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A multivariate study of the performance of an ultrasound-assisted madder dyes extraction and characterization by liquid chromatography-photodiode array detection

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Abstract

An extraction method of madder (*Rubia tinctorum*) roots dyes is established and optimized to obtain the original chemical composition. A central composite design (CCD) was developed to specify the importance of the three major factors studied (time, temperature and solvent composition) affecting the ultrasound-assisted extraction of this matrix. A preliminary granulometric study of madder roots is realized in the aim to determine the optimal particles size corresponding to the best ultrasound effects. A comparison with the classical extraction method of madder dyes by reflux is described. The identification of the constituents of *R. tinctorum* is carried out by liquid chromatography coupled with a photodiode array detector (LC-PDA). Anthraquinonic aglycone and heterosidic dyes compounds are characterized by retention time and UV spectrum: alizarin (1,2-dihydroxyanthraquinone), purpurin (1,2,4-trihydroxyanthraquinone), lucidin (1,3-dihydroxy-2-hydroxymethylanthraquinone), rubiadin (1,3-dihydroxy-2-methylanthraquinone),

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xanthopurpurin (1,3-dihydroxyanthraquinone), pseudopurpurin (1,2,4-trihydroxy-3-carboxyanthraquinone), lucidin primeveroside, ruberythric acid (alizarin primeveroside), galiosin (pseudopurpurin primeveroside) and rubiadin primeveroside. The optimal experimental conditions are 18 min, 36°C and 37/63 MeOH/H_2O (v/v).

Keywords : Anthraquinone; Ultrasound; LC-PDA; Madder (*Rubia tinctorum*); Extraction.

1. Introduction

Madder is a tinctorial plant belonging to the Rubiaceae family. There are several species of madder; the two main ones are *Rubia tinctorum* and *R. peregrina* growing from Mediterranean Europe to Asia. Two "Indian-type" of madder: *R. cordifolia* and *R. sikkimensis* are growing from Asia to Indonesia and, there is also an endemic species to Japan, *R. akane* [1]. *R. tinctorum* corresponds to the most known and used madder [1, 2]. This last species has been widely employed since ancient time for dyeing textiles (cotton, wool or silk) [3-9] and for painting [10]. Nowadays, the madder term seems to be reserved to *R. tinctorum* [6]. Madder roots contain dyes with an anthraquinonic (anthracen-9,10-dione) skeleton corresponding to heterosidic and aglycone molecules. The aglycone compounds are alizarin (1,2-dihydroxyanthraquinone), purpurin (1,2,4-trihydroxyanthraquinone), pseudopurpurin (1,2,4-trihydroxy-3-carboxyanthraquinone), lucidin (1,3-dihydroxy-2-hydroxymethylanthraquinone), xanthopurpurin (1,3-dihydroxyanthraquinone) and rubiadin (1,3-dihydroxy-2-methylanthraquinone). The heterosidic dyes are composed by molecules with an anthraquinonic part (aglycone) and a primeverose one (6-*O*-β-D-xylopyranosyl-β-D-glucose). The major heterosidic dyes are lucidin primeveroside, ruberythric acid (alizarin primeveroside), galiosin (pseudopurpurin primeveroside) and rubiadin primeveroside (Table 1).
Several screening methods of anthraquinones, based on reversed-phase liquid chromatography (RP-LC) and capillary electrophoresis (CE) have been described in the literature [11-15]. This paper deals with the high performance liquid chromatographic analysis of anthraquinonic compounds of madder extracted by several extraction processes. LC-PDA analyses are optimized and performed in order to characterize madder dyes by retention time and UV spectrum.

Nowadays, the classical extraction method of madder dyes is a reflux of roots with a water-alcohol mixture during more than one hour [16, 17]. So, this research work is axed to a novel process using ultrasounds to extract dyes originally biosynthesized by the plant. Power ultrasound is now well known to have significant effects on the rate of various physical and chemical processes. Much attention has been given to the application of ultrasound for the extraction of natural products

that usually needed hours or days with conventional methods. Using ultrasound, full extractions can now be completed in some minutes with high reproducibility, reducing the quantity of solvent and simplifying manipulation. Several groups of chemical components such as aromas, pigments, antioxidants, and other organic and mineral compounds have been extracted and efficiently analyzed from a variety of matrices (mainly animal tissues, food and plant materials) [18-25]. Ultrasound technique was also used to assist the combination between dye, metal ions and fibre during the dyeing of different matters [26-27]. Previous work was performed using microwaves for the extraction of dye compounds in Rubiaceae plants [28]. Moreover, a central composite design (CCD) is developed to specify the importance of the three major studied factors (time, temperature and solvent composition) affecting the ultrasound-assisted extraction of madder roots. A preliminary granulometric study of madder roots is realized to determine the optimal particles size corresponding to the best ultrasound effects. The yield of each madder sample extract resulting from all the experiments is considered. The comparison between CCD experiments and traditional one by reflux is realized to validate the novel extraction studied method.

The aim of this study is (i) to characterize madder roots dyes, (ii) to develop a simple method for the detection of such molecules by LC, (iii) to establish and to optimize an exhaustive extraction method of madder roots dyes and, (iv) to obtain the best yield of extraction in comparison with dry matrix, preserving of the native chemical population of madder.

2. Experimental

2.1. Materials

Solvent and reagents were all of analytical grade from Merck (Darmstadt, Germany). Alizarin and purpurin were purshased from Acros Organics (Geel, Belgium). Lucidin primeveroside ruberythric acid, rubiadin primeveroside and rubiadin have been kindly furnished by Pr. V. Golicov (Russian Research Institute for Cultural and Natural Heritage, Moscow, Russia). Lucidin primeveroside was hydrolysed (HCl) to obtain lucidin which its structure was characterized on the

basis of chemical and spectral evidence including two dimensional NMR experiments (COSY and NOESY ^{1}H-^{1}H, HMQC and HMBC) and mass spectrometric techniques (EI, HR-MS). *Rubia tinctorum* roots were purchased from Okhra (Roussillon, France).

2.2. Ultrasound apparatus and procedure

Ultrasounds were applied by means of a PEX 3 (R.E.U.S., Contes, France) sonifier (25 kHz, 150 W), composed by an inox jug with a maximum capacity of 3 L (Fig. 1). The actual ultrasonic power dissipated to the system was experimentally determined and more details are given in section 3.1. 20 g of crushed madder roots were extracted with 500 mL methanol-water mixture. 1 mL of the filtered extract was taken for the LC-PDA analysis, and the remaining phase was evaporated to dryness to determinate the corresponding yield.

2.3. Reflux procedure

In accord with specialized literature [17], 6 g of pulverised madder roots were extracted with 150 mL methanol-water (80:20, v/v) applying a reflux condenser (1 h). As previously, 1 mL of the filtered extract was taken for the LC-PDA analysis, and the remainder was evaporated.

2.4. Granulometric apparatus

A granulometric apparatus was used (i) to obtain a homogenous powder and (ii) to study the consequence of the granulometric size of madder powder to resulting extraction. Madder roots were crushed and the separation of the obtained powder was carried out with a sieve shakers Fritsch (Idar-Oberstein, Germany) including various granulometric sizes sieves (125 µm to 1.25 mm), (Prolabo, Paris, France).

2.5. Liquid Chromatography-photodiode-array detection

The LC-PDA analysis was carried out using a Waters liquid chromatography consisting of a quaternary pump Waters 600, an in-line vacuum degasser, a Rheodyne 7125 injector equipped with a 20 µL loop and a photodiode array detection system Waters 2996. The system was equipped with a C_{18}-column (Symmetry Shield RP18, Waters 5 µm, 4.6 × 250 mm) and controlled by Empower 2 software.

The LC separation was performed at 35°C with a binary elution mixture composed of acetonitril (A) and bidistilled water (B) containing 0.01% trifluoroacetic acid (TFA). The chromatographic analysis was carried out for 30 min at a continuous flow-rate of 0.7 mL/min. The gradient program was as follows: 0-5 min, 30% A and 70% B; 5-10 min, 30-70% A and 70-30% B; 10-20 min, 70% A and 30% B; 20-25 min, 70-100% A and 30-0% B; 25-30 min, 100% A. All chromatograms were acquired at 450 nm. Each sample was injected in triplicate.

2.6. Experimental design

A Box-Wilson central composite design, commonly called a central composite design (CCD) has been established to study the performance of the ultrasonic extraction. A multivariate method was chosen to optimise the number of experiments and allow identification of interactions between variables. This CCD comprises a three-level full factorial design (+1, –1), superimposed by the centre point (coded 0), and the star points (+α, –α). The star points allow estimation of the curvature in the model and establish new extremes for the low and high settings for all factors. The precise value of α depends on certain properties desired for the design and on the number of factors involved. In this study the design point describes a circle circumscribed about the factorial square. Usually, for three factors, the central composite circumscribed (CCC) design points describe a sphere around the factorial cube.

Each of the three studied variables (time, temperature, and solvent composition) has levels set at five separate coded levels: -α (= –1.68), -1, 0, +1, +α (= +1.68) as showed in Table 2. These

values were used to create a CCC design and the interpretation of data obtained was analysed by a statistical experimental design computer programs [29,30].

3. Results and discussion

3.1. Ultrasonic power measurement

A common problem in the sonochemical literature is that the power delivered to the system (as quoted by the manufacturer) is mentioned, but the actual power dissipated (P_{diss}) in the extraction mixture is rarely reported. One of the most common methods of measuring P_{diss}, introduced by Lorimer *et al.* [31], is to use the equation:

$$P_{diss} = \frac{dT}{dt} \sum m_i Cp_i$$

where m_i and Cp_i are the mass and heat capacity of the solvent, respectively, and dT/dt is the initial slope of the graph of temperature of the extraction mixture versus the time of exposure to ultrasound as shown in Fig.2. This equation is based on the use of calorimetry and assumes that all of the power entering the extraction mixture is dissipated as heat.

The power actually dissipated to the system was calculated to be 42 W whereas the maximum available ultrasonic output power quoted by the manufacturer, Pg, is 150 W.

3.2. Preliminary study

A preliminary study consisting of various experiments was carried in order to determine the role of the factors involved in the ultrasound-assisted extraction of madder dyes. The main factors are the size of the madder roots, the extraction time, the temperature and the solvent composition.

The roots size is an important parameter for the ultrasound extraction, because the efficacy of ultrasounds depends on it. The more size of the root increases, the more its contact surface decreases in comparison with its weight. However, a smaller root stays in the solvent surface during the extraction, so the ultrasonic effects are not optimized. So it is necessary to determine the best

particle size corresponding to the best effects of ultrasound. In the aim to optimize this parameter, a study was carried out in extracting, with the ultrasonic apparatus, six different roots sizes in the same experimental conditions arbitrarily determined (15 min, 25°C and 80% MeOH). After crushing, the corresponding madder roots powder was separated in function of its granulometric size. Several sieves were employed corresponding to 0.125, 0.25, 0.5, 0.8, 1 and 1.25 mm. The obtained results translate that the optimal sieve corresponding to the best yield is 0.5 mm (Fig. 3). Thus, this size of the granulometry has been used to continuate of this study.

The extraction of madder roots was realized with a water-methanol mixture. The proportions of these two solvents must be optimised to obtain the best conditions corresponding to an extract with the largest population of compounds. The temperature is also an important factor during the extraction of madder roots. In fact, the high sensibility of madder dyes, more particularly the heterosidic compounds, does not allow an extraction at high temperature. Moreover, it is important to note that the ultrasound effects decrease when temperature increases, so all of the experiments were realized at moderated temperature ($10°C \leq T \leq 50°C$). Finally, the extraction time must be optimised in order to obtain the highest efficiency of the extraction without affecting chemical structure of dyes. The classical extraction process by reflux is performed in 60 min, so we try to reduce this time factor using an ultrasonic apparatus extraction to validate the new technology.

3.2 Central composite design results

Responses obtained in the CCD experiments and the overall design are showed in Table 3. The yield corresponds to the weight of relative extracted dyes of madder roots reported to the weight of dry sample. The yields of all the experiments are included between 56.3% and 64.0%, except for experiment no 17 (38.1%). This last experiment was carried out in triplicate and this low value of yield was confirmed. An analysis of variance (ANOVA) was performed on the design to assess the significance of the model with the initial summary of the model statistics given by Table 4. The *F*-ratio in this table is the ratio of the mean square error to the pure error obtained from the

replicates at the design centre. The significance of the *F*-value depends on the number of degrees of freedom (DF) in the model, and is showed in the *P*-value column (95% confidence level). The Standardized Pareto Chart reveals two significant coefficients affecting the extraction, which are the squared term of extraction mixture (CC) and extraction mixture (C). The AB cross-product term is also important and corresponds to the interaction between the extraction time and the temperature in the studied area.

The second-order polynomial of the response surface obtained is as follows:

Yield of dye extracted by ultrasound (%) = $60.530 + 0.581t - 0.427T - 2.639S - 1.213tT + 0.012tS - 0.562TS + 0.073t^2 + 0.250T^2 - 3.391S^2$,

where *t* denotes extraction time (min), *T* temperature (°C) and *S* extraction mixture (% MeOH). The response surface for this polynomial is represented in Fig. 4 where a maximum at the positive extremes is clearly showed. Solvent composition is the major factor affecting the yield of the ultrasonic extraction of madder dyes. Indeed, the yield varies only when the extraction mixture changes and, remains stable when this factor is not modified. Extraction time and temperature also affect the yield in the same way as the extraction mixture.

3.3. Optimal conditions

It is possible to derive the optimal conditions for extraction from the first derivatives of the second order polynomial. The procedure involves equalling the derivatives to 0 and then to solve the resulting equations system. The optimal values of the variables affecting the ultrasound extraction are 18 min for the extraction time, 36°C, 37% of MeOH for the solvents mixture and 0.5 mm of granulometric size sieve, with a yield of 64.3%. This process was compared to the classical one corresponding to a refluxed method of madder roots during 60 min. The yield of this last experiment was 58,3%. Finally, a madder roots extraction was carried out in the optimal conditions without ultrasounds (control), with a yield of 56.2% (Table 5). This last experiment permitted to show the ultrasound effects on the extraction.

3.4. Composition of madder's extract

Initially, madder plant biosynthesises heterosidic compounds which are also the aglycone dyes precursors. From the third year, the plant is considered as enough mature to give the best coloration [32]. Then, madder carries out an enzymatic hydrolysis of the precursors to give aglycone compounds. These dyes are thermosensitive compounds, in particular the heterosidic precursors. Indeed, during the extraction process a high temperature may accelerate this enzymatic hydrolysis or cause degradation of any compounds as galiosin. The presence of purpurin could be explained by a double degradation process of galiosin via an enzymatic hydrolysis of the heterosidic precursor synthesizing pseudopurpurin which undergone a decarboxylation to obtain purpurin. This alteration can modify the chemical composition, and thus falsify analysis with erroneous results. So, the extraction temperature is an important factor to conserve the native chemical composition of dye compounds.

Each of twenty samples of madder extracts obtained during the CCD experiments was analysed by liquid chromatography (Table 6) and the corresponding results were compared. Peaks in the chromatograms of madder dyes were identified on the basis of the retention times and UV-Visible absorption spectra of the references molecules injected in the same conditions. The main anthraquinonic dyes of madder were identified: lucidin primeveroside (**1**), ruberythric acid (**2**), galiosin (**3**), rubiadin primeveroside (**4**), lucidin (**6**), alizarin (**7**), purpurin (**9**) and rubiadin (**10**). Pseudopurpurin (**5**) and xanthopurpurin (**8**) were not systematically detected in all the chromatograms. The compound eluted at 25.8 min is unknown and shows generally a very low peak area.

The principal compounds present in these madder roots chromatograms were three heterosidic precursors and one aglycone in smaller relative proportion. Lucidin primeveroside (**1**), ruberythric acid (**2**) and galiosin (**3**) were represented by peaks with a large area at 450 nm; they indicated, in relative proportion, more than 80% of all dyes (respectively 36.4%, 41.2% and 4.4%

on average for all the experiments in relative percentage). Alizarin (**7**) was the main aglycone compound and its relative proportions varied in madder extracts between 5.5% and 24.6%, directly depending on the extraction conditions. This compound, the most known of madder dyes, is also a degradation product [33]. The presence of alizarine could be the result of its original presence in madder roots but its presence could also be the consequence of a degradation process of its precursor compound named ruberythric acid. So it is difficult to interpret the origin and the relative proportion of this kind of molecule. In order to determine the reasons of these variations in relative percentage, all the compounds areas were put, as response, in the CCD. Table 7 allows to define the factors influencing anthraquinonic compounds extraction and reveals the extraction mixture (C) as a significant coefficient affecting the compounds extraction. But time (A) and temperature (B) must also be considered. Indeed, these parameters influence the extraction of aglycone compounds which result from the hydrolysis of precursors. The more time and/or temperature increase during extraction, the more the denaturation of precursors, which are thermosensitive molecules, is important.

The chemical composition of the optimal experiment and the classical method one were compared. The two obtained chromatograms showed a very similar chemical composition and translated a same chromatographic fingerprint. Heterosidic compounds (**1**, **2**, **3** and **4**) and aglycones ones (lucidin (**5**), alizarin (**7**) and purpurin (**9**)) were detected. Xanthopurpurin (**8**) was only present in extract coming from the classical method. In this point of view, it is difficult to carry out a qualitative interpretation of theses results. So it is necessary to introduce a ratio to determine the state of degradation according to the proportion of the chemical composition. The ratio between one of the most important precursors (ruberythric acid (**2**)) and its corresponding aglycone (alizarin (**7**)) was established. The more the ratio increases, the more the precursor proportion is important and less this compound is degraded. The chromatogram obtained by the optimal CCD conditions (Fig. 5) traduced a ratio, in relative percentage, between ruberythric acid (**2**) and alizarin (**7**) R = 7.3. The same ratio was calculated for classical method and the corresponding value was R = 4.4.

The comparison of the two ratios showed that the degradation state is less important for the optimal experiment than the classical method. Indeed, in the first extraction the relative percentage in precursor is more important than the aglycone compound, so it is characteristic to an extraction with a lower chemical denaturation.

3.5. Cost and energy

The ultrasound-assisted madder dyes extraction permits to reduce cost of the experiment. Indeed, the proposed method is advantageous for energy and time. Classical process required an extraction time of 60 min. The ultrasound method needs only 18 min. The energy required to perform the two extraction methods are respectively 0.2 kWh for conventional extraction (electrical energy for heating and boiling) and 0.1 kWh for ultrasound extraction (electrical energy for ultrasound supply). The power consumption has been determined with a Wattmeter at the ultrasound extractor entrance and the electrical heater power supply. Calculations were carried out, for the two processes, with the same quantities of solvent and madder roots. Ultrasound process can be considered as a “green” process preserving energy for a lasting development.

4. Conclusion

This multivariate study of ultrasound-assisted extraction based on a central composite design has permitted to considerably reduce the number of the experiments in comparison with a traditional optimization method in varying simultaneously three parameters: only 20 experiments were performed against 125 ones in usual conditions. The interpretation of the results showed that the optimal values of the variables affecting the ultrasound effects were 18 min for the extraction time, a temperature of 36°C, a composition of solvents in MeOH/H_2O 37/63 (v/v) and a madder roots granulometric size of 0.5 mm. This process gave a dye yield of 64.3%. Moreover, this method was compared with the classical one (reflux) and the optimal conditions without ultrasounds, with respective yields of 58.3% and 56.2%. Liquid chromatographic study of extracts showed

quantitative and qualitative differences of chemical dyes composition in function of the experimental conditions used. So, the comparison between the optimal experiment obtained by CCD and the classical one by reflux showed that they present the same qualitative chemical composition, but in our experimental extraction conditions it could seem that the relative proportion between precursor and aglycone compounds was preserve. So, the optimal experiment corresponded to the best extraction process in comparison with the other one.

The ultrasonic process permitted to reduce extraction time (18 min versus 1 h) and energy cost, to give a better yield and to preserve the dyes population by using soft extraction parameters values. This study could be very important to promote these substances and more especially in the "natural colour" dyeing process applied to the textile industry.

Acknowledgments

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References

[1] D. Cardon, Le monde des teintures naturelles, Editions Belin, Paris, 2003.

[2] N.P. Mischenko, S.A. Fedoreyev, V.P. Glazunov, G.K. Chernoded, V.P. Bulgakov, Y.N. Zhuravlev, Fitoterapia 70 (1999) 552-557.

[3] J. Orska-Gawrys, I. Surowiec, J. Kehl, H. Rejniak, K. Urbaniak-Walczak, M. Trojanowicz, J. Chromatogr. A 989 (2003) 239-248.

[4] P. Novotná, V. Pacáková, Z. Bosáková, K. Štulík, J. Chromatogr. A 863 (1999) 235-241.

[5] B. Szostek, J. Orska-Gawrys, I. Surowiec, M. Trojanowicz, J. Chromatogr. A 1012 (2003) 179-192.

[6] C. Clementi, W. Nowik, A. Romani, F. Cibin, G. Favaro, Anal. Chim. Acta 596 (2007) 46-54.

[7] J. Wouters, L. Maes, R. Germer, Stud. Conserv. 35 (1990) 89-92.

[8] I. Surowiec, A. Quye, M. Trojanowicz, J. Chromatogr. A 1112 (2006) 209-217.

[9] M. Trojanowicz, J. Orska-Gawryś, I. Surowiec, B. Szostek, K. Urbaniak-Walczak, J. Kehl, M. Wróbel, Stud. Conserv. 49 (2004) 115-130.

[10] C. Miliani, A. Romani, G. Favaro, Spectrochim. Acta Part A 54 (1998) 581-588.

[11] K. Krizsán, G. Szókán, Z.A. Tóth, F. Hollósy, M. László, A. Khlafulla, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 2295-2314.

[12] Z.A. Tóth, O. Raatikainen, T. Naaranlathi, S. Auriola, J. Chromatogr. A 630 (1993) 423-428.

[13] A.H. Lodhi, A.E.G. Sant'Ana, B.V. Charlwood, Phytochem. Anal. 5 (1994) 261-265.

[14] G.C.H. Derksen, T.A. van Beek, Æ. de Groot, A. Capelle, J. Chromatogr. A 816 (1998) 277-281.

[15] W.C. Weng, S.J. Sheu, J. High Resolut. Chromatogr. 23 (2000) 143-148.

[16] G.C.H. Derksen, H.A.G. Niederländer, T.A. van Beek, J. Chromatogr. A 978 (2002) 119-127.

[17] I. Boldizsár, Z. Szűcs, Zs. Füzfai, I. Molnár-Perl, J. Chromatogr. A 1133 (2006) 259-274.

[18] M.C. Herrera, M.D. Luque de Castro, J. Chromatogr. A 1100 (2005) 1-7.

[19] A. Jiménez, G. Beltrán, M. Uceda, Ultrason. Sonochem. 14 (2007) 725-731.

[20] I. Jerković, J. Mastelić, Z. Marijanović, Ž. Klein, M. Jelić, Ultrason. Sonochem. 14 (2007) 750-756.

[21] C.C. Nascentes, M. Korn, and M.A.Z. Arruda, Microchem. J. 69 (2001) 37-43.

[22]. A. Moreno-Cid, M.C. Yebra, S. Cancela, R.M. Cespón, Anal. Bioanal. Chem. 377 (2003) 730-734.

[23] H. Li, B. Chen, S. Yao, Ultrason. Sonochem. 12 (2005) 295-300.

[24] M.A. Rostagno, M. Palma, C.G. Barroso, J. Chromatogr. A 1012 (2003) 119-128.

[25] A.H. Goli, M. Barzegar, M.A. Sahari, Food Chem. 92 (2005) 521-525.

[26] V. Sivakumar, P.G. Rao, Environ. Sci. Technol. 38 (2004) 1616-1621.

[27] M.M. Kamel, R.M. El-Shishtawy, B.M. Youssef, H. Mashaly, Dyes Pigm. 73 (2007) 279-284.

[28] M. Dabiri, S. Salimi, A. Ghassempour, A. Rassouli, M. Tabeli, J. Sep. Sci. 28 (2005) 387-396.

[29] Nemrod, version 2000. (2003). LPRAI, Marseille, France.

[30] Statgraphics Plus, version 5.1. (2000). Statistical Graphics Corporation, Rockville, USA.

[31] V. Sivakumar, P.G. Rao, Ultrason. Sonochem. 10 (2003) 85-94.

[32] L.G. Angelini, L. Pistilli, P. Belloni, A. Bertoli, S. Panconesi, Ind. Crop. Prod. 6 (1997) 303-311.

[33] A.R. Burnett, R.H. Thomson, J. Chem. Soc. (1968) 2437-2441.

Figure 1

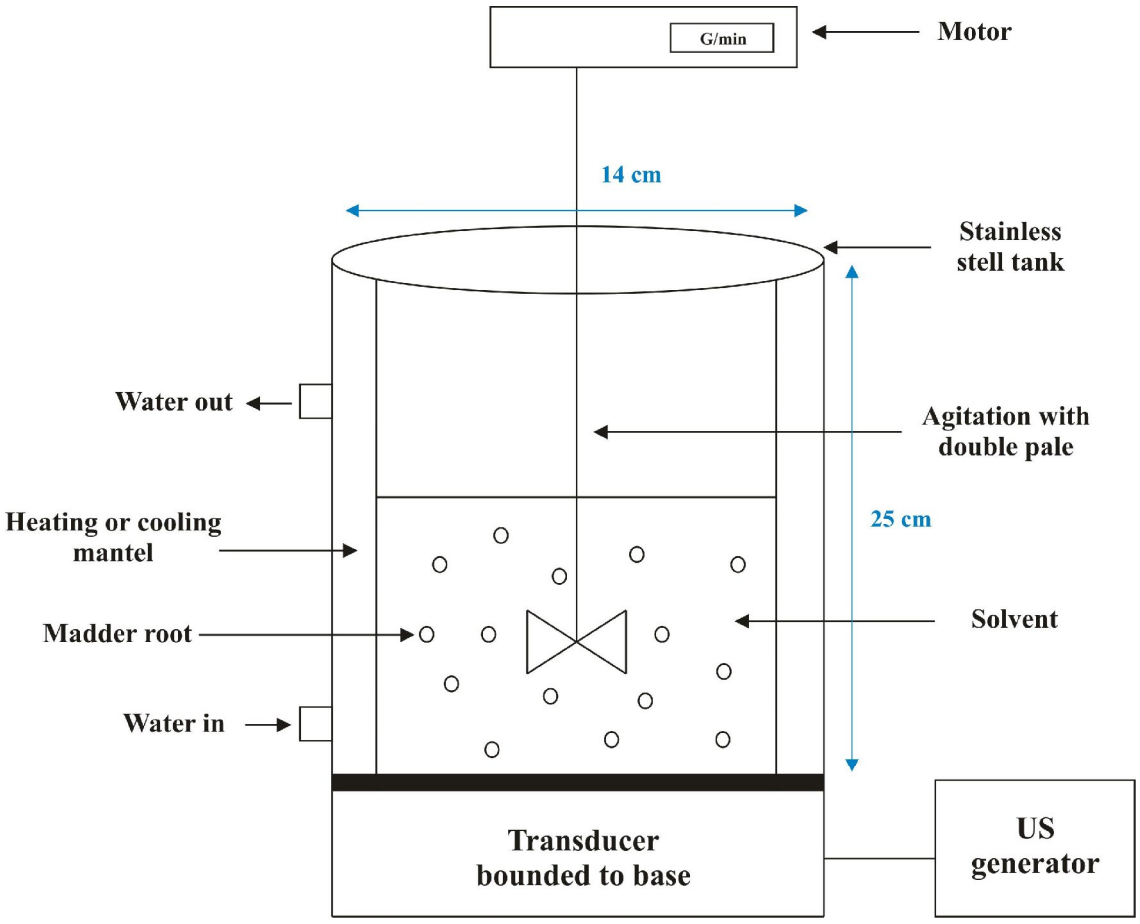

Fig. 1. PEX sonifier used to the madder extraction

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Figure 2

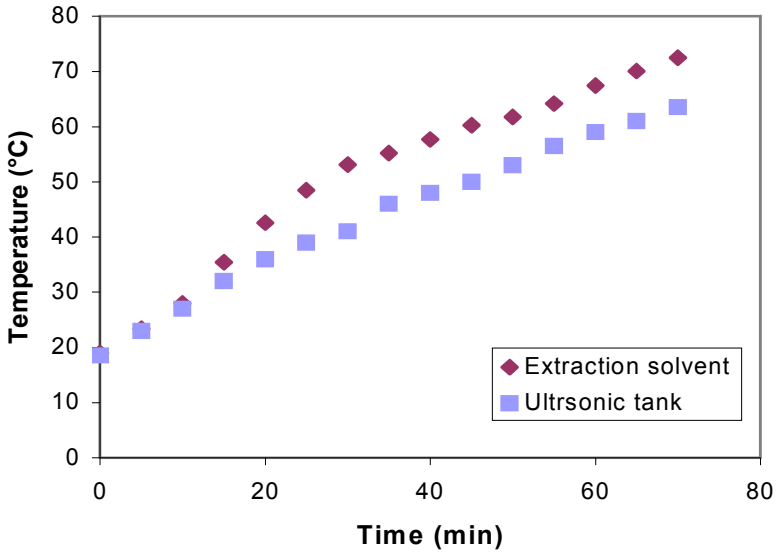

Fig. 2. Determination of power dissipated in the system from the temperature raise in the bath

Figure 3

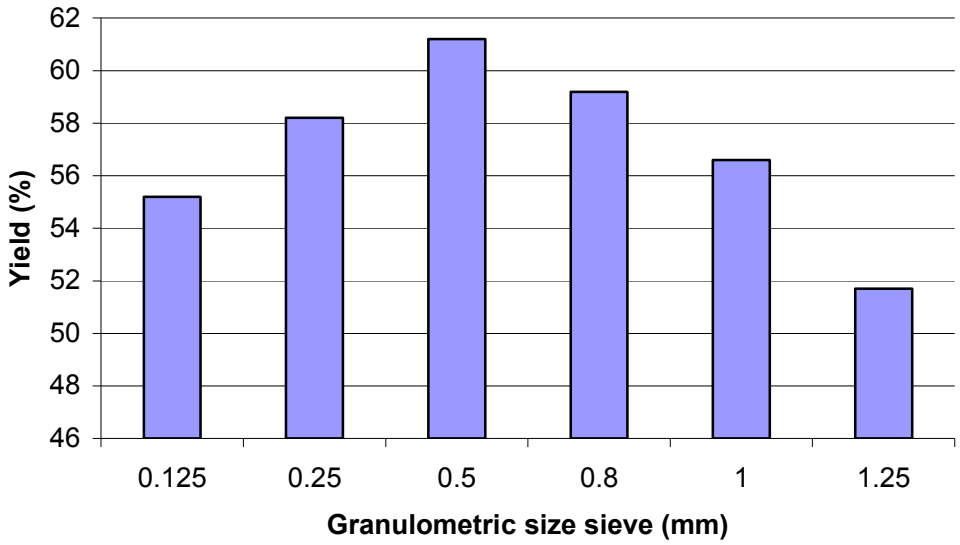

Fig. 3. Granulometric effect on the extraction yield

Figure 4

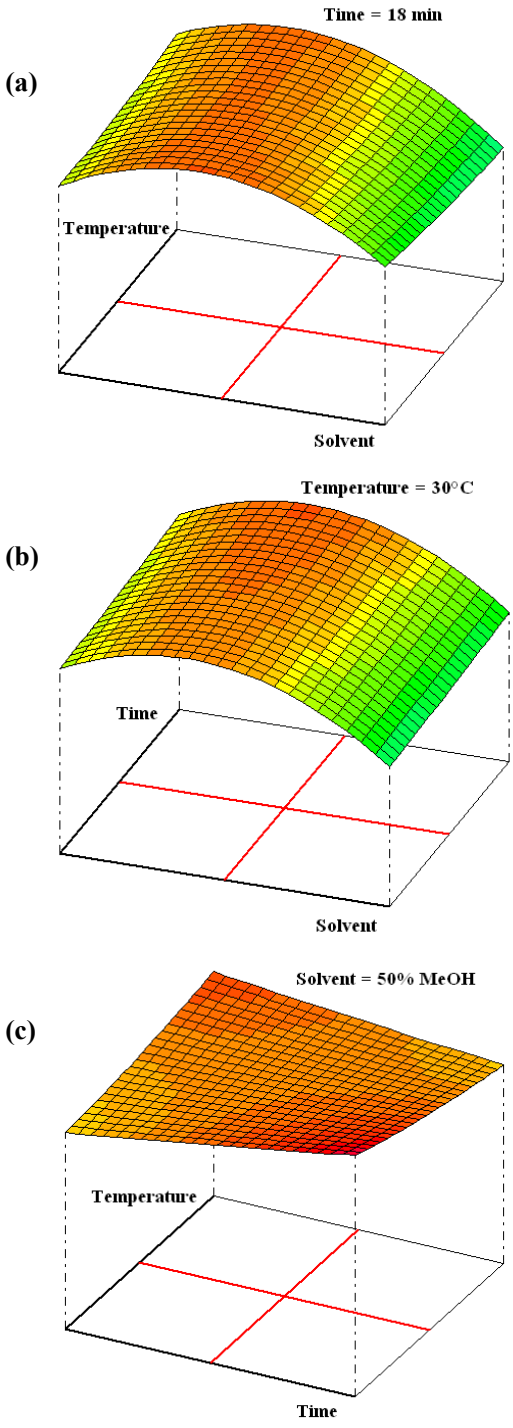

Fig. 4. Surfaces obtained with the CCD: (a) estimated percentage-solvent-temperature response surface, (b) estimated percentage-solvent-time response surface, (c) estimated percentage-time-temperature response surface

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Figure 5

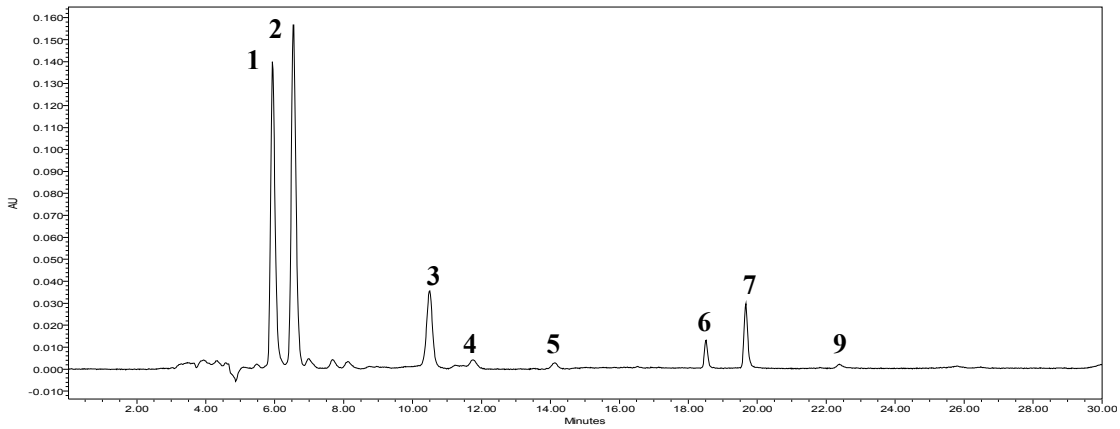

Fig. 5. LC-PDA chromatogram at 450 nm of madder extract obtained by the optimal CCD conditions

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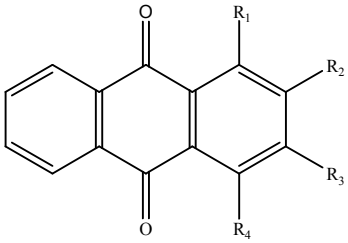
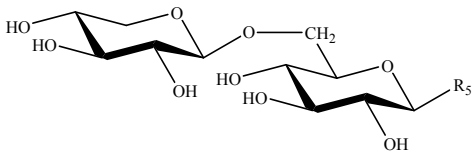
Table 1
Chemical dyes composition of madder roots



Anthraquinonic nucleus



Primeveroside R_5=O-aglycone

Common name	Structure	Absorption maxima (nm)
lucidin primeveroside (**1**)	R_1=OH, R_2=CH_2OH, R_3=O-primeveroside, R_4=H	203, 265, 406
ruberythric acid (**2**)	R_1=OH, R_2=O-primeveroside, R_3=H, R_4=H	199, 261, 334, 418
galiosin (**3**)	R_1=O-primeveroside, R_2=OH, R_3=COOH, R_4=OH	201, 255, 288, 434
rubiadin primeveroside (**4**)	R_1=OH, R_2=CH_3, R_3=O-primeveroside, R_4=H	203, 269, 412
pseudopurpurin (**5**)	R_1=OH, R_2=OH, R_3=COOH, R_4=OH	203, 253, 439
lucidin (**6**)	R_1=OH, R_2=CH_2OH, R_3=OH, R_4=H	203, 245, 280, 414
alizarin (**7**)	R_1=OH, R_2=OH, R_3=H, R_4=H	199, 248, 428
xanthopurpurin (**8**)	R_1=OH, R_2=H, R_3=OH, R_4=H	200, 245, 281, 416
purpurin (**9**)	R_1=OH, R_2=H, R_3=OH, R_4=OH	203, 256, 294, 481
rubiadin (**10**)	R_1=OH, R_2=CH_3, R_3=OH, R_4=H	203, 244, 278, 410

Table 2
Values of the variables at five levels used with the design

Level	Time (min)	Temperature (°C)	Solvent (% MeOH)
-α	5	10	0
-1	10	18	20
0	18	30	50
+1	25	42	80
+α	30	50	100

Table 3
Fully coded central composite design and corresponding yield

Run order	Time (min)	Temperature (°C)	Solvent (% MeOH)	Yield (%)
1	+1	+1	+1	56.3
2	-1	-1	+1	57.8
3	0	0	-α	58.4
4	-1	+1	+1	58.4
5	0	-α	0	57.4
6	+1	-1	-1	61.6
7	-1	-1	-1	58.9
8	+1	-1	+1	64.0
9	0	0	0	62.4
10	+1	+1	-1	59.6
11	0	+α	0	59.7
12	0	0	0	60.1
13	-α	0	0	58.1
14	-1	+1	-1	58.3
15	0	0	0	60.9
16	0	0	0	60.1
17	0	0	+α	38.1
18	0	0	0	60.0
19	+α	0	0	58.0
20	0	0	0	60.6

Table 4
Summary of the ANOVA model statistics

Effect	Sum of squares	DF	Mean squares	*F*-ratio	*P*-value
A: time	4.607	1	4.607	0.22	0.6491
B: temperature	2.490	1	2.490	0.12	0.7373
C: solvent	95.110	1	95.110	4.54	0.0589
AA	0.078	1	0.078	0.00	0.9526
AB	11.761	1	11.761	0.56	0.4708
AC	0.001	1	0.001	0.00	0.9940
BB	0.903	1	0.903	0.04	0.8397
BC	2.531	1	2.531	0.12	0.7353
CC	165.744	1	165.744	7.92	0.0184
Total error	209.372	10	20.937		
Total	498.505	19			

R^2=58.0%; R^2 (adjusted for d.f)=20.20%

Table 5
Optimal conditions and yield for ultrasonic and classical processes

	Root size (mm)	Time (min)	Temperature (°C)	Solvent (% MeOH)	Yield (%)
Ultrasounds	0.5	18	36	37	64.3
Control	0.5	18	36	37	56.2
Reflux	Powder	60	75	80	58.3

Table 6
Dye composition of madder extracts

Extraction conditions	Experiment	Relative percentage of compounds (%)								
t (min), *T* (°C), *S* (% MeOH)	no	lucidin prim.	ruberythric ac.	galiosin	rubiadin prim.	lucidin	alizarin	purpurin	unk. t_R = 25.8	rubiadin
25-42-80	1	35.4	40.1	10.8	1.8	2.8	7.1	0.8	1.0	0.2
10-18-80	2	36.1	40.7	11.8	1.8	0.9	5.6	0.7	2.2	0.1
18-30-0	3	29.9	37.1	0.8	1.4	2.9	13.5	0.8	12.4	1.1
10-42-80	4	34.4	38.3	13.6	1.7	2.9	7.0	1.0	0.8	0.2
18-10-50	5	38.9	43.1	4.2	1.8	1.1	7.7	0.9	2.3	0.0
25-18-20	6	33.4	39.6	2.7	2.0	0.7	10.8	0.6	9.8	0.4
10-18-20	7	38.4	44.6	1.1	1.8	0.5	6.9	0.7	5.6	0.4
25-18-80	8	36.7	41.4	11.4	1.6	2.3	5.7	0.5	0.2	0.2
18-30-50	9	39.4	44.2	1.6	1.9	0.4	7.8	0.8	3.4	0.5
25-42-20	10	25.2	27.4	2.2	2.7	4.8	24.6	0.6	11.6	0.8
18-50-50	11	37.3	40.8	1.0	1.6	0.7	8.2	0.7	8.7	1.1
18-30-50	12	39.9	44.9	1.6	2.0	0.4	7.7	0.7	2.6	0.3
5-30-50	13	37.5	42.3	10.3	1.6	1.4	5.5	0.5	0.8	0.0
10-42-20	14	34.1	39.9	2.0	0.4	1.3	12.9	0.6	8.3	0.4
18-30-50	15	40.2	44.8	1.4	1.0	0.3	8.2	1.5	2.7	0.0
18-30-50	16	40.8	45.4	0.3	0.5	0.4	8.1	0.8	3.2	0.2
18-30-100	17	36.7	39.7	7.4	1.7	3.3	7.8	0.7	0.0	0.0
18-30-50	18	39.5	44.6	1.5	1.8	0.5	8.5	0.9	2.5	0.2
30-30-50	19	39.4	43.6	0.9	1.9	0.2	9.9	0.8	3.1	0.2
18-30-50	20	40.0	44.3	1.7	1.9	0.4	8.0	0.7	2.8	0.1
	Reflux Exp.	31.6	38.4	12.4	1.3	1.8	8.7	1.1	0.4	0.0

Table 7
Parameter effects of each compounds

Compounds	A:Time	B:Temperature	C:Solvent	AA	BB	CC	AB	AC	BC
Precursors									
lucidin prim.	-1.20	-2.40	+3.03	-1.86	-2.08	-5.23	-0.61	+2.68	+1.64
ruberythric ac.	-1.54	-2.94	+1.61	-1.72	-2.31	-4.42	-1.00	+3.13	+2.07
galiosin	-1.67	-0.36	+4.80	+2.79	+1.33	+2.03	-0.48	-0.62	+0.10
rubiadin prim.	+1.57	-0.51	+0.28	+0.70	+0.57	+0.17	+1.7	-1.84	+0.56
Aglycone									
lucidin	+0.80	+1.81	+0.61	+0.82	+0.95	+3.88	+0.63	-0.84	-0.84
alizarin	+2.74	+2.77	-4.67	+0.24	+0.38	+1.97	+1.21	-2.39	-2.64
purpurin	+0.01	+0.18	+0.36	-1.46	-0.66	-0.92	+0.14	-0.43	+0.99
unk. t_R=25.8 min	+2.77	+4.23	-14.91	-1.07	+3.99	+5.00	+0.50	-3.48	-1.93
rubiadin	+0.99	+2.79	-3.74	-0.94	+1.71	+1.71	+0.47	-0.47	-0.47