suitable source of betacyanins with strong pigmentation features, together with many other desirable bioactive properties. Thus, the aim of this study was to enhance the antimicrobial and antifungal activities of a pigmented extract obtained from *G. globosa* flowers by ultrasound assisted extraction (UAE). The procedure was supported with the application of the response surface methodology, a robust optimization technique that allows to study jointly the effects of several variables and responses. To enhance the antimicrobial (*Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium*) and antifungal (*Aspergillus flavus*, *Aspergillus niger*, *Penicillium ochrochloron* and *Penicillium verrucosum*) activities, the responses were evaluated in terms of the concentrations needed to obtain minimum inhibitory (MIC), minimum bactericidal (MBC) and minimum fungicidal (MFC) concentrations. It was found that the optimal UAE conditions were 10.8 min, 410.5 W, 57.8% of ethanol, and 5 g L^{-1} of the solid–liquid ratio providing the following response values: (1) from the studied species of bacteria, the MIC ranged from ~0.15 to 0.35 g L^{-1} and the MBC ranges were ~0.30 to 0.65 g L^{-1}; and (2) from the studied fungus species, the MIC ranged from ~0.20 to 0.30 g L^{-1} and the MFC ranges were ~0.40 to 0.65 g L^{-1}. The antibacterial activity dose levels were lower than the antifungal ones. In conclusion, the results obtained in this study highlight extracts from *G. globosa* flowers as natural sources of betacyanins with application as food colorants with important antimicrobial and antifungal activities.

1. Introduction

Infectious diseases caused by bacteria and fungus are the cause of serious illness and death of a high number of people every year.[1] Although the world statistical information shows over 50 000 deaths every year, the numbers could be higher

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due to the reduced number of reports available in developing countries.[2,3] The antimicrobial qualities of plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s.[4] The new advances have aided a more quick characterization and confirmed the potential efficacy of plant-based extracts against various bacterial and fungal pathogens.[5,6] Although most of the compounds found in plant-based extracts are not of much help in fighting the microbial diseases directly, they could be helpful at the industrial level as food additives to prevent spoilage, contamination and dissemination of microbial diseases (foodborne diseases). Industrial preservation and decontamination processes are applied to prevent the growth of microorganisms in food and facilitate its storage and transportation.[7–9] Therefore, some of the attention of the research community in food science has shifted to screen out natural food additives with a potential composition that could enhance antimicrobial protection in food products.

Nowadays, it is well known that a diverse diet based on a daily intake of fruits and vegetables is able to diminish the risk of developing chronic diseases.[10] This is due to the rich variety of bioactive compounds (such as vitamins, minerals, phenolics, among others) present in these foods, which are known to help maintain the redox homeostasis and play an important role in health promotion and disease prevention.[11]

Among these compounds are the natural pigments, which in addition to their coloring attributes display strong bioactive properties, especially antimicrobial capacity. Most well-known plant natural pigments consist of carotenoids, chlorophylls, anthocyanins, and betalains. Although the first two types are deposited in particular plastids in plant cells, the last two are located in the vacuoles.[12] Betalains are water-soluble vacuolar chromoalkaloids, that are subdivided according to their chemical structure into red-violet betacyanins and yellow betaxanthins.[13]

Betacyanins have been described as possessing many desirable properties, such as hypoglycaemic and hypolipidemic action, antimicrobial activity (antibacterial and antifungal),[14,15] and the ability to inhibit cell growth and induce ultra-structural changes and cell fragmentation on carcinogenic cells.[16,17] These red-purple pigments constitute an optimal example of natural additives used as food colorants, displaying noticeable coloring attributes, as well as various *in vitro* and *in vivo* biological properties.[18]

The most common source of betacyanins is beetroot (*Beta vulgaris* L.); nonetheless, the flowers of *Gomphrena globosa* L. are a suitable alternative source of these compounds despite being less explored.[19] This plant has been associated with several health promoting properties, being used in the treatment of several diseases, such as respiratory system diseases, diabetes, jaundice, hypertension, urinary system conditions, and kidney and prostate problems.[20] Nonetheless, due to its intense coloration of *G. globosa* flowers, it is also an excellent candidate for the recovery of compounds with coloring capacity. A recent study demonstrated that the ultrasound assisted extraction (UAE) technique was one of the most successful and efficient ways to produce a rich colorant extract for industrial applications.[21] In consequence, the potential natural additive derived from the flowers of *G. globosa* could be optimized in order to enhance the antimicrobial activity.

When developing natural additives, the costs of the processes is as relevant as many other factors, and could be a handicap that limits it applications due to the low production costs of artificial additives.[22,23] In consequence, to make the extraction process as economically viable as possible, optimizing the operational conditions is a crucial basic research step. Variables like time, energy and solvent proportion need to be jointly optimized in order to obtain productive results at an industrial level. Additionally, it is important to emphasize that the betacyanin concentrations used to achieve functional benefits are significantly different (normally higher) from those used for colorant purposes[24] and therefore, the variable

solid-to-liquid ratio becomes an essential part of the analytical work to be performed. In this regard the statistical approach of the response surface methodology (RSM) is a commonly used tool, improving and optimizing processes with multiple variables and/or factors.[25]

In conclusion, the aim of this study was to evaluate the possibility of obtaining extracts rich in betacyanins with enhanced antimicrobial activity using the flowers of *G. globosa*. In order to achieve this goal, the extracts were obtained by ultrasound assisted extraction (UAE) in order to maximize the antimicrobial activity, with the support of RSM.

2. Materials and methods

2.1 Sample collection

Gomphrena globosa L. was purchased from Ervital (Portugal), settled in a high diverse mountain region. The harvested plants were processed using in-storage and low temperature drying methods as previously described by the authors.[21]

2.2 Ultrasound assisted extraction

The UAE was carried out using an ultrasonic device (QSonica sonicators, model CL-334, Newtown, CT, USA), equipped with a digital timer, and working in the range of 100 to 500 W, at a frequency of 20 kHz. The used solvent volume was settled at 50 mL. The samples were extracted according to different conditions regarding: time (t, in min), power (P, in W), water–ethanol proportion (S, in %) and solid-to-liquid ratio (S/L, in g L^{-1}), as defined by the RSM design (Table A1, at the ESI†), and as previously published.[26] These extracts have also been formerly characterized and quantified in terms of their betacyanin profile.[21]

2.3 Evaluation of antimicrobial properties

2.3.1 Antibacterial activity. The antibacterial activity was assayed using a procedure previously described.[27] Two categories of bacteria were used: (1) Gram-positive bacteria, *Bacillus cereus* (clinical isolate, coded in this study as B$_1$) and *Listeria monocytogenes* (NCTC7973, B$_2$), and (2) Gram-negative bacteria, *Escherichia coli* (ATCC 35210, B$_3$) and *Salmonella typhimurium* (ATCC 13311, B$_4$).

2.3.2 Antifungal activity. The antifungal activity was assayed using the procedure previously described.[28] The following microfungi were used: *Aspergillus flavus* (ATCC1022, F$_1$), *Aspergillus niger* (ATCC6275, F$_2$), *Penicillium ochrochloron* (ATCC9112, F$_3$) and *Penicillium verrucosum* var. *cyclopium* (food isolate, F$_4$).

2.3.3 Microbial inhibition analysis. Bacterial and fungal organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Sinisa Stanković", University of Belgrade, Serbia.

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by methodologies previously described.[29] Streptomycin was used as the positive control for bacteria growth, while ketoconazole was used as the control for fungi growth. All the response variables were uniformed in grams of the extracted material per liter of the solution applied in the microbial tests (g L^{-1}). In all cases the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD).

2.4 Experimental design, model analysis and statistical evaluation

2.4.1 Experimental design. The influence of different independent variables was investigated using one-factor-at-a-time to select the significant ones and determine the preliminary range of the processing variables. Based on these experimental results (data not shown), the selected significant variables for the RMS design were: X_1 (time in min), X_2 (power in W), X_3 (ethanol content in %) and X_4 (solid/liquid ratio in g L^{-1}). Therefore, the combined effect of these four variables on the antimicrobial activity was studied using circumscribed central composite design (CCCD) as proposed by Box.[30] The responses were solved using 28 independent combinations and four replicates as the center of the experimental design. In this design, the points of experiments are generated on a sphere around the center point. The center point is supposed to be an optimum position for the response and is repeated to maximize the prediction.[21] This design also requires five levels for each factor. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. A detailed description of the mathematical expressions to calculate the design distribution and to decode and code the ranges of the tested variables is presented in Table A1 (ESI†).

2.4.2 Mathematical model and responses used. The response surface models were fitted by means of least-squares calculation using the following second-order polynomial equation:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2 \quad (1)$$

where Y is the dependent variable (response variable) to be modelled, X_i and X_j define the independent variables, b_0 is the constant coefficient, b_i is the coefficient of linear effect, b_{ij} is the coefficient of interaction effect, b_{ii} is the coefficient of quadratic effect and n is the number of variables. The dependent variable responses comprise: (1) the antibacterial criteria (MIC and MBC, in g L^{-1}) for all the bacteria strains (B$_{1-4}$), and (2) the antifungal criteria (MIC and MFC, in g L^{-1}) for all fungal strains (F$_{1-4}$).

2.4.3 Procedure to optimize the variables to a maximum response. For response optimization, a maximization process was applied using a simple method to solve non-linear

problems.[31,32] Constraints were imposed to the variable coded values to avoid unnatural conditions (*i.e.*, times lower than 0).

2.5 Fitting procedures and statistical analysis

Fitting procedures, coefficient estimates and statistical calculations were performed as previously described.[33] In brief, (a) the coefficient measurement was performed using the non-linear least-squares (quasi-Newton) method provided by the macro "*Solver*" in Microsoft Excel,[34] which allows minimizing the sum of the quadratic differences between the observed and model-predicted values; (b) the coefficient significance was evaluated using the "*SolverAid*" to determine the parametric confidence intervals.[35] The non-statistically significant terms (*p*-value > 0.05) were dropped to simplify the model; and (c) the model reliability was verified using the following criteria: (i) the Fisher *F*-test ($\alpha = 0.05$) was used to determine whether the constructed models were adequate to describe the observed data; (ii) the "*SolverStat*" macro was used for the assessment of parameter and model prediction uncertainties;[36] and (iii) the R^2 was interpreted as the proportion of variability of the dependent variable explained by the model.

3. Results and discussion

3.1 Optimization of the extraction of compounds by RSM for enhancing antimicrobial activity

3.1.1 Development of the theoretical response surface models and statistical verification. Descriptive empirical mathematical models were developed to assist the comprehension of the potential cause of variation in the response, and to explain how much each factor contributes to that variation.[34,37] This was achieved by statistically relating variation in the response variable to the variation in the explanatory variables, and obtaining the best fit by minimizing the deviations between the predicted and the actual response.[37] Fitting the models for the selected responses is crucial to elucidate how precisely the second-order polynomial model with interactions of eqn (1) (standard model used in RSM analysis) can predict the ideal variances. The models for each response were built by fitting the model of eqn (1) (independent variables in coded values) to the experimental values (Table 1) through nonlinear least-squares estimations. The parametric coefficient values of the resulting models are statistically assessed to determine its significant or non-significant (ns) effect using the "*SolverAid*" and "*SolverStat*" macros in Microsoft Excel at a 95% confidence level ($\alpha = 0.05$).[35,36] Those that showed ns coefficient estimations are removed and the fitting procedure is restarted; afterwards the process is repeated until all the parameters show significant coefficient estimations ($\alpha = 0.05$). The final parametric values achieved for all experimental responses of Table 1 are presented in Table 2. The parametric values translate the response patterns and show the complexity of the possible interactions between variables. Under RSM analysis, the obtained model coefficients (Table 2) are empirical and cannot be associated with physical or chemical significance.

Table 1 Results of the RSM experimental plan for the optimization process and independent variables of time (t), ethanol content (S), power (P) and solid–liquid ratio (S/L) of the ultrasound system. Responses comprise the antibacterial criteria (MIC and MBC) for all the bacteria strains (*Bacillus cereus* (B₁), *Listeria monocytogenes* (B₂), *Escherichia coli* (B₃) and *Salmonella typhimurium* (B₄)) and antifungal criteria (MIC and MFC) of all fungal strains (*Aspergillus flavus* (F₁), *Aspergillus niger* (F₂), *Penicillium ochrochloron* (F₃) and *Penicillium verrucosum* var. *cyclopium* (F₄))

	Experimental design								Antibacterial activity								Antifungal activity							
	Coded values				Natural values				MIC				MBC				MIC				MBC			
	X_1	X_2	X_3	X_4	X_1 t, min	X_2 P, W	X_3 S, %	X_4 S/L, g L⁻¹	B_1, g L⁻¹	B_2, g L⁻¹	B_3, g L⁻¹	B_4, g L⁻¹	B_1, g L⁻¹	B_2, g L⁻¹	B_3, g L⁻¹	B_4, g L⁻¹	F_1, g L⁻¹	F_2, g L⁻¹	F_3, g L⁻¹	F_4, g L⁻¹	F_1, g L⁻¹	F_2, g L⁻¹	F_3, g L⁻¹	F_4, g L⁻¹
1	−1	−1	−1	−1	7	200	25	15	0.3	0.4	0.3	0.3	0.5	0.8	0.5	0.5	0.5	0.4	0.3	0.3	0.7	1.4	0.4	0.6
2	1	−1	−1	−1	17	200	25	15	0.4	0.5	0.3	0.3	0.6	1.0	0.6	0.5	0.5	0.4	0.3	0.6	0.8	1.2	0.5	1.0
3	−1	1	−1	−1	7	400	25	15	0.3	0.4	0.3	0.4	0.4	0.7	0.5	0.5	0.4	0.4	0.3	0.3	0.7	1.6	0.4	0.6
4	1	1	−1	−1	17	400	25	15	0.3	0.4	0.3	0.3	0.5	0.8	0.5	0.5	0.5	0.5	0.3	0.5	0.7	1.5	0.5	0.8
5	−1	−1	1	−1	7	200	75	15	0.3	0.3	0.3	0.3	0.4	1.1	0.6	0.5	0.5	0.3	0.4	0.5	0.7	1.3	0.6	1.0
6	1	−1	1	−1	17	200	75	15	0.3	0.4	0.4	0.3	0.5	1.1	0.9	0.5	0.3	0.3	0.4	0.6	0.7	1.2	0.5	1.1
7	−1	1	1	−1	7	400	75	15	0.3	0.4	0.3	0.4	0.4	0.7	0.4	0.5	0.4	0.4	0.5	0.5	0.8	1.1	0.7	0.9
8	1	1	1	−1	17	400	75	15	0.3	0.3	0.3	0.4	0.4	0.7	0.6	0.6	0.5	0.3	0.5	0.5	0.8	0.9	0.5	1.0
9	−1	−1	−1	1	7	200	25	35	0.4	0.4	0.3	0.4	0.6	0.7	0.7	0.5	0.5	0.5	0.3	0.2	0.9	1.4	0.3	0.6
10	1	−1	−1	1	17	200	25	35	0.4	0.4	0.3	0.4	0.6	0.7	0.5	0.5	0.4	0.5	0.3	0.4	0.8	1.5	0.4	0.8
11	−1	1	−1	1	7	400	25	35	0.6	0.6	0.5	0.3	0.8	1.1	1.0	0.5	0.8	1.0	0.5	0.5	1.3	2.5	0.7	1.1
12	1	1	−1	1	17	400	25	35	0.6	0.5	0.4	0.3	0.8	1.1	0.8	0.6	0.9	1.0	0.5	0.8	1.3	2.8	0.9	1.4
13	−1	−1	1	1	7	200	75	35	0.4	0.3	0.5	0.4	0.7	1.1	0.8	0.5	0.6	0.6	0.6	0.6	1.0	1.7	1.0	1.0
14	1	−1	1	1	17	200	75	35	0.3	0.4	0.4	0.4	0.6	0.9	0.6	0.5	0.6	0.6	0.5	0.7	1.0	1.8	0.9	1.0
15	−1	1	1	1	7	400	75	35	0.5	0.5	0.5	0.4	0.8	1.2	0.8	0.6	0.9	0.8	0.9	0.9	1.2	1.8	1.4	1.4
16	1	1	1	1	17	400	75	35	0.5	0.4	0.4	0.3	0.8	1.1	0.7	0.5	0.9	0.8	0.9	0.9	1.3	2.0	1.3	1.5
17	−2	0	0	0	2	300	50	25	0.5	0.4	0.3	0.5	0.7	0.8	0.6	0.6	0.8	0.7	0.4	0.5	1.0	1.8	0.5	0.9
18	2	0	0	0	22	300	50	25	0.5	0.4	0.3	0.4	0.7	0.8	0.6	0.6	0.8	0.6	0.4	0.8	1.0	1.9	0.6	1.3
19	0	−2	0	0	12	100	50	25	0.3	0.5	0.4	0.3	0.5	1.0	0.9	0.4	0.4	0.5	0.3	0.4	1.0	1.6	0.8	0.8
20	0	2	0	0	12	500	50	25	0.5	0.6	0.4	0.3	0.6	1.0	0.9	0.6	0.7	0.8	0.5	0.6	1.4	2.2	1.2	1.0
21	0	0	−2	0	12	300	0	25	0.4	0.5	0.5	0.4	0.6	1.0	0.8	0.6	0.7	0.7	0.5	0.6	1.2	1.6	0.6	1.1
22	0	0	2	0	12	300	100	25	0.3	0.2	0.6	0.5	0.5	1.2	0.8	0.6	0.6	0.5	1.0	0.9	1.0	1.1	1.2	1.6
23	0	0	0	−2	12	300	50	5	0.2	0.3	0.2	0.3	0.4	0.8	0.4	0.6	0.3	0.2	0.3	0.3	0.5	1.0	0.3	0.7
24	0	0	0	2	12	300	50	45	0.5	0.4	0.4	0.3	0.9	1.0	0.6	0.5	1.3	2.2	0.8	1.2	1.3	2.2	0.8	1.2
25	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9
26	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9
27	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9
28	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9
29	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9
30	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9
31	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9
32	0	0	0	0	12	300	50	25	0.3	0.3	0.3	0.3	0.5	0.8	0.5	0.4	0.4	0.3	0.4	0.5	0.8	1.2	0.5	0.9

Table 2 Estimated coefficient values obtained by the theoretical second order polynomial models of eqn (1), parametric intervals and numerical statistical criteria for each parametric response criteria of the antimicrobial activity tested. Responses comprise the antibacterial criteria (MIC and MBC) for all the bacteria strains (*Bacillus cereus* (B₁), *Listeria monocytogenes* (B₂), *Escherichia coli* (B₃) and *Salmonella typhimurium* (B₄)) and antifungal criteria (MIC and MFC) of all fungal strains (*Aspergillus flavus* (F₁), *Aspergillus niger* (F₂), *Penicillium ochrochloron* (F₃) and *Penicillium verrucosum* var. *cyclopium* (F₄))

Antibacterial activity

Parametric values		MIC				MBC			
		B₁	B₂	B₃	B₄	B₁	B₂	B₃	B₄
Intercept	b_0	0.38 ± 0.02	0.32 ± 0.04	0.33 ± 0.08	0.30 ± 0.02	0.58 ± 0.05	0.80 ± 0.04	0.69 ± 0.07	0.40 ± 0.07
Linear effect	b_1	ns	ns	ns	-0.02 ± 0.01	ns	ns	ns	ns
	b_2	0.04 ± 0.02	0.03 ± 0.01	ns	ns	0.03 ± 0.01	ns	ns	0.03 ± 0.01
	b_3	-0.03 ± 0.01	-0.05 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	-0.02 ± 0.01	0.05 ± 0.02	ns	ns
	b_4	0.07 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	0.01 ± 0.01	0.12 ± 0.03	0.05 ± 0.01	0.07 ± 0.02	ns
Quadratic effect	b_{11}	0.05 ± 0.02	0.02 ± 0.01	ns	0.04 ± 0.01	0.04 ± 0.01	ns	0.02 ± 0.01	0.04 ± 0.02
	b_{22}	0.02 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	ns	ns	0.05 ± 0.02	0.09 ± 0.03	0.02 ± 0.01
	b_{33}	ns	ns	0.05 ± 0.02	0.03 ± 0.01	ns	0.07 ± 0.02	0.06 ± 0.03	0.03 ± 0.01
	b_{44}	ns	ns	ns	ns	0.03 ± 0.01	0.02 ± 0.01	ns	0.05 ± 0.02
Interactive effect	b_{12}	ns	-0.04 ± 0.02	ns	ns	ns	ns	ns	ns
	b_{13}	ns	ns	ns	ns	ns	-0.03 ± 0.01	0.04 ± 0.01	ns
	b_{14}	ns	ns	-0.02 ± 0.01	ns	-0.02 ± 0.01	-0.03 ± 0.01	-0.07 ± 0.02	ns
	b_{23}	ns	ns	-0.02 ± 0.01	ns	ns	-0.06 ± 0.02	-0.05 ± 0.01	ns
	b_{24}	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	-0.04 ± 0.02	0.07 ± 0.02	0.14 ± 0.03	0.09 ± 0.02	ns
	b_{34}	ns	ns	ns	ns	ns	ns	-0.04 ± 0.01	ns
Statistics (R^2)		0.9235	0.9074	0.8768	0.8615	0.9576	0.9753	0.9623	0.8376

Antifungal activity

Parametric values		MIC				MBC			
		F₁	F₂	F₃	F₄	F₁	F₂	F₃	F₄
Intercept	b_0	0.44 ± 0.06	0.30 ± 0.07	0.40 ± 0.02	0.55 ± 0.04	0.80 ± 0.13	1.82 ± 0.13	0.70 ± 0.07	1.07 ± 0.08
Linear effect	b_1	ns	ns	ns	0.07 ± 0.02	ns	ns	ns	0.09 ± 0.02
	b_2	0.08 ± 0.04	0.10 ± 0.04	0.07 ± 0.02	0.06 ± 0.02	0.10 ± 0.03	0.17 ± 0.05	0.11 ± 0.05	0.09 ± 0.03
	b_3	ns	-0.05 ± 0.02	0.11 ± 0.02	0.09 ± 0.03	ns	-0.15 ± 0.05	0.17 ± 0.05	0.11 ± 0.02
	b_4	0.13 ± 0.04	0.15 ± 0.04	0.09 ± 0.02	0.08 ± 0.03	0.19 ± 0.05	0.31 ± 0.09	0.16 ± 0.05	0.11 ± 0.03
Quadratic effect	b_{11}	0.09 ± 0.03	0.08 ± 0.04	ns	0.03 ± 0.01	0.03 ± 0.01	0.15 ± 0.03	ns	0.04 ± 0.01
	b_{22}	0.03 ± 0.01	0.08 ± 0.04	ns	ns	0.08 ± 0.02	0.17 ± 0.03	0.11 ± 0.05	ns
	b_{33}	0.05 ± 0.02	0.05 ± 0.01	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	ns	0.09 ± 0.05	0.08 ± 0.02
	b_{44}	0.03 ± 0.01	0.06 ± 0.02	ns	ns	ns	0.09 ± 0.02	ns	ns
Interactive effect	b_{12}	0.04 ± 0.02	ns	ns	ns	ns	ns	ns	ns
	b_{13}	ns	ns	ns	-0.05 ± 0.02	ns	ns	-0.06 ± 0.02	-0.05 ± 0.02
	b_{14}	ns	ns	ns	ns	ns	0.09 ± 0.01	ns	ns
	b_{23}	ns	-0.03 ± 0.01	0.03 ± 0.01	ns	ns	-0.18 ± 0.01	ns	ns
	b_{24}	0.09 ± 0.05	0.08 ± 0.03	0.06 ± 0.03	0.09 ± 0.03	0.08 ± 0.01	0.18 ± 0.01	0.10 ± 0.06	0.15 ± 0.03
	b_{34}	0.04 ± 0.01	ns	0.04 ± 0.02	0.05 ± 0.02	ns	ns	0.11 ± 0.06	ns
Statistics (R^2)		0.9482	0.9532	0.9734	0.9525	0.8954	0.9819	0.9755	0.9513

However, they are useful for predicting the results of untested extraction conditions and for optimization procedures (maximization or minimization).[38] The sign and the weight of the parametric value informs the effect and the performance of the response. In this way, when a factor has a positive effect, the response is higher at the high level, and when a factor has a negative effect, the response is lower at the high level. The higher the absolute value of a coefficient, the more important

the weight of the corresponding variable. Next all mathematical models produced are presented.

For the antibacterial activity responses:

(a) when the analysis is performed in terms of the MIC responses:

$$Y_{B_1}^{MIC} = 0.38 + 0.04x_2 - 0.03x_3 + 0.07x_4 + 0.05x_1{}^2 + 0.02x_2{}^2 + 0.05x_2x_4 \tag{2}$$

$$Y_{B_2}^{MIC} = 0.32 + 0.03x_2 - 0.05x_3 + 0.03x_4 + 0.02x_1^2 + 0.06x_2^2$$
$$+ 0.04x_1x_2 + 0.04x_2x_4$$

$$(3)$$

$$Y_{B_3}^{MIC} = 0.33 + 0.02x_3 - 0.05x_4 + 0.02x_2^2 + 0.05x_3^2 - 0.02x_1x_4$$
$$- 0.02x_2x_3 + 0.03x_2x_4$$

$$(4)$$

$$Y_{B_4}^{MIC} = 0.30 + 0.02x_1 + 0.01x_3 + 0.01x_4 + 0.04x_1^2 + 0.03x_3^2$$
$$- 0.04x_2x_4$$

$$(5)$$

(b) when the analysis is performed in terms of the MBC responses:

$$Y_{B_1}^{MBC} = 0.58 + 0.03x_2 + 0.02x_3 + 0.12x_4 + 0.04x_1^2 + 0.03x_4^2$$
$$- 0.02x_1x_4 + 0.07x_2x_4$$

$$(6)$$

$$Y_{B_2}^{MBC} = 0.8 + 0.05x_3 + 0.05x_4 + 0.05x_2^2 + 0.07x_3^2 - 0.03x_1x_3$$
$$- 0.03x_1x_4 - 0.06x_2x_3 + 0.14x_2x_4$$

$$(7)$$

$$Y_{B_3}^{MBC} = 0.69 + 0.07x_4 + 0.09x_4^2 + 0.06x_3^2 + 0.04x_1x_3 - 0.07x_1x_4$$
$$- 0.05x_2x_3 + 0.09x_2x_4 + 0.04x_3x_4$$

$$(8)$$

$$Y_{B_4}^{MBC} = 0.40 + 0.03x_2 + 0.04x_1^2 + 0.02x_2^2 + 0.03x_3^2 + 0.05x_4^2$$

$$(9)$$

For the antifungal activity responses:
(a) when the analysis is performed in terms of the MIC responses:

$$Y_{F_1}^{MIC} = 0.44 + 0.08x_2 + 0.13x_4 + 0.09x_1^2 + 0.03x_2^2 + 0.05x_3^2$$
$$+ 0.04x_1x_2 + 0.09x_2x_4 + 0.04x_3x_4$$

$$(10)$$

$$Y_{F_2}^{MIC} = 0.3 + 0.1x_2 + 0.05x_3 + 0.15x_4 + 0.08x_1^2 + 0.08x_2^2$$
$$+ 0.05x_3^2 + 0.06x_4^2 - 0.03x_2x_3 + 0.08x_2x_4$$

$$(11)$$

$$Y_{F_3}^{MIC} = 0.4 + 0.07Px_2 + 0.11x_3 + 0.09x_4 + 0.07x_3^2 + 0.03x_2x_3$$
$$+ 0.06x_2x_4 + 0.04x_3x_4$$

$$(12)$$

$$Y_{F_4}^{MIC} = 0.55 + 0.07x_1 + 0.06x_2 + 0.09x_3 + 0.08x_4 + 0.06x_3^2$$
$$- 0.05x_1x_3 + 0.09x_2x_4 + 0.05x_3x_4$$

$$(13)$$

(b) when the analysis is performed in terms of the MFC responses:

$$Y_{F_1}^{MFC} = 0.8 + 0.1x_2 + 0.19x_4 + 0.03x_1^2 + 0.08Px_2^2 + 0.05x_3^2$$

$$(14)$$

$$Y_{F_2}^{MFC} = 1.82 + 0.17x_2 - 0.15x_3 + 0.31x_4 + 0.15x_1^2 + 0.17x_2^2$$
$$- 0.09x_4^2 + 0.09x_1x_4 - 0.18x_2x_3 + 0.18x_2x_4$$

$$(15)$$

$$Y_{F_3}^{MFC} = 0.7 + 0.11x_2 - 0.17x_3 + 0.16x_4 + 0.11x_2^2 + 0.09x_3^2$$
$$- 0.06x_1x_3 + 0.1x_2x_4 + 0.11x_3x_4$$

$$(16)$$

$$Y_{F_4}^{MFC} = 1.07 + 0.09x_1 + 0.09x_2 + 0.11x_3 + 0.11x_4 + 0.04x_1^2$$
$$+ 0.08x_3^2 - 0.05x_1x_3 + 0.15x_2x_4$$

$$(17)$$

where X_1 (t, min), X_2 (P, W), X_3 (S, %) and X_4 (S/L, g L^{-1}) are the variables, Y is the response, sub-indices indicate the microbial strains used and the super-indices indicate the response criteria used to evaluate the results. The mathematical models of the antibacterial activity are displayed in eqn (2)–(5) for the MIC and eqn (6)–(9) for the MBC. Meanwhile, the mathematical models of the antifungal activity are displayed in eqn (10)–(13) for the MIC and eqn (14)–(17) for the MFC. Confidence intervals of the parametric values are presented in Table 2. For both types of antimicrobial responses (antibacterial and antifungal activity), the linear effect was the most significant one, followed closely by the quadratic and interactive effects.

In all cases, the statistical correlation coefficients (R^2) proved to be a good agreement between the experimental results and the predicted patterns, showing values higher than 0.84 and in most of the cases showing values higher than 0.98 (Table 2). These workable models were applied in the subsequent prediction and optimization steps. In all cases, the MIC values showed lower response values than the MBC and MFC values.

3.1.2 Detailed description of one specific case to illustrate the analysis applied to evaluate the antibacterial and antifungal activity. Fig. 1 shows an illustration of a representative case to exemplify the procedure applied to enhance the antimicrobial activity of the extracts by optimizing the conditions of UAE using the RSM technique. The figure shows the results of the antifungal activity of *Aspergillus niger* (F_2) in terms of the MIC values obtained. Fig. 1 is divided into three separate parts (A, B and C) and each of them shows specific meaningful features of the response. By definition, the lower the MIC values the higher is the inhibitory activity; therefore, to display the responses in an informative and visual form, the axis of the results presented in Fig. 1 was inverted.

Part A of Fig. 1 shows the graphical analysis of the responses as a function of all variables assessed (X_1 to X_4) in two different forms: (1) top diagonal part that shows the responses in a 3D format using surface plots, and (2) the bottom diagonal part that shows the 2D format using contour plots. The figures are produced with the polynomial model of eqn (11) that predicts the behavior of the responses displayed in Table 1 with $R^2 = 0.9532$. The model shows significant parametric values of linear, quadratic and interactive effects

A) GRAPHICAL ANALYSIS
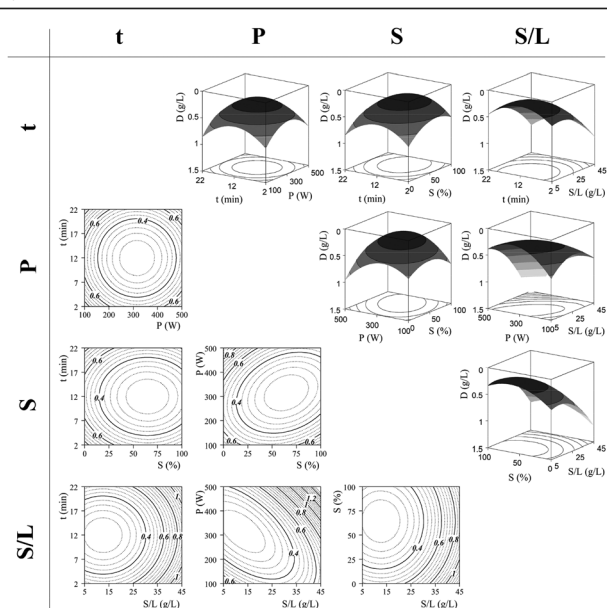
B) STATISTICAL ANALYSIS
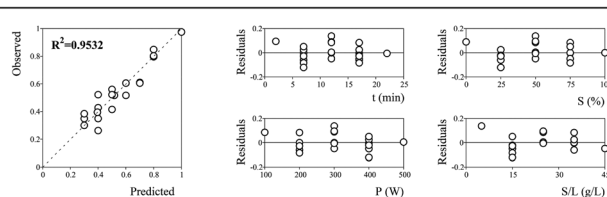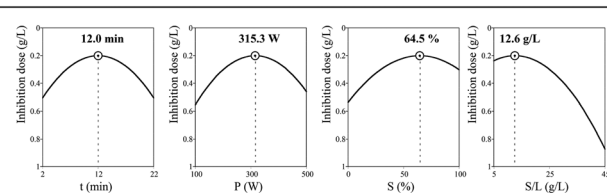
C) OPTIMUM VALUES

Fig. 1 Illustration of the procedure applied for the analysis of the antimicrobial responses of the UAE extracts obtained in the RSM experimental design applied. As a representative case, the figure shows the results of the antifungal activity in terms of the MIC of *Aspergillus niger* (F$_2$). **Part A**: The graphical analysis of the responses as a function of all variables assessed in two different forms (top diagonal part shows the 3D surfaces and the bottom diagonal part shows the contour plots of the same responses). Note that the Z axis of the 3D surfaces is inverted to display the results in an informative form. **Part B**: Illustration of the goodness of fit through two graphical statistical criteria, namely the ability to simulate response changes between the observed and predicted values and the residual distribution as a function of each variable. **Part C**: Individual responses as a function of all the variables assessed. The dots (⊙) presented alongside each line highlight the location of the optimum value. Lines and dots are generated by the theoretical second order polynomial models of eqn (1). The variables in each of the graphs of part A and C were positioned at the optimal values of the others (Table 3). Note that the Z axis of the 3D surfaces in part A and the Y axis of the 2D graphs in part 3 are inverted to display the results in an informative form.

(Table 2). The non-significant parametric effects detected were the linear parametric value of b_1 (accounting for the effect of t) and the interactive parametric values of b_{12}, b_{13}, b_{14} and b_{34} (accounting for the effects of $t \times P$, $t \times S$, $t \times$ S/L and $S \times$ S/L, respectively). Since the parametric values are expressed in coded values, the weight of each parameter represents, in comparative terms, the real influence of the response. The orders of the statistically significant parametric values as a function of their weight were as follows: b_4 (S/L, 0.15) $\gg b_2$ (P, 0.10) > b_{11} (t^2, 0.08) $\approx b_{22}$ (P^2, 0.08) $\approx b_{24}$ ($P \times$ S/L, 0.08) > b_{44} (S/L^2, 0.06) > b_{33} (S^2, 0.05) $\approx b_3$ (S, −0.05) > b_{23} ($P \times S$, −0.03). The relevance of the significant parametric values can be ordered as a function of the variables involved in a decreasing form as S/L > $P \gg S > t$. Part A of Fig. 1 shows that the UAE extracts have a relevant antifungal activity with low MIC values in a variety of different extraction conditions, generally at low S/L values, and medium t, P and S values.

Part B of Fig. 1 illustrates the goodness of fit through two graphical statistical criteria. The first one shows the ability to simulate response changes between the observed and predicted values and, the second one shows the residual distribution as a function of each variable. These changes illustrate how precisely the predictions are given by the observed experimental responses. If this is true, then the relationship between the two can be summarized with a straight line. Such a linear relation can be evaluated through the coefficient of determination R^2, which in this illustrative case showed a value of 0.9532, providing a measure of how well the observed outcomes are replicated by the multivariable model of eqn (11) applied. Regarding the graphical illustrations of the residual distribution, it is known that a residual value is the difference between the actual observed value and the predicted one. The smaller the residual values, the better the fitting procedure. In all cases, it can be perceived that the residual values obtained showed values lower than ±5% of variance.

Finally, part C of Fig. 1 shows the individual responses of a function of all the variables assessed. Lines in each graph show the response pattern as a function of the variable assessed. The dots (⊙) presented alongside each line highlight the location of the optimum value. Lines and dots are generated by the theoretical second order polynomial models of eqn (11). The optimum condition values that maximize the MIC response were t = 12.0 min, P = 315.3 W, S = 64.5% and S/L = 12.6 g L^{-1}.

In consequence, once an example has been used as an illustrative case, in the following paragraphs all antimicrobial responses assessed described in Table 2 (antibacterial and antifungal) will be analyzed. Although the same analytical procedure was applied in all other responses, the illustrative material presented is simplified to the minimum graphical material.

3.1.3 Effects of the extraction variables on the response criteria that are used to evaluate the antibacterial activity and antifungal activity. The antibacterial responses MIC and MBC (Table 1) for all the bacteria strains (B$_{1-4}$) analyzed are illus-

trated in Fig. 2. The illustration is divided into two sections, one for the MIC results and another for the MBC ones. The representation of responses is made by 2D contour plots generated by the mathematical models of eqn (2)–(5) for the MIC and eqn (6)–(9) for the MBC. The obtained parametric fitting values and statistical coefficients are described in Table 2. Linear, quadratic and interactive effects showed significant values, in which the quadratic effects showed the strongest relevance, and the interactive effects were the ones that has a reduced influence on the responses. These findings are characterized in the response illustration displayed in Fig. 2, for each bacteria strains ($B_{1–4}$) in terms of the MIC or MBC values. Each of the 6 counter plots generated for each bacteria strain involves two variables out of the four analyzed and one response, in which the other two variables were excluded, in each of the graphs, is positioned at the optimal values of the others (Table 3). The course of the maximization patterns is highlighted with trajectory lines to summarize and guide the interpretation of the figures. For the B_1 strain the tendency shows nearly identical MIC and MBC responses, with maximization values of inhibition towards low S/L, medium t and high P and S. Meanwhile for the other strains ($B_{2–4}$) the responses are different, and only the B_4 strain does not present uniformity between MBC and MIC tendencies.

The antifungal activity (in terms of MIC and MFC) of the fungus strains ($F_{1–4}$) was evaluated and the graphical illustration is presented in Fig. A1 (ESI†). The analysis of Fig. A1† is the same as in the antibacterial cases (Fig. 2). The mathematical models of the antifungal activity used are described in eqn (10)–(13) for the MIC and eqn (14)–(17) for the MFC and the full parametric information and its statistical results can be seen in Table 2. As in the antibacterial cases the paramedic values that describe the antifungal activity show that the interactive effects continue to be those that are less frequently occurring, and the quadratic effects are those that present a stronger expression to characterize the growth inhibition, either in MIC or MFC responses. For all the strains assessed ($F_{1–4}$) the MIC and MFC responses show nearly similar patterns with a different dose level of response. The general tendencies show maximization values of inhibition towards low S/L, medium S, medium-high t and high P.

3.2 Optimal extraction conditions for maximizing the response criteria

By applying a simplex algorithm methodology to solve non-linear problems, the optimum individual, relative and global
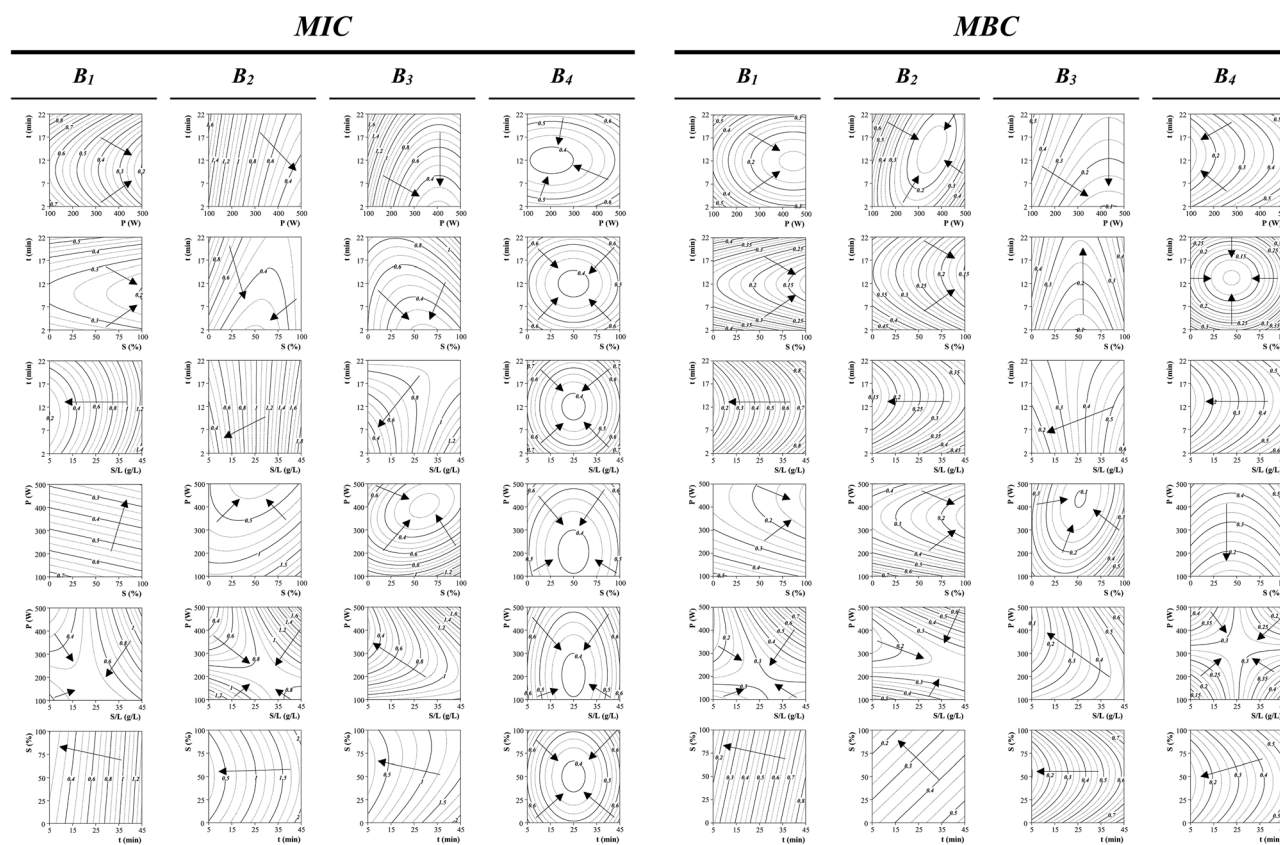

Fig. 2 Contour plots for all the antibacterial criteria (MIC and MBC) of all bacteria strains used: *Bacillus cereus* (B_1), *Listeria monocytogenes* (B_2), *Escherichia coli* (B_3) and *Salmonella typhimurium* (B_4). For representation purposes, the variables excluded in each of the graphs were positioned at the optimal values of the others (Table 3). The obtained parametric fitting values are presented in Table 2.

Table 3 Operating conditions that maximize the extraction of compounds with antimicrobial activity from *Gomphrena globosa* and optimal response values for all the parametric response criteria. Responses comprise the anti-bacterial criteria (MIC and MBC) for all the bacteria strains (*Bacillus cereus* (B$_1$), *Listeria monocytogenes* (B$_2$), *Escherichia coli* (B$_3$) and *Salmonella typhimurium* (B$_4$)) and antifungal criteria (MIC and MFC) of all fungal strains (*Aspergillus flavus* (F$_1$), *Aspergillus niger* (F$_2$), *Penicillium ochrochloron* (F$_3$) and *Penicillium verrucosum* var. *cyclopium* (F$_4$))

	Optimal extraction conditions				
	X_1: t (min)	X_2: P (W)	X_3: S (%)	X_4: S/L (g L^{-1})	Response optimum
Individual optimal responses					
Antibacterial activity					
MIC$_{B_1}$	12.0	446.6	100.0	5.0	0.125 ± 0.019 g L^{-1}
MIC$_{B_2}$	14.3	357.1	100.0	5.0	0.148 ± 0.018 g L^{-1}
MIC$_{B_3}$	2.0	435.0	52.8	5.0	0.097 ± 0.014 g L^{-1}
MIC$_{B_4}$	13.3	100.0	44.3	5.0	0.113 ± 0.004 g L^{-1}
MBC$_{B_1}$	9.9	500.0	100.0	5.0	0.197 ± 0.033 g L^{-1}
MBC$_{B_2}$	2.0	500.0	49.6	5.0	0.254 ± 0.042 g L^{-1}
MBC$_{B_3}$	2.0	405.6	60.8	5.0	0.244 ± 0.033 g L^{-1}
MBC$_{B_4}$	12.0	205.9	50.0	25.0	0.386 ± 0.027 g L^{-1}
Antifungal activity					
MIC$_{F_1}$	9.8	495.1	68.4	5.0	0.180 ± 0.030 g L^{-1}
MIC$_{F_2}$	12.0	315.3	64.5	12.6	0.201 ± 0.021 g L^{-1}
MIC$_{F_3}$	16.4	500.0	32.5	5.0	0.102 ± 0.017 g L^{-1}
MIC$_{F_4}$	4.1	500.0	34.7	5.0	0.131 ± 0.019 g L^{-1}
MFC$_{F_1}$	12.0	345.0	50.0	5.0	0.414 ± 0.048 g L^{-1}
MFC$_{F_2}$	15.0	467.4	100.0	5.0	0.728 ± 0.113 g L^{-1}
MFC$_{F_3}$	22.0	340.2	76.2	5.0	0.273 ± 0.031 g L^{-1}
MFC$_{F_4}$	2.3	500.0	19.0	5.0	0.245 ± 0.041 g L^{-1}
Relative optimal responses					
Antibacterial activity					
MIC$_{B_1}$	9.0	500.0	64.2	5.0	0.194 ± 0.024 g L^{-1}
MIC$_{B_2}$					0.432 ± 0.064 g L^{-1}
MIC$_{B_3}$					0.184 ± 0.018 g L^{-1}
MIC$_{B_4}$					0.474 ± 0.054 g L^{-1}
MBC$_{B_1}$					0.232 ± 0.046 g L^{-1}
MBC$_{B_2}$					0.342 ± 0.087 g L^{-1}
MBC$_{B_3}$					0.495 ± 0.121 g L^{-1}
MBC$_{B_4}$					0.754 ± 0.291 g L^{-1}
Antifungal activity					
MIC$_{F_1}$	11.6	383.5	58.2	5.0	0.218 ± 0.039 g L^{-1}
MIC$_{F_2}$					0.240 ± 0.048 g L^{-1}
MIC$_{F_3}$					0.219 ± 0.022 g L^{-1}
MIC$_{F_4}$					0.298 ± 0.033 g L^{-1}
MFC$_{F_1}$					0.432 ± 0.179 g L^{-1}
MFC$_{F_2}$					1.429 ± 0.140 g L^{-1}
MFC$_{F_3}$					0.383 ± 0.104 g L^{-1}
MFC$_{F_4}$					0.699 ± 0.171 g L^{-1}
Global optimal responses					
MIC$_{B_1}$	10.8	410.5	57.8	5.0	0.186 ± 0.023 g L^{-1}
MIC$_{B_2}$					0.278 ± 0.041 g L^{-1}
MIC$_{B_3}$					0.190 ± 0.018 g L^{-1}
MIC$_{B_4}$					0.378 ± 0.043 g L^{-1}
MBC$_{B_1}$					0.335 ± 0.066 g L^{-1}
MBC$_{B_2}$					0.500 ± 0.127 g L^{-1}
MBC$_{B_3}$					0.454 ± 0.111 g L^{-1}
MBC$_{B_4}$					0.667 ± 0.258, g L^{-1}
MIC$_{F_1}$					0.206 ± 0.037 g L^{-1}
MIC$_{F_2}$					0.265 ± 0.053 g L^{-1}
MIC$_{F_3}$					0.208 ± 0.021 g L^{-1}
MIC$_{F_4}$					0.259 ± 0.029 g L^{-1}
MFC$_{F_1}$					0.454 ± 0.188 g L^{-1}
MFC$_{F_2}$					1.494 ± 0.187 g L^{-1}
MFC$_{F_3}$					0.421 ± 0.115 g L^{-1}
MFC$_{F_4}$					0.625 ± 0.153 g L^{-1}

conditions for maximizing the antimicrobial activity (in terms of MIC and MBC or MFC) were determined. Fig. 3 and Table 3 show the patterns and numerical values of the variable conditions that maximize the responses assessed.

Table 3 presents the optimal operation values for the individual, relative and global maximization purposes of the antimicrobial activity (in terms of MIC and MBC or MFC). In the first part of Table 3 the optimal individual responses (MIC and MBC or MFC) to the tested bacteria and fungi strains are displayed. Thus, in the second part of Table 3, the relative operating conditions that maximize the MIC and MBC or MFC responses for the bacteria and fungi strains studied are presented. Finally, in the third part of Table 3 the optimal global conditions that maximize all responses are shown.

More specifically, Fig. 3 shows the 2D individual response patterns as a function of all the variables assessed for the antibacterial strains (B$_{1-4}$, under MIC and MBC criteria) and antifungal strains (F$_{1-4}$, under MIC and MBC criteria). The variables in each of the 2D graphs were positioned at the optimal values (Table 3) and the dots (⊙) presented alongside each line highlight the location of the optimum value. The lines and dots are generated by the theoretical second order polynomial models of eqn (1).

Antimicrobial results (Fig. 3 and Table 3), either in terms of inhibition (MIC) or bactericidal/fungicidal activity (MBC or MFC) show low values of S/L, medium-low values of t, medium values of S and high values of P. Except for power, which shows that higher potency values may be required to increase the antibacterial activity, all other results seem to be as planned in the RSM, within the variable range used. In fact, most of the responses were optimized, finding clear optimal values in the centre of the experimental design proposed. Such results have been found in previous studies,[39] in which the authors found that strong energies in UAE may improve the extraction efficiency by increasing the extraction of compounds from active sites of the plant matrix used, due to the improvement of the solvent penetration and disruption of the cell membranes. The UAE global optimal condition results were 10.8 min, 410.5 W, 57.8% of ethanol and 5 g L^{-1} of the solid–liquid ratio providing the following response values: (1) from the studied bacteria species, the MIC ranged from ~0.15 to 0.35 g L^{-1} and regarding its MBC the ranges were ~0.30 to 0.65 g L^{-1}; and (2) from the studied fungus species, the MIC ranged from ~0.20 to 0.30 g L^{-1} and regarding its MFC the ranges were ~0.40 to 0.65 g L^{-1}. The antibacterial activity dose levels were lower than the antifungal ones.

UAE is an eco-friendly alternative to conventional techniques. The findings of this work are in accordance with the ones of Tomšik *et al.*,[40] which stated that UAE enhances the extraction process by increasing the mass transfer between the solvent and the plant material. The main benefits are the time and energy reduction, and type of applied solvents, consequently minimizing industrial emissions,[41] which is an objective of the sustainable "green" chemistry. The collision of cavi-

ANTIBACTERIAL ACTIVITY   ## ANTIFUNGAL ACTIVITY

MIC   MBC   MIC   MFC
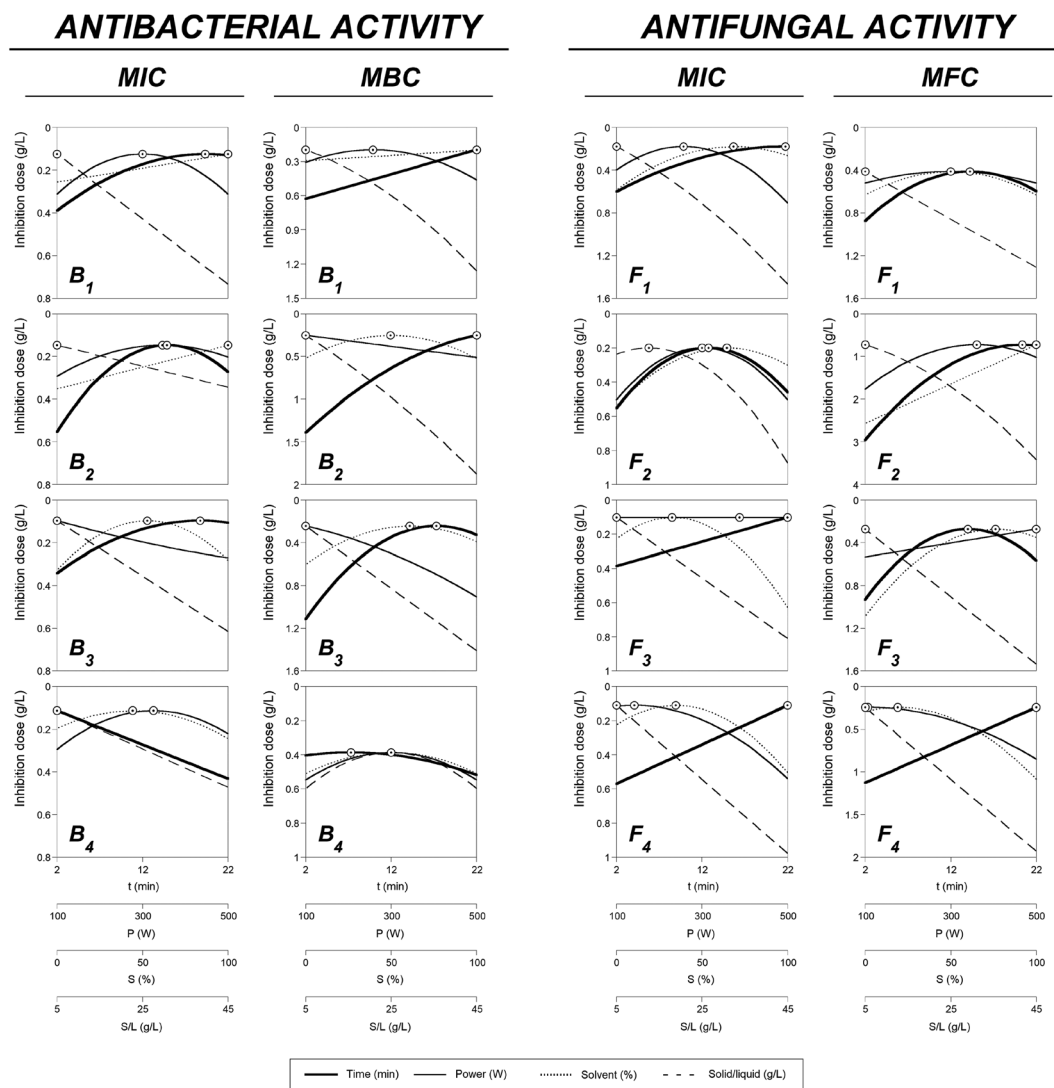

Fig. 3 Individual response criteria as a function of all the variables assessed. Responses comprise the antibacterial criteria (MIC and MBC) for all the bacteria strains (*Bacillus cereus* (B$_1$), *Listeria monocytogenes* (B$_2$), *Escherichia coli* (B$_3$) and *Salmonella typhimurium* (B$_4$)) and antifungal criteria (MIC and MFC) of all fungal strains (*Aspergillus flavus* (F$_1$), *Aspergillus niger* (F$_2$), *Penicillium ochrochloron* (F$_3$) and *Penicillium verrucosum* var. *cyclopium* (F$_4$)). The variables in each of the 2D graphs were positioned at the optimal values of the others (Table 3). The dots (⊙) presented alongside each line highlight the location of the optimum value. Lines and dots are generated by the theoretical second order polynomial models of eqn (1).

tation bubbles leads to a better cell disruption facilitating the release of extractable compounds, allowing a greater penetration of the solvent into the sample matrix, thus increasing the contact surface area between the solid and liquid phase.[40,41] The results herein obtained are in accordance with conclusions previously found.[41–43]

3.3 Industrial relevance

The antimicrobial dose-level activity of the extracts is one of the most effective techniques for preserving the phytochemical composition and bioactive properties at the industrial level, to assure quality and reduce losses associated with microbial contamination. For the different industrial sectors,

the use of suitable treatments is critical to obtain high quality products.

In this regard, different compounds/extracts with antimicrobial properties have been used as food additives to prevent deterioration processes.[44] However, due to the limitation of the use of artificial compounds and enhanced public awareness of health issues, there is an increasing need to develop and use health-promoting natural ingredients in foods with antimicrobial properties.[45] Despite the antimicrobial activity of the extracts being much lower compared with the evaluated commercial artificial ones, it is important to note that the extracts from *G. globosa* are a natural alternative colorant composed of different biomolecules, while the commercial antimicrobials are isolated pure compounds.

Based on these optimized processing parameters it is possible to produce a food colorant ingredient with antimicrobial properties and thus add value to *G. globosa* extracts.

4. Conclusions

There is a current trend toward consuming more natural and healthier products at the expense of the counterparts with artificial additives. Thus, some studies are focused on finding new natural components that have a similar or better effect than artificial additives that also maintain the food quality and extend the shelf life, without changing the sensory characteristics of the products. There is a wide diversity of natural pigments that can be exploited for their incorporation into foods as colorants providing a variety of bioactive properties important for industrial applications. Recent studies have also shown that a diverse diet based on natural pigments present in fruits and vegetables is capable of acting as an antibacterial and antifungal agent.

The aim of this study was to evaluate the antimicrobial activity of extracts obtained from *G. globosa*, with the support of the RSM adapted to the UAE. The required extraction time was 10.8 min, the power was 410.5 W, with a percentage of ethanol of 57.8%, and the solid–liquid ratio was 5 g L^{-1}. In general, all responses were optimized, finding clear optimal values in the centre of the experimental design proposed. Although the UAE was conducted at strong energies, increased power values may improve the extraction efficiency of the compounds with antimicrobial potential from *G. globosa* flowers.

Thus, we have provided evidence that the extracts obtained from *G. globosa* that contain betacyanins, if used as natural colorants, would also retard microbial contamination, and therefore reduce the onset of spoilage. In addition to these favorable features, small quantities would be required for this effect. Therefore, these extracts could be considered as a good alternative to be applied in the food industry as natural colorants and preservative ingredients.

Conflicts of interest

The authors declare that no conflict of interest exists.

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