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Modern extraction techniques optimized to extract betacyanins from *Gomphrena globosa* L.

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ABSTRACT

Gomphrena globosa L. is a source of betacyanins, molecules with high colorant power. In order to replace conventional extraction techniques, microwave (MAE) and ultrasound assisted extraction (UAE) processes were optimized by the response surface methodology (RSM) to maximize the recovery of betacyanins from *G. globosa*. A five-level full factorial design of 24 combinations and 4 replicates was implemented for MAE and UAE. The optimum processing conditions for MAE ($t = 8$ min; $T = 60$ °C; $Et = 0\%$; and $S/L = 5$ g/L) conducted to an extraction yield of 39.6 ± 1.8 mg/g dry weight, while for UAE ($t = 22$ min; $P = 500\%$; $Et = 0\%$; and $S/L = 5$ g/L) a value of 46.9 ± 4.8 mg/g was achieved, validating the UAE more suitable to obtain the target compounds. This technique can provide extracts rich in betacyanins and with high potential to be used as natural colorants.

1. Introduction

Alkaloids are a group of metabolites that can be found in a wide variety of natural sources. These compounds are characterized by the presence of, at least, one nitrogen, commonly in a cyclic structure, representing the main factor of the displayed pharmacological activity. Despite of this common element, alkaloids can present a huge range of different chemical structures, for instance in plants, they can be divided into more than 25 different subgroups. Within their chemical diversity, which includes a wide range of structures, several biological and pharmacological properties can be observed (Klein-júnior et al., 2016). Betalains, pigments belonging to a class of secondary metabolites synthesized from tyrosine, are a group of chromoalkaloids, which are divided in betacyanins (red-violet pigments) and betaxanthins (yellow pigments) (Junqueira-Goncalves et al., 2011). Although structurally related to alkaloids, betacyanins do not present toxic effects.

These pigments have received great attention because of their proven antiviral and antimicrobial activities, among others (Delgado-Vargas et al., 2000). Moreover, these compounds, especially regarding their use as colorants in the food industry, offer the possibility to replace the chemical synthetic counterparts, such as azo-dyes. Addi-tionally, these compounds can confer, over the colouring power, also functional properties (Gengatharan et al., 2015).

Even though betacyanins are associated mostly with *Beta vulgaris* L., several plants such as *Hylocereus polyrhizus* (Weber) Britton & Rose, *Opuntia ficus-indica* [L.] Miller, *Opuntia stricta* (Haw.) Haw., *Rivina humilis* L. and *G. globosa*, (Martins et al., 2016), present these compounds in their composition. Despite not being widely studied, *G. globosa* is particularly rich in betacyanins, making this plant an excellent source for obtaining this type of compounds.

For pigment extractions, several techniques can be applied, from traditional ones such as maceration, a good example since the achieved extraction yields are very acceptable (Roriz et al., 2017) to more contemporary approaches. In this context, in the last few years, several methods have emerged for the extraction of plant bioactive compounds, such as enzyme assisted extraction, accelerated extraction methods, microwave assisted extraction (MAE), and ultrasound assisted extraction (UAE) (Chavan and Singhal, 2013). When compared with tradi-tional techniques, they feature a variety of advantages, such lower solvent consumption and increased extraction efficiency (Heleno et al., 2016). For instance, MAE, a green extraction methodology, is gaining relevance due to its high extraction efficiency, and achievement of
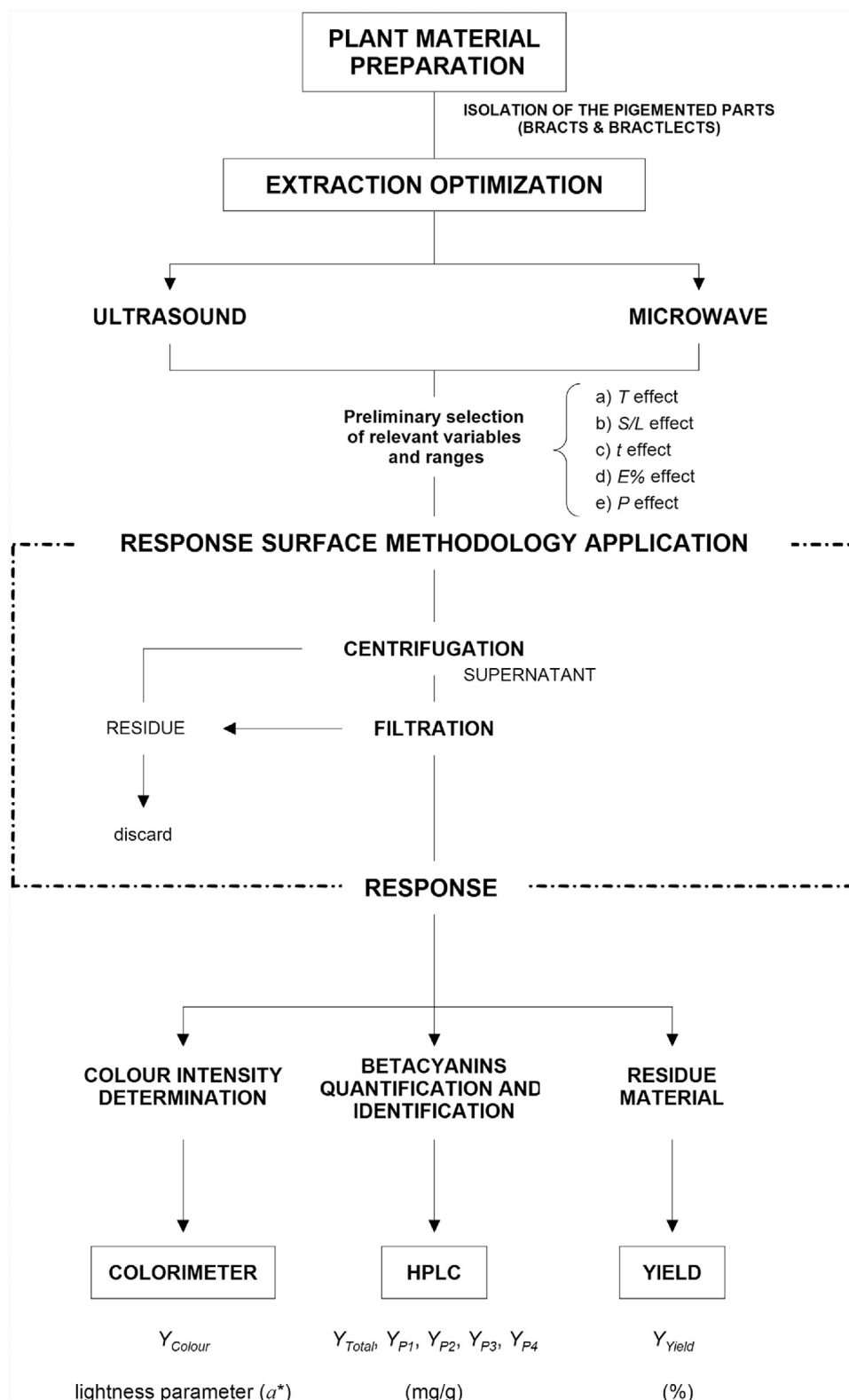

Fig. 1. Diagram of the different steps carried out for the extraction of betacyanin compounds from *G. globosa* Independent variables of time (*t*), temperature (*T*), ethanol content (*Et*), power (*P*) and solid-liquid ratio (*S/L*) of the MAE and UAE systems. Response criteria comprise the following: extraction yield of the obtained residue (Y_{Yield}); individual content of the betacyanins compounds of gomphrenin II (Y_{P1}), gomphrenin III (Y_{P2}), isogomphrenin II (Y_{P3}) and isogomphrenin III (Y_{P4}); total betacyanin content (Y_{Total}); and colour intensity (Y_{Colour}).

products with superior quality at a much lower cost (Pinela et al., 2016b). Among all the mentioned advantages, UAE offers particular advantages, such as mechanical effects, which allow a greater solvent penetration within the matrix, thus increasing the contact area solid/liquid (Hossain et al., 2014).

Several parameters can be controlled to increase MAE and UAE extraction efficiency, including several process variables that require individual consideration, independently of the applied extraction technic to recover the target compounds from natural matrices. Therefore, it is essential to identify the main variables prior to the

optimization process, and maximize their responses considering the minimum time, energy and solvent consumption to design the most cost-effective and profitable extraction system (Dai and Mumper, 2010). The most frequent form to carry out an optimization is to measure, independently, the influence of each variable whereas all the others are fixed. Nevertheless, the application of mathematical models such as the one of response surface methodology (RSM) is gaining visibility within the scientific community. The RSM design allows to optimize, simultaneously, all the variables and to predict the most efficient conditions. This is achieved by using second order polynomial models with interactions, which are able to describe and maximize the selected response criteria, based on the tested experimental range (Bezerra et al., 2008; Kalil and Maugeri, 2000; Pinela et al., 2016b).

Therefore, the aim of this work was to study the association of MAE and UAE, and use RSM to obtain and identify the optimal points for the extraction of betacyanins from *G. globosa*, a promising source of novel alternative natural colorant additives for the food industrial sector.

2. Material and methods

2.1. Samples

The plant material, *G. globosa*, was acquired from a Portuguese company, Ervital, which is based in Castro Daire, a highland area full of diversity. This company has its own harvesting based on organic farming principles. They commercialize several certified plant materials from great plant diversity. Ana Maria Carvalho, person in charge of the medicinal plant collection of the Herbarium of the Agriculture School of the Polytechnic Institute of Bragança (Trás-os-Montes, Portugal), was the responsible for the botanical identification of the plant material. The plants were subjected to mechanical separation to isolate the flowers, bracts and bracteoles, the pigmented parts of the plant, from the inflorescence, in order to increase the extraction efficiency. The flowers, bract and bracteoles, separated from the bract-like leaf-pair and heads, were put into a rotary sieve device to be blended and sieved (2 μm). The obtained powder (pigmented parts) was further mixed to guarantee sample homogeneity and stored, protected from light, in a desiccator at room temperature (average 25 °C), until analysis.

2.2. Standards and reagents

Acetonitrile of HPLC grade and ethanol p.a. were purchase from Fisher Scientific (Lisbon, Portugal). The standard (betalain) was acquired from Sigma (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA). All other chemicals and solvents were of analytical grade and purchased from common suppliers.

2.3. Betacyanin extraction

2.3.1. Microwave-assisted extraction

The MAE process was performed in a Biotage Initiator Microwave (Biotage® Initiator⁺, Uppsala, Sweden) in closed vessels of high-precision glass. The solvent volume, an ethanol-water mixture, was fixed at 20 mL. The samples were extracted according to different conditions regarding time (t), temperature (T), ethanol content (Et) and solid/liquid ratio (S/L), defined as the independent variables by the RSM design (supplementary material, Fig. 1 and Table A1). During processing, samples were stirred at 600 rpm using a magnetic stirring bar. After applying the established microwave power and time, the mixture in the extraction vessel was quickly cooled in the processing chamber.

2.3.2. 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 100–500 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 t, power (P), Et and S/L, as defined by the RSM design (Fig. 1 and Table A1).

After the microwave and ultrasonic extractions, the mixtures were centrifuged at 14000 rpm for 10 min. The solid was discarded and the supernatant divided into two parts. One part was used to quantify the extraction yield and measure the light intensity using a colorimeter (Minolta spectrophotometer, Konica Minolta Sensing, Inc., Chroma Meter CR-400, Japan). The second part was carefully collected into a glass vial, filtered through 0.2 μm nylon filters (Whatman), and used directly to quantify the total betacyanin content by HPLC-DAD analysis.

2.3.3. Extraction yield determination

The extraction yield was determined gravimetrically. For that, a sample (10 mL) of the filtered solution was dried in an oven, firstly at 60 °C to evaporate the ethanol, then at 100 °C to evaporate the residual water. After complete sample dryness, the achieved solid residue was removed from the oven and allowed to cool before weighting. Extraction yield was thereafter expressed as the ratio between the mass of the achieved residue (determined by difference) and the mass of the corresponding original plant material.

2.3.4. Colour parameters analysis

A Minolta spectrophotometer (Konica Minolta Sensing, Inc., Chroma Meter CR-400, Japan) was used to measure the colour of the extract powder. Using the illuminaçnt C and a diaphragm aperture of 8 mm, the CIE $L*a*b*$ colour space values were reported through the computerized system using the colour data software Spectra Magic Nx (version CM-S100W 2.03.0006, Konica Minolta Company, Japan). Average values were considered to determine the colour coordinates, where $L*$ represents lightness, $a*$ represents chromaticity on a green (−) to red (+) axis, and $b*$ represents chromaticity on a blue (−) to yellow (+) axis. Since the colour of interest was the red, only parameter $a*$ measurements were taken into account for further analysis. The instrument was calibrated with standard white tiles before analysis (Spectra Magic NX Instruction Manual, Konica Minolta Sensing, Inc. (ver. 2.0), 2009, Japan).

2.3.5. Betacyanins analysis and quantification

For betacyanin determination HPLC-DAD analyses were performed with a Dionex Ultimate 3000 UPLC instrument (Thermo Scientific, San Jose, CA, USA) coupled with a diode-array detector. A quaternary pump, an autosampler maintained at 5 °C, a degasser a photodiode-array detector and an automatic thermostatic column compartment constitute the chromatographic system. The separation was carried out in a Waters Spherisorb S3 ODS-2 C18, (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column operating at 35 °C. The solvents used were: (A) 0.1% trifluoracetic acid (TFA) in water and (B) acetonitrile. The gradient elution programing, performed using a flow rate of 0.5 mL/min, was as follows: 10% B for 3 min, from 10 to 15% B for 12 min, 15% B for 5 min, from 15 to 18% B for 5 min, from 18 to 30% B for 20 min, from 30 to 35% B for 5 min, and from 35 to 10% B for 10 min, resulting in a total run time of 60 min, plus 10 min for column reconditioning. Betacyanins maximum absorbance is 530 nm, therefore this was preference wavelength used to recorded all chromatograms.

The identification of these compounds was previously performed by authors (Roriz et al., 2014) and confirmed in this study. Four betacyanin compounds were found: gomphrenin II (P1), gomphrenin III (P2), isogomphrenin II (P3) and isogomphrenin III (P4). For quantitative analysis, a calibration curve was obtained based on gomphrenin III, and results were expressed as mg per g of plant dry weight (dw).

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 (temperature in °C or power in W, if it applies MAE or UAE, respectively), X_3 (ethanol content in%) and X_4 (solid/liquid ratio in g/L). Therefore, the combined effect of these four variables on betacyanins extraction was studied using *circumscribed central composite design* (CCCD) as proposed by Box and Hunter (1957). The responses were solved using 24 independent combinations and four replicates as the centre of the experimental design. In this design, the points of experiments are generated on a sphere around the centre point. The centre point is supposed to be an optimum position for the response and is repeated to maximize the prediction (Box et al., 2005). 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 tested variable's ranges is presented in the supplementary material section (Fig. 1 and Table A1).

2.4.2. Mathematical model

The response surface models were fitted by means of least-squares calculation using the following second-order polynomial equation:

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

(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} the coefficient of quadratic effect and n is the number of variables. The dependent variable responses, for each extraction technique (MAE and UAE), were the following: extract yield (Y_{Yield}), determined gravimetrically, colour (Y_{Colour}), determined as the analytical colour parameter a^*, total content of betacyanins (Y_{Total}), and individual content of each betacyanin P1–P4 (Y_{P1}–Y_{P4}). The two last responses were evaluated based on the quantification achieved by HPLC-DAD analysis.

2.4.3. Procedure to optimize the variables to a maximum response

For response optimization, a maximization process was applied using a simple method tool to solve non-linear problems (Heleno et al., 2016; Pinela et al., 2016a). 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

The fitting procedures applied to the responses were performed on a Microsoft Excel spreadsheet. Coefficient estimations and statistical calculations of the proposed experimental equations were carried out in three phases:

- Coefficients estimation was obtained by minimization the sum of quadratic differences between the observed and model-predicted values, using the nonlinear least-squares (quasi-Newton) method provided by the macro *Solver* in *Microsoft Excel* (Kemmer and Keller, 2010).
- The significance of the coefficients of the parametric confidence intervals was calculated using the "*SolverAid*" (Prikler, 2009). The model was simplified by dropping terms, which were not statistically significant (*p*-value > 0.05).

- The uniformity of the model was checked by applying the following statistical assessment criteria: a) The Fisher *F*-test (α = 0.05) was used to determine whether the constructed models were consistent to describe the observed data; b) The 'SolverStat' macro was used for the assessment of the parameter and model prediction uncertainties (Comuzzi et al., 2003); and c) R^2 was interpreted as the proportion of variability of the dependent variable explained by the model.

3. Results and discussion

3.1. Preliminary analysis to select, previously to the application of RSM optimization studies, the adequate ranges for the main MAE and UAE variables

The basic concept of the preliminary experiments, for MAE and UAE systems, is to assure that the experimental domain of the operating conditions, for the optimization procedure, will be properly defined to maximize the responses (Kala et al., 2016; Tiwari, 2015). It is well known that several variables can affect the extraction of compounds from natural sources (Bhuyan et al., 2015; Dahmoune et al., 2015; Routray and Orsat, 2012). However, different authors (Albuquerque et al., 2016; Heleno et al., 2016; Pinela et al., 2016b) are in agreement concerning the variables affecting the extraction responses in MAE and UAE systems, the main being identified as: X_1 (t, min), X_2 (T, °C or P, W, respectively for MAE and UAE), X_3 (Et, %) and X_4 (S/L, g/L). Therefore, to ensure a proper experimental domain, the relevant variables and ranges for MAE and UAE systems were selected based on the information gathered in the available bibliographical data and obtained by experimental results in one-variable-at-the-time-format. Results led to the following conclusions:

- The effect of t plays one of the most important and critical roles. If samples are exposed at t values lower or higher than the optimum one, compounds may not be properly extracted or, otherwise, degraded. In both extractions systems, the effect of t is closely related to the T (in case of MAE) and P (in case of UAE). Generally, the effect of T or P interacts with t positively (increasing the compounds extraction) or negatively (increasing the compounds degradation), compromising the achieved responses. In MAE the typical ranges for the t and T vary from small ranges, 0–160 s (Cardoso-Ugarte et al., 2014); 2–10 min, 25–55 °C (Thirugnanasambandham and Sivakumar, 2015) and to shorter t and higher T, 0–20 min, 60–180 °C (Pinela et al., 2016b). For the UAE, although the typical ranges are lower t but with higher P (5–15 min, 250–500 W, Heleno et al., 2016), there are cases in which lower P and longer t are used (15–45 min, 60–120 W, Maran et al., 2015).
- Regarding the type of solvent, and in the case of mixtures the used composition, authors also found important effects on the molecules to be extracted (Arnao et al., 2001; Sultana et al., 2009). Generally, the solvent extraction ability can be grouped in non-polar, polar aprotic and polar protic solvents (Huffman et al., 2012; Kislik, 2012). Water mixtures with polar solvents such as methanol, ethanol, acetone, among others, are the most commonly used in the extractions of natural compounds from plants (Prieto and Vázquez, 2014). Aqueous mixtures are preferable due to the range of the polarity degrees associated with the molecules to be extracted; it increases the microwave/ultrasound extraction efficiency, also allowing a better heat dissipation (Kala et al., 2016). In this sense, considering the principles of green chemistry, binary mixtures ethanol:water were selected as the extraction solvent. Therefore, the variable studied was the ethanol content in the ethanol:water mixture, which variation leads to significant differences (ranges from 0 to 100% were selected).
- Finally, the S/L ratio interacts, at a very low extent, with the other system variables, but is very important parameter from the indus-

trial point of view. It is well know that the contact surface of the sample with the extraction solvent will influences the efficiency of the extraction process (Kala et al., 2016; Tiwari, 2015). Lower S/L ratios can facilitate the extraction process, but generates a high waste of solvent (10–30 g/mL, Maran et al., 2015; Thirugnanasambandham and Sivakumar, 2015), demanding its recovery and recycling. At an industrial scale, higher ratios need to be defined based on a compromise between process efficiency (more productive process) and minimal solvent consumption (more sustainable process) (5–45 g/L, Pinela et al., 2016).

Therefore, the final ranges selected for the five level CCCD of the RSM analysis for the four relevant variables (Fig. 1 and Table A1) were:

- For MAE process: t (0–20 min), T (60–180 °C), Et (0–100%) and S/L (5–45 g/L);
- For UAE process: t (2–22 min), P (100–500 W), Et (0–100%) and S/L (5–45 g/L).

The processing conditions were optimized based on several responses (Y_{Yield}, Y_{Colour}, Y_{Total}, Y_{P1}, Y_{P2}, Y_{P3} and Y_{P4}). The proposed RSM design (Fig. 1 and Table A1) reduces the number of experimental trials, which otherwise would imply 625 possible combinations (five levels of four independent variables), to be solved in 24 independent combinations and four replicates at the centre of the experimental design. The experimental results for the 28 runs are presented in Table 1.

3.2. Optimization of the responses by RSM

3.2.1. Development of theoretical response surface models and statistical verification

Fitting the models for the selected responses is crucial to elucidate, how precisely the RSM mathematical model can predict ideal variances. The models for each response were built by fitting the second-order polynomial model of Eq. (1) (independent variables in coded values) to the experimental values (Table 1) through nonlinear least-squares estimations. The resulting models are presented below.

For MAE:

When the extraction yield response was considered:

$$Y_{Yield}^{MAE} = 38.97 + 0.64x_1 + 6.25x_2 - 3.94x_3 - 2.24x_4 - 0.91x_1^2 - 0.66x_2^2$$
$$- 2.80x_4^2 + 0.77x_3^2$$
$$- 1.69x_2x_3 - 1.22x_3x_4 \tag{2}$$

When the betacyanin compounds (P1–4) and the total amount were considered:

$$Y_{P1}^{MAE} = 1.0 - 0.04x_1 - 0.45x_2 - 0.33x_3 + 0.15x_2^2 + 0.09x_3^2 - 0.38x_2x_3 \tag{3}$$

$$Y_{P2}^{MAE} = 1.1 - 0.08x_1 - 1.12x_2 - 0.94x_3 + 0.43x_2^2 + 0.34x_3^2 + 1.08x_2x_3 \tag{4}$$

$$Y_{P2}^{MAE} = 1.1 - 0.08x_1 - 1.12x_2 - 0.94x_3 + 0.43x_2^2 + 0.34x_3^2 + 1.08x_2x_3 \tag{5}$$

$$Y_{P2}^{MAE} = 1.1 - 0.08x_1 - 1.12x_2 - 0.94x_3 + 0.43x_2^2 + 0.34x_3^2 + 1.08x_2x_3 \tag{6}$$

$$Y_{P4}^{MAE} = 1.0 - 0.13x_1 - 0.83x_2 - 0.65x_3 + 0.13x_1^2 + 0.28x_2^2 + 0.21x_3^2$$
$$+ 0.75x_2x_3 \tag{7}$$

$$Y_{TOTAL}^{MAE} = 3.12 - 2.84x_2 - 2.15x_3 - 1.95x_4 + 1.11x_2^2 + 0.75x_3^2 + 1.28x_4^2$$
$$+ 2.51x_2x_3 \tag{8}$$

When colour determination, based on spectrophotometric quantifi-

cation, was considered:

$$Y_{Colour}^{MAE} = 13.91 - 7.30x_2 - 4.09x_3 + 1.75x_1^2 + 1.74x_2^2 + 1.18x_3^2 + 3.35x_2x_3$$
$$\tag{8}$$

For UAE:

When the extraction yield response was considered:

$$Y_{Yield}^{UAE} = 36.38 + 5.90x_1 + 3.58x_2 - 7.36x_3 - 3.07x_4 + 0.25x_1^2 - 0.68x_2^2$$
$$- 1.04x_3^2 - 1.37x_4^2 \tag{9}$$

When the betacyanin compounds (P1–4) and the total amount were considered:

$$Y_{P1}^{UAE} = 1.80 + 0.13x_1 + 0.10x_2 - 0.92x_3 - 1.0x_4 + 0.11x_3^2 + 0.75x_4^2 \tag{10}$$

$$Y_{P2}^{UAE} = 2.40 - 2.68x_3 - 0.80x_4 + 0.40x_1^2 + 0.37x_2^2 + 0.93x_3^2 + 0.70x_4^2$$
$$\tag{11}$$

$$Y_{P3}^{UAE} = 2.42 + 0.08x_1 - 0.86x_3 - 0.17x_1^2 - 0.13x_2^2 \tag{12}$$

$$Y_{P4}^{UAE} = 2.46 - 1.60x_3 - 0.42x_4 + 0.19x_1^2 + 0.18x_2^2 + 0.68x_3^2 + 0.51x_4^2$$
$$\tag{13}$$

$$Y_{TOTAL}^{UAE} = 8.83 + 0.58x_1 - 6.12x_3 - 2.85x_4 + 0.36x_1^2 + 0.32x_2^2 + 1.85x_3^2$$
$$+ 2.19x_4^2 \tag{14}$$

When the colour determination was considered:

$$Y_{Colour}^{UAE} = 20.83 - 4.00x_1 - 3.93x_2 - 4.61x_3 - 4.41x_4 + 1.28x_1^2 \tag{15}$$

where X_1 (t, min), X_2 (T, °C or P, W), X_3 (Et, %) and X_4 (S/L, g/L), Y is the response, sub-indices indicate the responses used and the super-indices the technique applied. The mathematical models (Eqs. (2)–(8) for MAE and Eqs. (9)–(15) for UAE), and the parametric information present in Table 2, provides a complete summary of the effects caused by each of the involved variables defined for each extraction technique. In all cases, for both extraction methods, the linear effect was the most significant one, followed by the quadratic effect. Only the MAE procedure showed some interactive effects, consistently, between X_2 (T, °C) and X_3 (Et, %) variables.

In all cases, the found statistical correlation coefficients (R^2), proved the good agreement between the experimental results and the predicted patterns, showing values higher than 0.82. For the MAE system, the lowest value was 0.84 for the Y_{P2} response and the highest one was 0.97 for the Y_{Yield} response. For the UAE method, the lowest value was 0.82 for the Y_{Colour} response and the highest one was 0.97 for the Y_{Yield} response.

3.2.2. Effects of the extraction variables used to evaluate the response criteria

The patterns observed for the extractions can be explained by means of the parametric values of the second-order polynomial models described in Eqs. (2)–(8) for MAE and Eqs. (9)–(15) for UAE, or can be illustrated by graphical representations. Figs. 2–4 , Figs. A1 and A2 (Supplementary material) show the graphical results of the studied responses (Y_{Yield}, Y_{Colour}, Y_{Total}, Y_{P1}, Y_{P2}, Y_{P3} and Y_{P4}). For representation purposes, the variables excluded in each graph were positioned at the centre of their experimental domain. The obtained parametric fitting values are presented in Table 2.

Fig. 2 shows the comparative matrix combination for the 3D surface responses of the Y_{Yield} response for MAE and UAE processes. In the top diagonal part, the 3D surface responses of UAE are presented, while in the bottom diagonal, the ones presented are those derived from the MAE process. The effects can be summarized as follows:

- For MAE: The compound extraction increases at high T and intermediate t (180 °C, 11.7 min) showing patterns with linear and quadratic effects. When Et is higher than 20%, the extraction yield starts to be compromised, a fact that can be associated with the

Table 1

Results of the response surface experimental plan for the MAE and UAE optimization process. independent variables of time (t), temperature (T), ethanol content (Et), power (P) and solid-liquid ratio (S/L) of the microwave and ultrasound system. Response criteria comprise the following: extraction yield of the obtained residue (Y_{Yield}); individual content of the betacyanins compounds of gomphrenin II (Y_{P1}), gomphrenin III (Y_{P2}), isogomphrenin II (Y_{P3}) and isogomphrenin III (Y_{P4}); total betacyanin content (Y_{Total}); and colour intensity (Y_{Colour}).

| | Experimental Design | | | | Microwave Conditions | | | | Ultrasound Conditions | | | | MICROWAVE RESPONSES | | | | | | | Ultrasound Responses | | | | | | |
| | Coded Values | | | | | | | | | | | | Residue | BETACYANIN Content | | | | | Colour | Residue | Betacyanin content | | | | | Colour |
	X_1	X_2	X_3	X_4	X_1 t min	X_2 T °C	X_3 Et %	X_4 S/L g/L	X_1 t min	X_2 P W	X_3 Et %	X_4 S/L g/L	Y_{Yield} %	Y_{P1} mg/g	Y_{P2} %	Y_{P3} mg/g	Y_{P4} mg/g	Y_{Total} mg/g	Y_{Colour}	Y_{Yield} %	Y_{P1} mg/g	Y_{P2} mg/g	Y_{P3} mg/g	Y_{P4} mg/g	Y_{Total} mg/g	Y_{Colour}
1	−1	−1	−1	−1	5	90	25	15	7	200	25	15	31.49	2.7	5.4	2.5	4.3	15.0	32.32	34.13	3.8	7.5	3.2	5.8	20.3	33.94
2	1	−1	−1	−1	15	90	25	15	17	200	25	15	32.07	3.1	6.3	2.8	4.9	17.2	38.00	45.31	4.3	8.6	3.5	6.5	22.9	33.34
3	−1	1	−1	−1	5	150	25	15	7	400	25	15	45.82	1.2	1.2	1.2	1.2	4.7	6.60	40.57	4.2	8.5	3.4	6.4	22.5	35.43
4	1	1	−1	−1	15	150	25	15	17	400	25	15	48.47	1.2	1.2	1.2	1.2	4.7	11.07	56.57	4.5	9.2	3.7	7.0	24.3	20.09
5	−1	−1	1	−1	5	90	75	15	7	200	75	15	30.20	1.6	1.7	1.5	1.8	6.6	23.54	21.50	2.3	3.2	1.6	2.2	9.2	32.03
6	1	−1	1	−1	15	90	75	15	17	200	75	15	30.23	1.3	1.5	1.5	1.5	5.7	22.29	30.69	2.4	3.0	1.6	2.2	9.1	27.39
7	−1	1	1	−1	5	150	75	15	7	400	75	15	37.14	1.2	1.2	1.2	1.2	4.7	12.58	27.41	2.6	3.6	1.6	2.4	10.1	25.86
8	1	1	1	−1	15	150	75	15	17	400	75	15	41.24	2.5	5.7	2.2	4.4	14.7	15.22	39.00	2.6	3.2	1.6	2.3	9.7	15.04
9	−1	−1	−1	1	5	90	25	35	7	200	25	35	30.15	2.0	4.6	1.8	3.4	11.9	32.40	28.17	2.9	7.1	2.6	5.2	17.8	37.39
10	1	−1	−1	1	15	90	25	35	17	200	25	35	30.95	0.5	0.5	0.5	0.5	2.0	30.91	40.73	3.1	7.6	2.9	5.6	19.1	23.71
11	−1	1	−1	1	5	150	25	35	7	400	25	35	43.60	0.5	0.5	0.5	0.5	2.0	17.24	33.93	2.9	7.0	2.6	5.1	17.6	21.33
12	1	1	−1	1	15	150	25	35	17	400	25	35	45.95	0.5	0.5	0.9	0.5	2.0	17.98	49.46	3.4	8.4	3.0	6.1	20.9	15.19
13	−1	−1	1	1	5	90	75	35	7	200	75	35	24.65	0.8	1.0	0.9	1.1	3.8	13.96	15.57	0.7	1.2	0.9	1.2	4.1	29.17
14	1	−1	1	1	15	90	75	35	17	200	75	35	24.26	0.6	0.7	0.8	0.6	2.7	15.52	24.64	0.9	1.4	1.0	1.4	4.7	13.48
15	−1	1	1	1	5	150	75	35	7	400	75	35	31.74	0.5	0.5	0.5	0.5	2.0	11.35	20.74	0.9	1.5	1.0	1.4	4.7	7.80
16	1	1	1	1	15	150	75	35	17	400	75	35	31.41	0.5	0.5	0.5	0.5	2.0	9.03	32.31	0.8	1.3	0.9	1.3	4.3	6.16
17	−2	0	0	0	0	120	50	25	2	300	50	25	34.81	1.0	1.3	1.1	1.7	5.1	27.82	25.61	1.6	2.9	1.4	2.4	8.3	29.86
18	2	0	0	0	20	120	50	25	22	300	50	25	37.54	0.7	0.7	0.7	0.7	2.8	10.76	48.11	2.3	3.7	1.8	3.3	11.0	16.11
19	0	−2	0	0	10	60	50	25	12	100	50	25	22.51	2.1	4.0	2.3	3.0	11.4	36.08	26.49	1.5	2.8	1.7	2.5	8.6	24.20
20	0	2	0	0	10	180	50	25	12	500	50	25	51.87	0.7	0.7	0.7	0.7	2.8	2.45	39.82	2.0	3.5	1.8	3.1	10.5	18.79
21	0	0	−2	0	10	120	0	25	12	300	0	25	37.84	1.6	3.3	1.2	2.4	8.5	25.82	46.66	3.9	10.1	3.7	7.3	25.1	27.87
22	0	0	2	0	10	120	100	25	12	300	100	25	19.41	0.7	0.7	0.7	0.7	2.8	8.26	16.78	0.7	0.7	0.7	0.7	2.8	4.27
23	0	0	0	−2	10	120	50	5	12	300	50	5	47.85	3.5	3.5	3.5	3.5	14.0	4.36	36.43	8.1	6.5	4.8	6.0	25.4	28.53
24	0	0	0	2	10	120	50	45	12	300	50	45	37.91	0.4	0.4	0.4	0.4	1.6	16.79	24.35	1.7	2.5	2.2	2.3	8.7	10.05
25	0	0	0	0	10	120	50	25	12	300	50	25	39.48	0.7	0.7	0.7	0.7	2.8	14.25	36.41	1.9	2.5	1.9	2.5	8.8	21.98
26	0	0	0	0	10	120	50	25	12	300	50	25	38.05	0.7	0.7	0.7	0.7	2.8	12.81	36.35	1.6	2.2	2.8	2.2	8.8	16.28
27	0	0	0	0	10	120	50	25	12	300	50	25	38.98	0.7	0.7	0.7	0.7	2.8	14.19	36.38	1.8	2.5	1.9	2.5	8.8	20.53
28	0	0	0	0	10	120	50	25	12	300	50	25	39.38	0.7	0.7	0.7	0.7	2.8	16.10	36.36	1.9	2.5	1.9	2.6	8.9	21.30
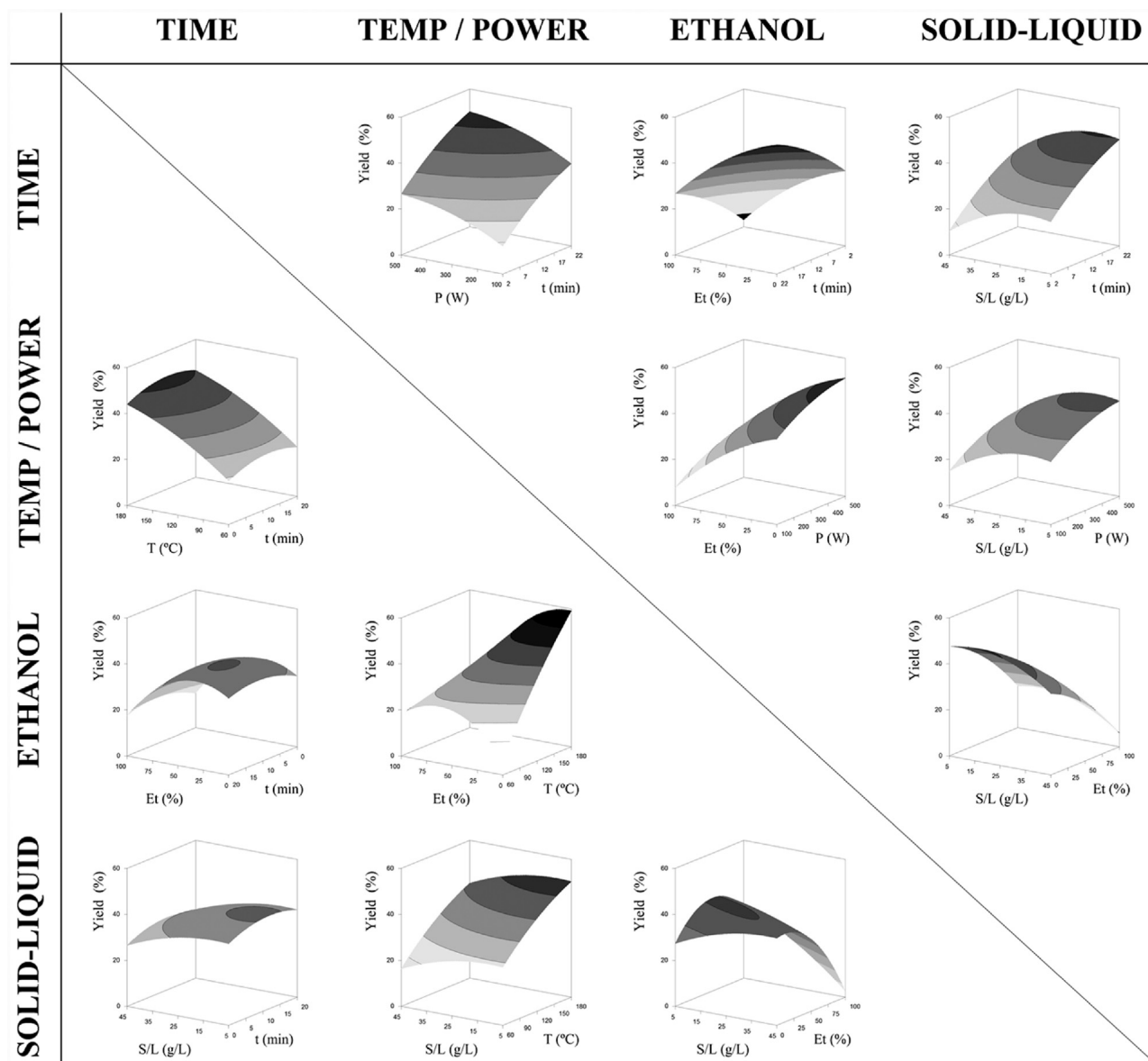
Fig. 2. Matrix combination for the surface Y_{Yield} responses MAE and UAE systems. In the top diagonal part is presented the response surface of UAE and in the bottom diagonal part is presented the response surface of MAE. For representation purposes, the variables excluded in each 3D graph were positioned at the centre of their experimental domain. The obtained parametric fitting values are presented in Table 2. Note that the independent variable X_2 in the ultrasound extraction is related to the power (W), meanwhile for the maceration and microwave extraction it is related with the temperature (°C).

compounds polarity, which show higher affinity for hydrophilic environments. Regarding the S/L ratio effect, low values favours optimal extraction yields (5 g/L). The Et and S/L variables showed complex sceneries with linear, quadratic and interactive effects.

- For UAE: The optimal extraction responses are produced at intermediate t, high P, low Et and low/medium values of S/L (22 min, 500 W, 0%, 13.7 g/L respectively). Only linear and quadratic effects were found.

Fig. 3 shows the graphical matrix combination of the responses Y_{Total} and Y_{Colour}. Each of the contour graphs represents the projection in XY plane of the theoretical three-dimensional response surface predicted with the second-order polynomial of Eq. (1). For the Y_{Total} the response effects can be summarized as follows:

- For MAE: The t variable did not show any relevant effect in the responses. This is a typical effect when analysing the response of various compounds due to the dissimilar time response effects of

each one. The optimal extraction responses are produced at high T, low percentage of Et and low values of S/L (180 °C, 28.2%, 5 g/L, respectively). Linear and quadratic effects, produced by models, governed the response. Only the variables T and Et showed interactive effects.

- UAE: The optimal extraction responses are produced at higher t, higher P, absence of Et and medium/low values of S/L (22 min, 500 W, 0%, 13.7 g/L, respectively). Only linear and quadratic effects were found.

For Y_{Colour} the response effects can be summarized as follows:

- For MAE: Simple response scenarios are found, in which only some linear and quadratic effects are found. The optimal extraction responses are produced at medium low t, low T, absence of Et and low values of S/L (8 min, 60 °C, 0%, 5 g/L, respectively).
- For UAE: The optimal extraction responses are produced at short t, low P, absence of Et and low values of S/L (2 min, 100 W, 0%, 5 g/L,
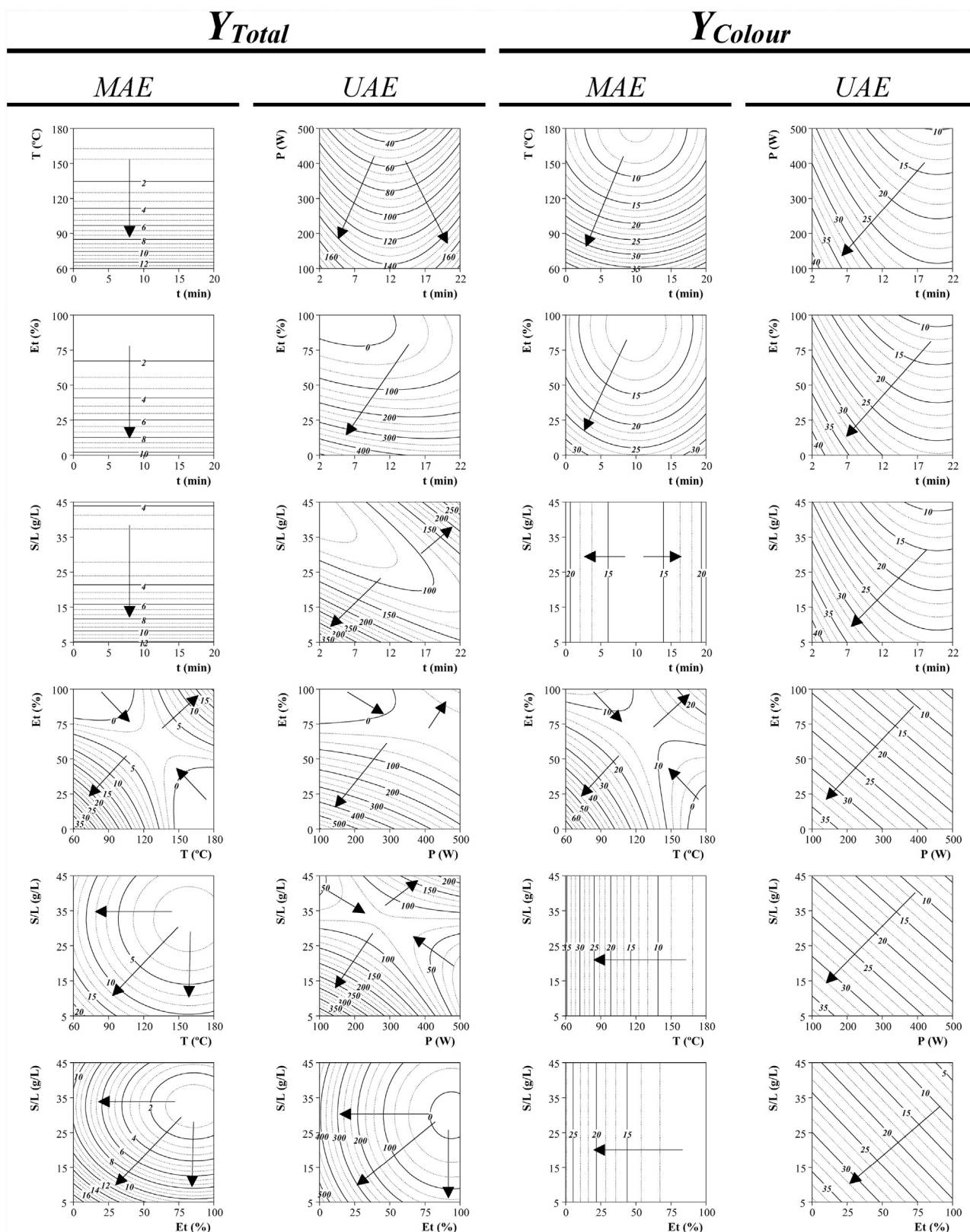
Fig. 3. Matrix combination of the response surfaces of the total betacyanin extraction content (Y_{Total}) and colour intensity from developed equations for the MAE and UAE system optimizations. For representation purposes, the variables excluded in each 3D graph were positioned at the centre of their experimental domain. The obtained parametric fitting values are presented in Table 3.

respectively).

Finally, Figs. A1 and A2 (Supplementary material) show the contour graphs of the individual betacyanin compounds content (Y_{P1}–Y_{P4}) for

MAE and UAE systems, respectively. The response effects can be summarized as follows:

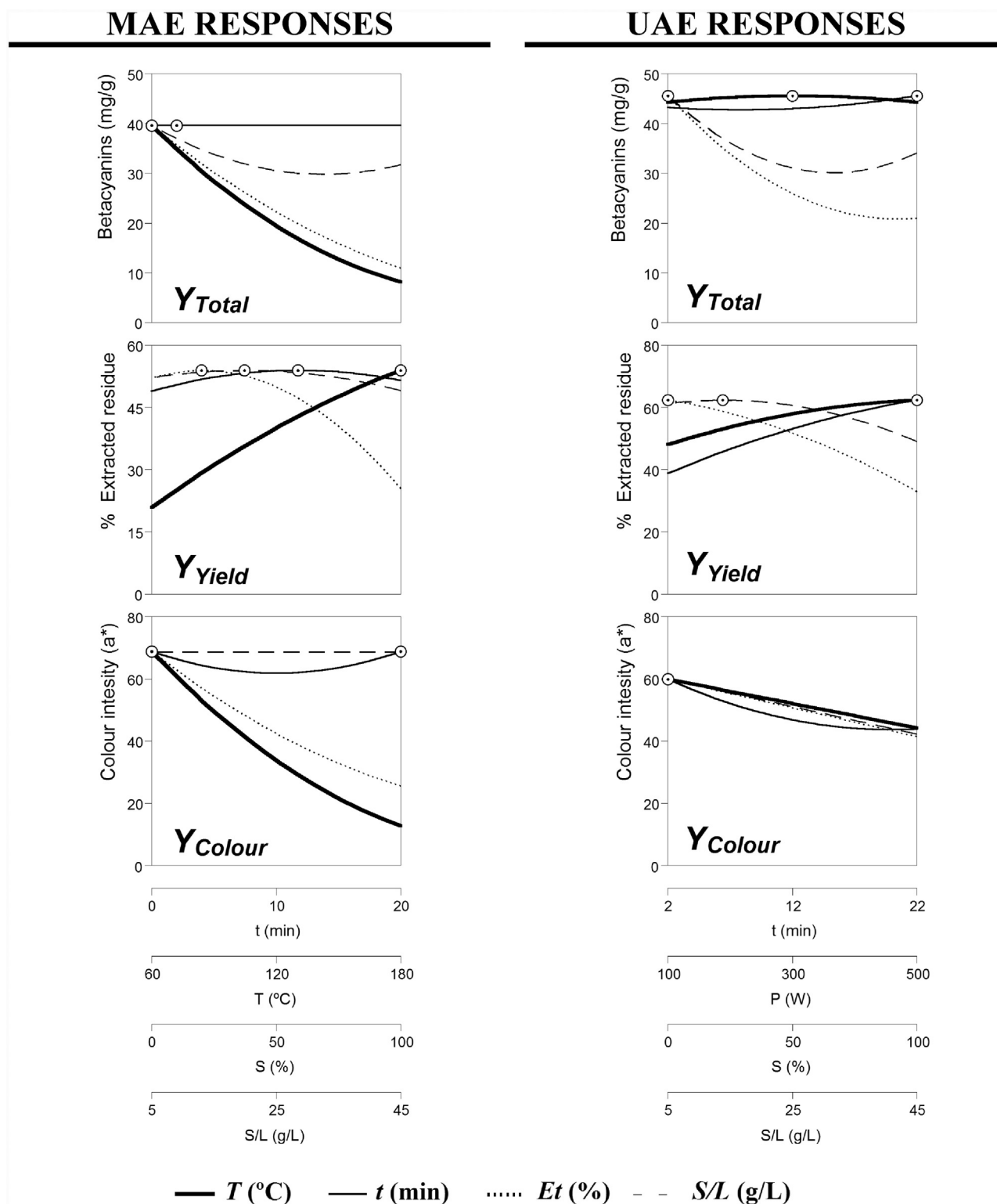- For MAE: The linear and quadratic effects are once again the

Fig. 4. Individual responses of Y_{Yield}, Y_{Colour} and Y_{Total} as a function of all the variables assessed. The variables in each of the 2D graphs were positioned at the optimal values of the others (Table 3). The obtained parametric fitting values are presented in Table 2. 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 Eqs. (2)–(8) for MAE and Eqs. (9)–(15) for UAE. The results for the responses of the individual values of each betacyanin compound identified (Y_{P1}, Y_{P2}, Y_{P3} and Y_{P4}) were excluded due to their pattern similarities with the Y_{Total}.

predominant effect for all compounds content (Y_{P1}, to Y_{P4}). The optimal extraction response is the same for all the compounds and produced at short t, low T, absence of Et and low values of S/L (8 min, 60 °C, 0%, 5 g/L, respectively).

- For UAE: This methodology showed a simpler response than MAE. It was noted that the response Y_{P3} showed different optimum values

compared with the other applied response criteria. Therefore, the optimal extraction response for Y_{P1}, Y_{P2} and Y_{P4} are produced at higher t and T, absence of Et, and low values of S/L (22 min, 500 W, 0%, 5 g/L, respectively), while Y_{P3} produce the optimal extraction response at medium values of t and T, absence of Et, and low values of S/L (13 min, 300, 0%, 5 g/L, respectively).

Table 2

Estimated coefficient values obtained from the Box polynomial model, parametric intervals and numerical statistical criteria for each parametric response criteria of the extractions systems tested (MAE and UAE). Response criteria comprise the following: extraction yield of the obtained residue (Y_{Yield}); individual content of the betacyanins compounds of gomphrenin II (Y_{P1}), gomphrenin III (Y_{P2}), isogomphrenin II (Y_{P3}) and isogomphrenin III (Y_{P4}); total betacyanin content (Y_{Total}); and colour intensity (Y_{Colour}).

Parameters		Residue	Betacyanin Content					Colour
		Y_{Yield}	Y_{P1}	Y_{P2}	Y_{P3}	Y_{P4}	Y_{Total}	Y_{Colour}
Microwave Asisted Extraction								
Intercept	b_0	38.97 ± 1.05	0.99 ± 0.35	1.11 ± 0.42	1.01 ± 0.34	1.01 ± 0.46	3.12 ± 0.85	13.91 ± 2.87
Linear effect	b_1	0.64 ± 0.61	−0.04 ± 0.27	−0.08 ± 0.03	−0.04 ± 0.00	−0.13 ± 0.11	ns	ns
	b_2	6.25 ± 0.61	−0.45 ± 0.27	−1.12 ± 0.32	−0.44 ± 0.26	−0.83 ± 0.31	−2.84 ± 0.56	−7.30 ± 1.89
	b_3	−3.94 ± 0.61	−0.33 ± 0.27	−0.94 ± 0.32	−0.24 ± 0.06	−0.65 ± 0.31	−2.15 ± 0.56	−4.09 ± 1.89
	b_4	−2.24 ± 0.61	ns	ns	ns	ns	−1.95 ± 0.56	ns
Quadratic effect	b_{11}	−0.91 ± 0.55	ns	ns	0.02 ± 0.00	0.13 ± 0.11	ns	1.75 ± 1.70
	b_{22}	−0.66 ± 0.55	0.15 ± 0.24	0.43 ± 0.29	0.17 ± 0.02	0.28 ± 0.27	1.11 ± 0.50	1.74 ± 1.70
	b_{33}	−2.80 ± 0.55	0.09 ± 0.24	0.34 ± 0.29	ns	0.21 ± 0.11	0.75 ± 0.50	1.18 ± 0.79
	b_{44}	0.77 ± 0.55	ns	ns	ns	ns	1.28 ± 0.50	ns
Interactive effect	b_{12}	ns	ns	ns	ns	ns	ns	ns
	b_{13}	ns	ns	ns	ns	ns	ns	ns
	b_{14}	ns	ns	ns	ns	ns	ns	ns
	b_{23}	−1.69 ± 0.74	0.38 ± 0.33	1.08 ± 0.39	0.30 ± 0.11	0.75 ± 0.37	2.51 ± 0.69	3.35 ± 2.31
	b_{24}	ns	ns	ns	ns	ns	ns	ns
	b_{34}	−1.22 ± 0.74	ns	ns	ns	ns	ns	ns
Statistics (R^2)		0.9766	0.9116	0.8456	0.8621	0.9588	0.9321	0.8975
Ultrasound Asisted Extraction								
Intercept	b_0	36.38 ± 0.85	1.80 ± 0.25	2.40 ± 0.50	2.42 ± 0.33	2.46 ± 0.50	8.83 ± 1.16	20.83 ± 1.90
Linear effect	b_1	5.90 ± 0.49	0.13 ± 0.06	ns	0.08 ± 0.03	ns	0.58 ± 0.27	−4.00 ± 1.74
	b_2	3.58 ± 0.49	0.10 ± 0.06	ns	ns	ns	ns	−3.93 ± 1.74
	b_3	−7.36 ± 0.49	−0.92 ± 0.19	−2.68 ± 0.29	−0.86 ± 0.37	−1.60 ± 0.29	−6.12 ± 0.67	−4.61 ± 1.74
	b_4	−3.07 ± 0.49	−1.00 ± 0.19	−0.80 ± 0.29	ns	−0.42 ± 0.29	−2.85 ± 0.67	−4.41 ± 1.74
Quadratic effect	b_{11}	0.25 ± 0.21	ns	0.40 ± 0.26	−0.17 ± 0.03	0.19 ± 0.11	0.36 ± 0.27	1.28 ± 1.55
	b_{22}	−0.68 ± 0.44	ns	0.37 ± 0.26	−0.13 ± 0.03	0.18 ± 0.11	0.32 ± 0.27	ns
	b_{33}	−1.04 ± 0.44	0.11 ± 0.03	0.93 ± 0.26	ns	0.68 ± 0.26	1.85 ± 0.60	ns
	b_{44}	−1.37 ± 0.44	0.75 ± 0.17	0.70 ± 0.26	ns	0.51 ± 0.26	2.19 ± 0.60	ns
Interactive effect	b_{12}	ns	ns	ns	ns	ns	ns	ns
	b_{13}	ns	ns	ns	ns	ns	ns	ns
	b_{14}	ns	ns	ns	ns	ns	ns	ns
	b_{23}	ns	ns	ns	ns	ns	ns	ns
	b_{24}	ns	ns	ns	ns	ns	ns	ns
	b_{34}	ns	ns	ns	ns	ns	ns	ns
Statistics (R^2)		0.9689	0.9206	0.9508	0.8639	0.9224	0.9588	0.8256

3.3. Optimal extraction conditions for maximizing the responses criteria

Although the effects described above provided a guiding range of conditions, where the responses are maximized, optimal values can be achieved using a simple method tool to solve non-linear problems (Heleno et al., 2016; Pinela et al., 2016a). The results of the application of this simple procedure are presented in Table 3, in which the operating conditions that maximize each of the responses, in individual and global terms, are presented. Additionally, Fig. 4 summarizes the information derived from the mathematical equations, presenting the 2D graphs as a function of all the accessed variables. The results for the responses of the individual values of each identified betacyanin compound (Y_{P1}, Y_{P2}, Y_{P3} and Y_{P4}) were excluded due to their pattern similarities with the Y_{Total}. The variables in each of the 2D graphs were positioned at the optimal values of the others (Table 3). If the optimal individual conditions achieved are not found in absolute terms and they are relative optimums when the optimal value may be outside of the experimental range studied, the conditions that maximize the responses are marked (*). The information can be summarized as follows (first part of Table 3):

- The maximum response values and optimal conditions for the MAE technique were: the Y_{Yield} with 58.6 ± 1.5% at the optimal conditions of 11.7 min, (*) 180 °C, 28.2% of ethanol and (*) 5 g/L; the Y_{Total} with 39.6 ± 1.8 mg/g at the optimal conditions of (*) 8 min, (*) 60 °C, 0% of ethanol and (*) 5 g/L; the Y_{Colour} with 68.8 ± 2.2 at the optimal conditions of 11.7 min, (*) 60 °C, 0% of ethanol and (*) 5 g/L.

- The maximum response values and optimal conditions for the UAE technique were: the Y_{Yield} with 65.9 ± 4.8% at the optimal conditions of (*) 22.0 min, (*) 500 W, 0% of ethanol and 13.7 g/L; the Y_{Total} with 46.9 ± 4.8 mg/g at the optimal conditions of (*) 22 min, 300 W, 0% of ethanol and (*) 5 g/L; the Y_{Colour} with 59.9 ± 1.6 at the optimal conditions of (*) 2 min, 100 W, 0% of ethanol and (*) 5 g/L.

When combining, the information produced from all response criteriás (Y_{Yield}, Y_{Colour}, Y_{Total}, Y_{P1}, Y_{P2}, Y_{P3} and Y_{P4}), the complete behaviour of each relevant variable influencing the responses is defined in global terms. The global optimizing results are presented in the second part of Table 3 and summarized below:

- For MAE response: the optimal global conditions were at (*) 20 min, (*) 60 °C, 0% and (*) 5 g/L, producing 8.0 ± 0.6% (Y_{Yield}), 39.6 ± 2.9 mg/g (Y_{Total}) and 68.7 ± 6.2 (Y_{Colour}). Regarding the responses of the compounds P1 to P4 the results in mg/g were 4.9 ± 1.1, 12.4 ± 1.5, 4.2 ± 0.9 and 9.2 ± 1.1, respectively.
- For UAE response: the optimal global conditions were at (*) 22 min, 257.8 W, 0% and (*) 5 g/L, producing 55.2 ± 5.1% (Y_{Yield}), 45.5 ± 6.2 mg/g (Y_{Total}) and 37.6 ± 2.5 (Y_{Colour}). Regarding the responses of the compounds P1 to P4 the results in mg/g were 9.3 ± 1.4, 17.5 ± 1.1, 3.6 ± 0.4 and 12.1 ± 1.2, respectively.

The global optimal conditions found were experimentally tested to confirm the accuracy of the presented results. Comparing the results of the extraction efficiencies among the techniques, UAE gave signifi-

Table 3

Operating conditions that maximize the extraction of betacyanin from *Gomphrena globosa* and optimal response values for the parametric response criteria. Response criteria comprise the following: extraction yield of the obtained residue (Y_{Yield}); individual content of the betacyanins compounds of gomphrenin II (Y_{P1}), gomphrenin III (Y_{P2}), isogomphrenin II (Y_{P3}) and isogomphrenin III (Y_{P4}); total betacyanin content (Y_{Total}); and colour intensity (Y_{Colour}). Note that the independent variable X_2 in the ultrasound extraction is related to the power (W), meanwhile for the maceration and microwave extraction is related to temperature (°C).

	Optimal Extraction Conditions				Response Optimum
	X_1:t (min)	X_2:T (°C) or P (W)	X_3:Et (%)	X_4:S/L (g/L)	
Individual Optimal Responses					
Microwave Assisted Extraction (MAE)					
Y_{Yield}	11.7	180.0	28.2	5.0	58.6 ± 1.5%
Y_{Total}	8.0	60.0	0.0	5.0	39.6 ± 1.8 mg/g
Y_{Colour}	8.0	60.0	0.0	5.0	68.8 ± 2.2
Y_{P1}	8.0	60.0	0.0	5.0	5.1 ± 0.9 mg/g
Y_{P2}	8.0	60.0	0.0	5.0	12.7 ± 1.1 mg/g
Y_{P3}	8.0	60.0	0.0	5.0	4.4 ± 0.7 mg/g
Y_{P4}	8.0	60.0	0.0	5.0	9.5 ± 0.6 mg/g
Ultrasound Assisted Extraction (UAE)					
Y_{Yield}	22.0	500.0	0.0	13.7	65.9 ± 4.8%
Y_{Total}	22.0	300.0	0.0	5.0	46.9 ± 4.8 mg/g
Y_{Colour}	2.0	100.0	0.0	5.0	59.9 ± 1.6
Y_{P1}	22.0	500.0	0.0	5.0	9.5 ± 1.1 mg/g
Y_{P2}	22.0	500.0	0.0	5.0	19.0 ± 3.3 mg/g
Y_{P3}	13.2	300.0	0.0	5.0	4.2 ± 0.7 mg/g
Y_{P4}	22.0	500.0	0.0	5.0	12.7 ± 2.6 mg/g
Global Optimal Responses					
Microwave Assisted Extraction (MAE)					
Y_{Yield}	20.0	60.0	0.0	5.0	8.0 ± 0.6%
Y_{Total}					39.6 ± 2.9 mg/g
Y_{Colour}					68.7 ± 6.2
Y_{P1}					4.9 ± 1.1 mg/g
Y_{P2}					12.4 ± 1.5 mg/g
Y_{P3}					4.2 ± 0.9 mg/g
Y_{P4}					9.2 ± 1.1 mg/g
Ultrasound Assisted Extraction (UAE)					
Y_{Yield}	22.0	257.8	0.0	5.0	55.2 ± 5.1%
Y_{Total}					45.5 ± 6.2 mg/g
Y_{Colour}					37.6 ± 2.5
Y_{P1}					9.3 ± 1.4 mg/g
Y_{P2}					17.5 ± 1.1 mg/g
Y_{P3}					3.6 ± 0.4 mg/g
Y_{P4}					12.1 ± 1.2 mg/g

cantly higher values than MAE. Regarding the extraction time, MAE was a faster extraction method. Although water is a polar solvent with greater interest in green processes, ethanol has a hydroxyl group and dissolves many ionic compounds, but also has a non-polar end, which will contribute to dissolve non-polar substances. However, in the extraction of betacyanin compounds, in terms of colour intensity (Y_{Colour}), or content (Y_{Total}, Y_{P1}, Y_{P2}, Y_{P3} and Y_{P4}), the presence of ethanol did not improve their final optimal values. The optimal conditions of the binary interactions of ethanol-water mixture were found generally preferable in pure water solutions. About the S/L factor, a broad range was tested, finding that lower values lead to an enhanced extraction yield, but also contributed to a significant waste of solvent. A higher S/L will result in lower extraction yields but in a better rationalization of raw materials consumption.

3.4. Comparing the betacyanin extraction results from G. globosa obtained by MAE and UAE with results from literature using other plant source materials

For *beta vulgaris*, one of the best source of betacyanins, different authors have shown optimal extraction values using different variable conditions: 0.26–1.99 mg/g dw (Vulić et al., 2012); 2.81–5.38 mg/g dw (Stagnari et al., 2014); and 9.6 mg/g dw (Pavlov and Bley, 2006). From

the Amaranthaceae family (Li et al., 2015; Silva et al., 2012), the *Amaranthus hypochondriacus* L. showed a maximum value of 16.90 mg/g dw, meanwhile the *Amaranthus caudatus* L. showed 20.93 mg/g dw. Using paper flower (*Bougainvillea glabra* Choisy) as a material source, Maran et al., 2015 reported an optimum response of 1.76 mg/g dw. Even when a optimization procedure is applied using RSM with beetroot as a source of betacyanin compounds (Cardoso-Ugarte et al., 2014), the best results found were 1.3 mg/g of freeze-dried beet root. For dragon fruit (*Hylocereus polyrhizus* (Weber) Britton & Rose) values of 1.8 mg/g dw were found (Thirugnanasambandham and Sivakumar, 2015). All of them are far beyond those produced by *G. globosa* in which it was possible to obtain 39.6 and 46.9 mg/g (expressed as plant dw) with MAE and UAE, respectively.

In general, UAE or MAE modern techniques, showed better responses than traditional ones (e.g. dynamic maceration). Although it was expected to extract higher amounts of betacyanin compounds, the working variables t and Et showed lower optimal values than usual, making these techniques an asset for industry.

4. Conclusions

When compared with traditional techniques, MAE and UAE, are powerful and modern extraction procedures that proved to be efficient for betacyanins extraction, with the advantage of reducing the extraction time. The combined effects of the independent variables t, T or P, Et and S/L on the extraction of couloring compounds from *G. globosa* were optimized using a five-level full factorial design of 24 combinations and four replicates at the centre to maximize the responses. The response criteriás were the extract yield (Y_{Yield}), the analytical colour parameter $a*$ (Y_{Colour}), the quantification of the total sum of the betacyanin compounds by HPLC-DAD analysis (Y_{Total}) and the quantification of the individual betacyanin compounds P1 to P4 (Y_{P1} to Y_{P4}). The experimental domain was successfully implemented for MAE and UAE optimization using RSM. The MAE and UAE conditions were optimized for each response, as well as for the combined responses. Under the global optimum conditions for MAE (t = 8 min, T = 60 °C, Et = 0%, and S/L = 5 g/L), the optimal response value was 39.6 ± 1.8 mg/g; for UAE (t = 22 min, P = 500 W, Et = 0%, and S/L = 5 g/L) being in this case the optimal response value of 46.9 ± 4.8 mg/g, validating the UAE as an ideal extraction technique for obtaining the compounds of interest. The high coefficient R^2 value for each extraction methodology, higher than 0.9 for most of the analysed responses, and the no-significant difference between predicted and experimental values demonstrated the validity of the proposed optimization model.

Finally, we can highlight that this study reports the use of *G. globosa* as a source of betacyanin compounds in an optimized UAE system that would assist in the production of extracts with important colourant properties, thus with high potential to be used as natural colorant additives.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.05.008.

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