Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 93 (2005) 417–423
Food Chemistry
www.elsevier.com/locate/foodchem

Optimization of accelerated solvent extraction of antioxidants from *Spirulina platensis* microalga

Miguel Herrero [a], Pedro J. Martín-Álvarez [a], F. Javier Señoráns [b], Alejandro Cifuentes [a], Elena Ibáñez [a,*]

[a] *Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain*
[b] *Área de Tecnología de Alimentos, Facultad de Ciencias, Univ. Autónoma de Madrid, Unidad asociada al IFI-CSIC, 28049 Cantoblanco, Madrid, Spain*

Received 28 June 2004; received in revised form 28 September 2004; accepted 28 September 2004

Abstract

An experimental design has been used to optimize the extraction of antioxidants from the microalga *Spirulina platensis* using accelerated solvent extraction (ASE) with four different solvents (hexane, petroleum ether, ethanol and water). The optimization of the main variables involved in the ASE process (extraction temperature and time) has been done by means of a full factorial (three levels) design using, as responses, the extraction yield and the antioxidant activity of the extracts (determined as EC_{50}, i.e., efficient concentration, using an in vitro assay based on a free radical method). The parameters of the model, for each response variable, were estimated by multiple linear regression (MLR). The statistical analysis of the results provided mathematical models that allowed prediction of the behaviour of the different responses selected, as a function of the main variables involved in the process. It was observed that the optimum conditions that maximize yield and minimize EC_{50} depend on the polarity of the solvent used to perform the extractions. Extraction temperature had an enormous influence in both responses while the effect of extraction time was almost negligible.

Ethanol was finally selected as the extracting solvent for its GRAS (Generally Recognized as Safe) status and because it provides higher yields with medium antioxidant activities. The results presented in this work show the possibility of using a fast and easy process to recover natural antioxidants from natural sources such as microalgae.

Keywords: ASE; Antioxidant compounds; Alga; Subcritical water; Experimental design; Optimization

1. Introduction

In recent years, there has been a growing interest in functional foods, that is, foods able to provide additional physiological benefits for human health, other than the basic nutritional and energetic requirements (Goldberg, 1996). Often, functional foods are traditional foods enriched with an ingredient able to provide or promote a specific beneficial action for human health. These are called functional ingredients. These ingredients are preferred to have a natural origin, such as plants or perhaps algae and/or microalgae. These types of marine sources are receiving increasing attention mainly for their content in, for example, polyunsaturated fatty acids (Mahajan & Kamat, 1995; Cohen & Vonshak, 1991), β-carotene and other pigments (antioxidants) (Madhava et al., 2000; Bhat & Madyastha, 2000) and sulphated polysaccharides (anti-virals) and sterols (antimicrobials) (Richmond, 1988; Otles & Pire, 2001; Xue et al., 2002). In this work, the microalga *Spirulina platensis* was investigated as a natural source of antioxidants because of its usefulness in food preservation and human health.

* Corresponding author. Tel.: +34 915 622 900(x)388; fax: +34 915 644 853.
E-mail address: elena@ifi.csic.es (E. Ibáñez).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.09.037

The traditional extraction methods used to obtain these type of products have several drawbacks. They are time consuming, laborious and exhibit low selectivity and/or extraction yields; moreover, they usually employ large amounts of organic solvents. At present, there is a renewed interest in developing new processes based on the use of sub- and supercritical fluids, that is, environmentally friendly processes that use GRAS solvents (ethanol, water) or small amounts of other organic solvents. These sub- and supercritical processes provide some additional benefits, such as higher selectivity and shorter extraction times. Among them, supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) are two of the most promising processes (King, 2000).

ASE has been used, with ethanol as a solvent, to study carotenoid extraction from microalgae *Haematococcus pluvialis* and *Dunaliella salina* (Denery, Dragull, Tang, & Li, 2004). The authors demonstrated that similar extraction yields could be obtained with this technique compared to traditional extraction techniques.

In a previous study, we demonstrated the possibilities of combining ASE, in vitro assays and micellar electrokinetic chromatography, along with diode array detection (MEKC-DAD), to investigate natural antioxidants from the microalga *Spirulina platensis* (Herrero, Ibáñez, Señoráns, & Cifuentes, 2004). In that paper, we presented the development of a new MEKC-DAD method able to provide a fast profile of the different components in the ASE-extracts; further, the extracts were functionally characterized by an in vitro assay and correlated with the obtained MEKC-DAD profile.

The objective of this work is to optimize, by means of an experimental design using a quadratic mathematical model, the process for extraction of antioxidant compounds from the microalga *Spirulina platensis.* Different extracting solvents have been tested in order to evaluate the influence of solvent polarity on the ability to extract natural antioxidant ingredients. Such optimization will provide, not only the right conditions (in terms of extraction yield and EC_{50} values, that is, the concentration of antioxidant needed to reduce, by 50%, the initial concentration of a free radical, DPPH) but also mathematical models able to properly predict the behaviour of the system, such as the influences of extraction temperature and time.

2. Material and methods

2.1. Samples and chemicals

Microalgae samples (*Spirulina platensis*) consisted of air-dried microalgae with 6% moisture by weight, from Algamar S.A. (Pontevedra, Spain). They were stored under dry and dark conditions.

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH, 95% purity) was obtained from Sigma–Aldrich (Madrid, Spain). Methanol and ethanol were obtained from Scharlau Chemie S.A. (Barcelona, Spain). Hexane (HPLC grade) from Lab Scan (Dublin, Ireland) and petroleum ether, purchased from Panreac Quimica S.A. (Barcelona, Spain), were also used, as well as Milli-Q Water (Millipore Corporation, Billerica, MA, USA).

2.2. Experimental design

A full factorial design (3 levels) with two factors (extraction temperature (Tem) and time (tim)) was used. A total of 12 experiments: 9 points of the factorial design, and 3 centre points to establish the experimental errors, were carried out in randomized run order. By using this design, the two variables were tested at 3 different levels: extraction temperatures at 60, 115 and 175 °C, and extraction times of 3, 9 and 15 min. The response variables selected were EC_{50} (i.e., efficient concentration, as a measure of antioxidant activity) and extraction yield (Yield).

Table 1 shows the experimental matrix design, with the experimental levels of the independent variables (factors), along with the results obtained for the response analyzed variables for each solvent (hexane, petroleum ether, ethanol and water). The quadratic model proposed for each response variable (Y_i) was

$$Y_i = ß_0 + ß_1\mathrm{Tem} + ß_2\mathrm{tim} + ß_{1,1}\mathrm{Tem}^2 + ß_{2,2}\mathrm{tim}^2 + ß_{1,2}\mathrm{Tem}\cdot\mathrm{tim} + \mathrm{error}, \quad (1)$$

where $ß_0$ is the intercept, $ß_1$ and $ß_2$ the linear coefficients, $ß_{1,1}$ and $ß_{2,2}$ the quadratic coefficients, $ß_{1,2}$ the interaction coefficient, and error is the error variable. The parameters of the model were estimated by multiple linear regression (MLR), using the programme MODDE 5.0, a Software for Design of Experiments and Optimization (Umetrics AB, Umeå, Sweden). This programme permits the creation and analysis of experimental designs. The goodness of fit of the model was evaluated by the coefficient of determination (R^2), the residual standard deviation (RSD) and the lack of fit test for the model from the ANOVA table. From the fitted model, the optimum conditions, that maximize the yield and minimize the EC_{50} response variables, were also provided by the programme. Contour plots were developed using the obtained fitted quadratic polynomial equation.

2.3. Accelerated solvent extraction

To perform the extractions with the four different solvents (namely, hexane, petroleum ether, ethanol and water), an accelerated solvent extraction system, ASE 200, equipped with a solvent controller unit from Dio-

Table 1
Experimental matrix design and results obtained for each of the response variables studied

Exp.	Temperature (°C)	Time (min)	Hexane		Petroleum ether		Ethanol		Water	
			EC_{50}(Y1)[a]	Yield(Y2)[b]	EC_{50}(Y3)	Yield(Y4)	EC_{50}(Y5)	Yield(Y6)	EC_{50}(Y7)	Yield(Y8)
1	60	3	70.5	0.25	108.0	0.34	100.5	6.88	303.9	1.55
2	60	9	82.7	0.5	82.7	0.43	98.8	7.28	350.7	1.62
3	60	15	72.6	0.58	80.5	0.44	84.2	7.21	353.8	1.81
4	115	3	74.7	1.43	76.8	1.32	84.8	12.33	354.6	3.28
5	115	9	74.6	1.73	82.5	1.7	85.8	11.81	333.9	5.0
6	115	9	71.1	1.74	77.5	1.51	87.1	11.26	370.3	4.61
7	115	9	74.8	1.82	70.7	1.64	89.9	11.4	335.5	4.2
8	115	9	72.0	1.74	67.9	1.46	83.2	11.71	348.1	4.41
9	115	15	72.9	1.77	74.9	1.66	89.2	11.94	317.5	4.19
10	170	3	103.2	3.85	117.9	3.28	91.1	19.62	257.2	7.16
11	170	9	110.3	4.28	109.0	2.94	100.1	19.7	257.2	8.22
12	170	15	107.8	4.3	110.3	4.01	98.6	17.14	247.2	10.12

[a] Efficient concentration (μg/ml).
[b] Extraction yield obtained from dry weight/total weight expressed in %.

nex Corporation (Sunnyvale, CA, USA) was used. Extractions were performed at three different extraction temperatures (60, 115 and 170 °C) and different extraction times (3, 9 and 15 min) according to the above experimental design. Prior to each experiment, an extraction cell heat-up was carried out for a given time, which changed according to extraction temperature. Particularly, 5 min heat-up was used when extraction temperature was set at 60 °C, 6 min at 115 °C and 8 min at 170 °C. Likewise, all extractions were performed in 11 ml extraction cells, containing 2.5 g of sample.

The extraction procedure was as follows: (i) sample is loaded into cell; (ii) cell is filled with solvent up to a pressure of 1500 psi; (iii) initial heat-up time is applied; (iv) a static extraction with all system valves closed is performed; (v) the cell is rinsed (with 60% cell volume using extraction solvent); (vi) solvent is purged from the cell with N_2 gas and (vii) depressurization takes place. Between extractions, a rinse of the complete system was made in order to overcome any extract carry-over. For solvent evaporation, a Rotavapor R-200 (from Büchi Labortechnik AG, Flawil, Switzerland) was used when the extracts were obtained with organic solvents and, for the case of water extracts, a freeze dryer (Unitop 400 SL, Virtis, Gardiner, NY, USA) was employed. Afterwards, different extract solutions were prepared using the same solvent as used during extraction, to known concentrations. When water was used as the extracting solvent, care had to be taken with regard to clogging of the extractor lines by the extracted material. To avoid clogging, the microalgae were placed inside a filter paper and the extraction procedure was performed as mentioned above.

2.4. Antioxidant activity determination (in vitro assay)

Antioxidant activity of all extracts was measured using a method based on a procedure described by Brand-Williams, Cuvelier, and Berset (1995). The procedure followed was: 23.5 mg of DPPH were weighed and dissolved in 100 ml methanol. This solution was stored at 4 °C. For antioxidant activity measurements, this stock solution was diluted 1:10 on methanol. Approximately 0.1 ml of extract solutions were added to 3.9 ml diluted DPPH solution to complete the final reaction medium (4 ml). Due to the coloration of the extracts, it was necessary to prepare a control (i.e., blank) which consisted of 0.1 ml of each solution added to 3.9 ml of methanol. The reaction was complete after 4 h at room temperature, and the absorbance was measured at 516 nm using a UV/VIS Lambda 2 spectrophotometer (Perkin–Elmer Inc., Wellesley, MA, USA). Methanol was used to adjust the spectrophotometric zero. The absorbance value was obtained by subtracting the blank absorbance measurement from the value given by the extract solutions. A calibration curve was obtained that correlates the concentration of DPPH with the absorbance measured at 516 nm. The calibration ($n = 7$; $r = 0.999$) gave the following equation: [DPPH] = (Abs + 0.0029)/0.0247. For each extract, a known solution concentration was prepared in order to obtain the remaining DPPH concentration upon completion of the reaction. The use of these values allowed the estimation of the extract concentration necessary to achieve a 50% reduction of the initial DPPH concentration. This value is known as EC_{50} (Efficient concentration, also called oxidation index) and was utilized to quantify the antioxidant activity.

3. Results and discussion

3.1. Solvents

In this study, different extracting solvents (covering a wide range of dielectric constants, 1.9 for hexane, 4.3 for

petroleum ether, 24.3 for ethanol and 78.5 for water) were tested in order to evaluate the influence of the solvent polarity in extracting the natural antioxidants from the microalga *Spirulina platensis*.

3.2. Effect of the factors

As mentioned in Section 2, Table 1 lists the values of EC_{50} and yield (response variables Y1 to Y8), obtained for all the experiments corresponding to the matrix design. MLR was applied to estimate the parameters of the proposed model in Eq. (1) for each of the eight response variables. A summary of these results is shown in Fig. 1, where the regression coefficient values for centred and scaled factors are shown as bar graphs for all the responses considered. From this, it is possible to compare the coefficients between responses; the corresponding values are normalized by dividing them by the standard deviation of their corresponding responses. Fig. 1 shows the importance of the different terms in the model for each of the responses evaluated. As can be seen, temperature (Tem) and its quadratic term (Tem*Tem) have the strongest influence in all response variables, with a positive influence in all of them except for Y7 (EC_{50} values using water as extracting solvent); the extraction time (tim) and its quadratic term (tim*tim), which have a lower influence and the temperature–time interaction term (Tem*tim) show only some effect for more polar extraction solvents (response variables from Y5 to Y8). As can be seen in Fig. 1, the factors that mostly influence extraction yields (responses Y2, Y4, Y6 and Y8) have similar patterns (showing an increase on the response by increasing the extraction temperature). This fact can be explained by the increase in the diffusion coefficient of the liquid solvent into the solid matrix with increasing extraction temperature, which favours the kinetics of desorption of the compounds from the matrix. On the other hand, stronger differences among the factors that influence the EC_{50} values (for the different solvents studied and considering that the highest antioxidant activity corresponds to the lowest EC_{50} values) were found, mainly when comparing organic solvents and water. This is probably due to the effect of the change in the dielectric constant of water with the temperature that favours the extraction of less polar compounds, that is, compounds similar to those extracted with medium–low polarity organic solvents. In a previous paper (Herrero et al., 2004), we demonstrated that the compounds that mostly contribute to the antioxidant activity of the microalgae extracts (thus decreasing the EC_{50} value) were non-polar compounds.

The statistical significance of the estimated regression coefficients were analyzed from the table of analysis of variance. The interaction and quadratic terms of the model, not significantly different from zero ($P > 0.10$), were excluded from Eq. (1), and the mathematical model was refitted by MLR. The new results are listed in Table 2, and they include the following information: the regression coefficients obtained, for unscaled factors, the determination coefficient (R^2), the RSD and the *P*-values from the lack of fit test for the model. From these

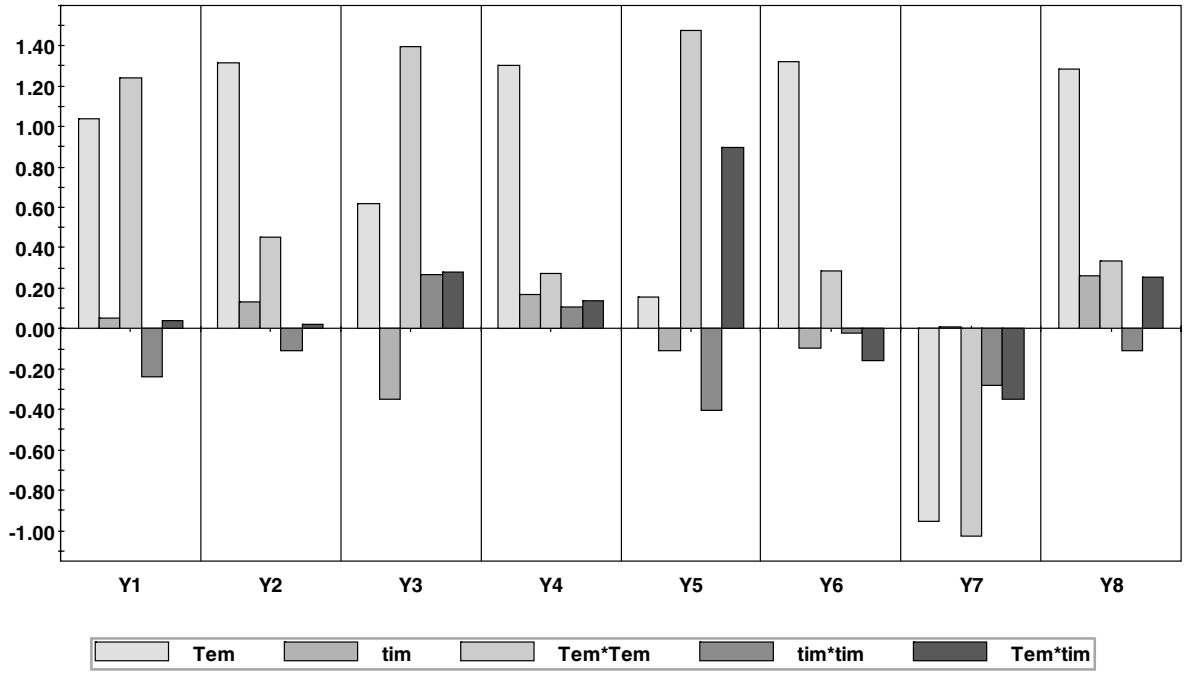

Fig. 1. Plot of normalized regression coefficient values, for centred and scaled factors, obtained from MLR, for the eight response variables (Y1 to Y8) studied.

results, the following conclusions can be drawn: (1) the eight estimated models were found adequate enough to describe the data (P-values of lack of fit test >0.05); (2) the determination coefficient (R^2), that is the fraction of variation of the response variable explained by the model, was higher than 0.80 for the EC_{50} response, and higher than 0.96 for the yield response; (3) the RSD of the fit for the EC_{50} was below 7.5 for three of the four solvents used and slightly higher than 20 for water extraction; (4) when considering the yield, the RSD values were below 0.55 for all the solvents tested. The RSD values, expressed as a percentage of the mean value of the response (RRSD = $RSD/\bar{Y}$), provides a measure of the relative error of the fit; values obtained are also shown in Table 2, all of them being below 10%, except the one corresponding to the Y4 response (Ether-Yield) that was slightly higher.

Fig. 2 shows the contour plots for the response variables, as a function of temperature and time. By analyzing the plots for the EC_{50} responses, and considering that, to maximize the antioxidant activity the response EC_{50} has to decrease, it can be seen that an increase of temperature drives toward an increase in EC_{50}. When organic solvents are considered (responses Y1, Y3, Y5), the optimum temperature can be found around the intermediate temperatures of the experimental region studied (that is, from 90 to 120 °C). It is also shown that the time factor has a very low influence in the final response. Thus, although similar antioxidant capacity seems to be obtained for the microalgae extracts obtained using hexane, petroleum ether and ethanol, it can be deduced that, in general, a slightly better antioxidant activity was obtained for hexane-microalgae extracts. This difference can be correlated with the higher amount of non-polar compounds (carotenoids, among others) that can be extracted using hexane, which contribute to the antioxidant activity of the extracts (Herrero et al., 2004). When using water as extracting agent, the behaviour is completely different, with decreasing EC_{50} values with increasing extraction temperatures. As mentioned above, heating water at high temperatures (while keeping it in the liquid state) produces a decrease in its dielectric constant, i.e., it becomes a less polar solvent (increasing the temperature from 25 to 170 °C reduces the dielectric constant of water by half, from 80 to 40).

The analysis of the surface plots for the yield (responses Y2, Y4, Y6 and Y8) shows typical behaviour, increasing the response by raising the extraction temperature. This is also true when increasing the extraction time for all the solvents except ethanol, although the effect of time on ethanol is less important. The predicted yield values are higher for the more polar solvents, ethanol and water, reaching, when ethanol is considered, values up to 18% at the highest temperature (170 °C). This result can be explained by the composition of Spirulina (Richmond, 1988). This microalga is composed of 50–70% of protein and about 15% of carbohydrates (Richmond, 1988). Therefore, it is expected that, by using more polar solvents, these polar compounds can be extracted to a higher extent, in this way increasing the yield of extract obtained.

As can be inferred from the comments about the extraction conditions, to optimize both EC_{50} and yield, it will be very difficult to obtain large amounts of extracts (high yield) together with high antioxidant activity, because the highest yields were obtained at the highest temperatures. Therefore, from the point of view

Table 2
Regression coefficients, for unscaled factors, and statistics for the fit, obtained from MLR

Terms of the model	Regression coefficients							
	Hexane		Petroleum ether		Ethanol		Water	
	EC_{50}(Y1)	Yield(Y2)	EC_{50}(Y3)	Yield(Y4)	EC_{50}(Y5)	Yield(Y6)	EC_{50}(Y7)	Yield(Y8)
Constant	116.81	0.05948	176.5	−0.32090	143.07	3.32247	217.387	1.70554
Tem	−1.0665***	−0.01493*	−1.8035**	8.814910^{-5}***	−0.81861	0.03197***	2.92579***	−0.01693***
Tim	0.1361	0.10674*	−1.0278	0.0325	−2.19570	0.17426	0.07778	−0.12050**
Tem∗Tem	0.00589***	0.00021*	0.00871***	0.00012*	0.00294**	0.00041**	−0.01597**	0.00026*
tim∗tim		−0.00420*						
Tem∗tim					0.01803*	−0.00213*		0.00204*
Statistics for goodness of fit of the model								
R^2	0.954	0.999	0.879	0.967	0.802	0.991	0.838	0.980
RSD	3.866	0.046	7.243	0.245	3.695	0.537	20.381	0.467
P	0.080	0.425	0.435	0.068	0.257	0.073	0.342	0.235
RRSD (%)	4.70	2.30	8.21	14.16	4.06	4.34	6.38	9.98

R^2, determination coefficient; RSD, residual standard deviation; P, P-value of the lack of fit test for the model; RRSD, the residual standard deviation expressed as a percentage of the mean value of the response.

* Regression coefficient significantly different from zero $P < 0.05$.

** Regression coefficient significantly different from zero $P < 0.01$.

*** Regression coefficient significantly different from zero $P < 0.001$.

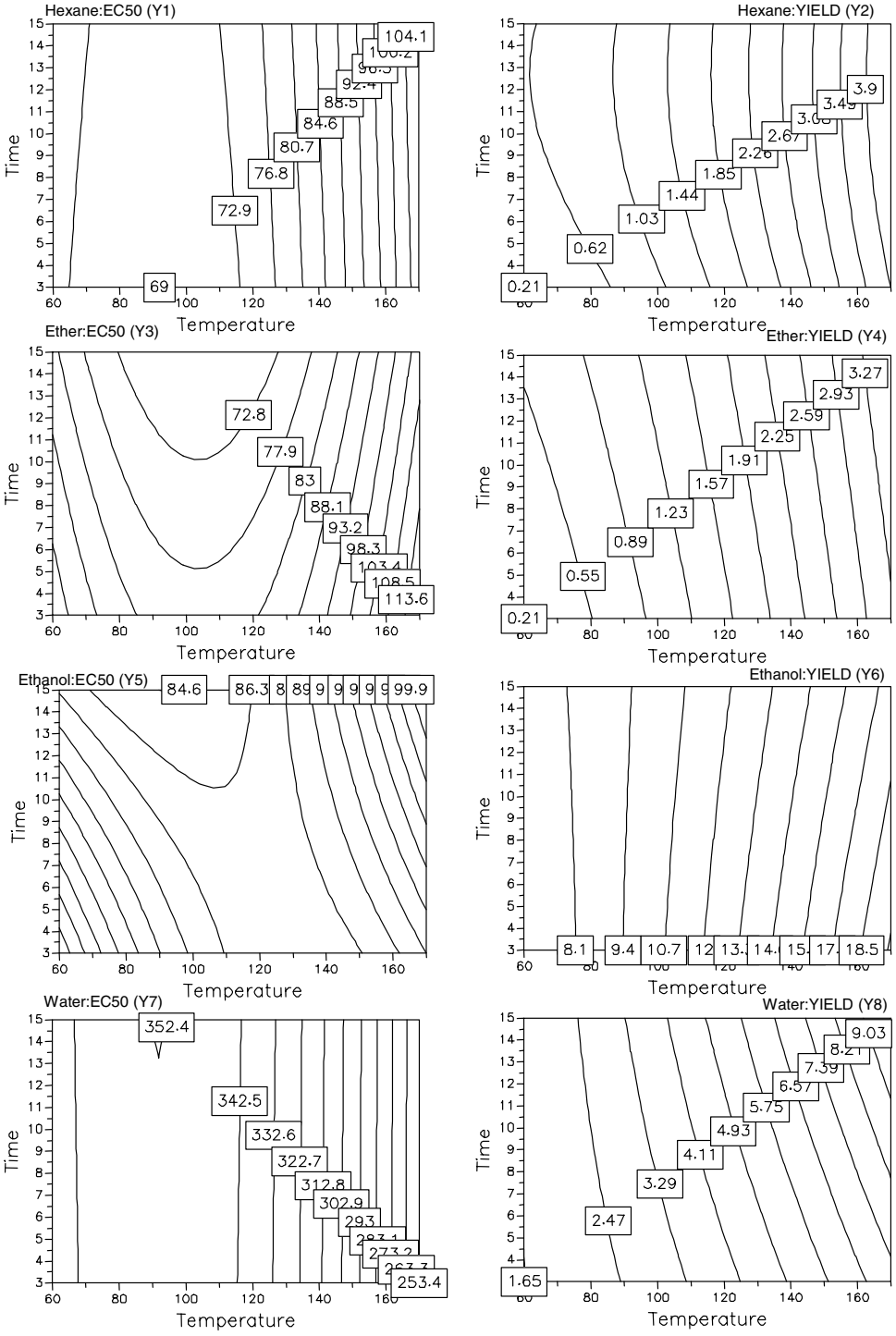

Fig. 2. Contour plot for the EC_{50} and yield, as a function of temperature and time, for each of four studied conditions.

of the whole process, it will be necessary to reach a compromise between the two responses.

Table 3 shows the optimum conditions of the extraction process provided by the statistical programme, and the predicted value for the response variables using the fitted model in Table 2. As can be seen, when minimizing EC_{50}, the optimum temperature depends on the solvent polarity (being highest for water at 170 °C). When trying to maximize the extraction yield, the optimum temperature was always the highest experimental value, that is, 170 °C. As for the extraction time, the optimum was the highest experimental value tested (that is, 15 min) for 5 of the 8 responses analyzed. Nevertheless, we must consider that the effect of the extraction time was not as

Table 3
Optimum conditions (min EC50 and max yield), provided by the statistical programme, and the predicted value for the response variables, using the fitted model in Table 2

		Optimum conditions		Predicted		Predicted value for the other responses of the process for these ideal conditions
		Temperature (°C)	Time (min)	Value	95% Confidence interval	
Hexane	EC_{50}Y1	90	3	69.0	64.0, 74.0	Yield(%) = 0.71 (0.64,0.78)
	Yield(%) Y2	170	13	4.30	4.23, 4.37	EC_{50} = 108 (102, 113)
Petroleum ether	EC_{50}Y3	103	15	67.7	58.2, 77.3	Yield(%) = 1.43 (1.11,1.76)
	Yield(%) Y4	170	15	3.60	3.20, 4.00	EC_{50} = 106 (94.4, 118)
Ethanol	EC_{50} Y5	111	15	85.5	80.4, 90.5	Yield(%) = 10.95 (10.22, 11.68)
	Yield(%) Y6	170	3	19.94	18.85, 21.05	EC_{50} = 91.4 (83.8, 99.0)
Water	EC_{50} Y7	170	3	253	220, 287	Yield(%) = 7.14 (6.18, 8.09)
	Yield(%) Y8	170	15	9.86	8.91, 10.82	EC_{50} = 254 (221, 288)

important as the extraction temperature on the final response (yield) (see Table 2).

Interestingly, from the optimum values given in Table 3, it can be also deduced that ethanol extracts possess good antioxidant activity, but slightly worse than that obtained with hexane and petroleum ether. This property can be used as an additional advantage taking into account that ethanol, unlike hexane or petroleum ether, is generally considered GRAS and therefore can be used as a safe solvent for the food industry. Moreover, the yields obtained with ethanol are the highest, providing a good efficiency of the extraction process.

Acknowledgements

M.H. thanks MCYT for a FPI grant. Authors thank MCYT (Project AGL2002-04621-C02-02) for financial support.

References

Bhat, V. B., & Madyastha, K. M. (2000). C-phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro. *Biochemical and Biophysical Research Communications, 275*, 20–25.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensittel Wissenchaft und Technologie, 28*, 25–30.

Cohen, Z., & Vonshak, A. (1991). Fatty acid composition of *Spirulina* and *Spirulina*- like cyanobacteria in relation to their chemotaxonomy. *Phytochemistry, 30*, 205–206.

Denery, J. D., Dragull, K., Tang, C. S., & Li, Q. X. (2004). Pressurized fluid extraction of carotenoids from *Haematococcus pluvialis* and *Dunaliella salina* and kavalactones from *Piper methysticum*. *Analytica Chimica Acta, 501*, 175–181.

Goldberg, I. (1996). *Functional foods. Designer foods, pharmafood, nutraceuticals*. London, UK: Chapman and Hall, p. 3.

Herrero, M., Ibáñez, E., Señoráns, F. J., & Cifuentes, A. (2004). Accelerated solvent extracts from *Spirulina Platensis* microalga: determination of their antioxidant activity and analysis by micellar electrokinetic chromatography. *Journal of Chromatography A, 1047*, 195–203.

King, J. W. (2000). Advances in critical fluid technology for food processing. *Food Science and Technology Today, 14*, 186–191.

Madhava, C., Bhat, V. B., Kiranmai, G., Reddy, M. N., Reddanna, P., & Madyastha, K. M. (2000). Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliproptein from *Spirulina* platensis. *Biochemical and Biophysical Research Communications, 277*, 599–603.

Mahajan, G., & Kamat, M. (1995). γ-Linoleic acid production from *Spirulina platensis*. *Applied Microbiology and Biotechnology, 43*, 466–469.

Otles, S., & Pire, R. (2001). Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. *Journal of AOAC, 84*, 1708–1714.

Richmond, A. (1988). Spirulina. In M. A. Borowitzka & L. J. Borowitzka (Eds.), *Micro-algal biotechnology* (pp. 96–100). Cambridge, UK: Cambridge University Press.

Xue, C., Hu, Y., Saito, H., Zhang, Z., Li, Z., Cai, Y., et al. (2002). Molecular species composition of glycolipids from *Spirulina platensis*. *Food Chemistry, 277*, 9–13.