Experimental and modeling studies on microwave-assisted extraction of mangiferin from *Curcuma amada*

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Abbreviations: ANOVA, analysis of variations; **MAE,** microwave-assisted extraction; **OFAT,** one-factor-at-a-time; **HPLC,** High Performance Liquid Chromatography; **FTIR**, Fourier transform infrared spectroscopy; **SCFE,** Supercritical fluid extraction; **UAE,** Ultrasound assisted extraction; **HRE,** heat reflux extraction.

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Abstract

Mangiferin, a bioactive compound having potent nutraceutical, strong antioxidant and pharmacological significance has been extracted using microwave-assisted extraction (MAE) technique from *Curcuma amada*, commonly known as mango ginger. The extraction solvent ethanol is eco-friendly, nontoxic and reduces the risk of environmental hazards. The influence of several independent variables such as microwave power, ethanol concentration, extraction (irradiation) time and pre-leaching time has been studied under varying conditions using one-factor-at-a-time analysis to obtain an optimal extraction ratio. The maximum mangiferin content of 1.1156 mg/g is obtained at microwave power of 550W and extraction time of 50s with 80% ethanol as a solvent and pre-leaching time of 20 min. The results indicate that microwave power and ethanol concentration have the most significant effect on the yield of mangiferin content. The presence of mangiferin in final *Curcuma amada* extract is confirmed through High Performance Liquid Chromatography (HPLC) and the functional groups are identified through Fourier transform infrared spectroscopy (FTIR) analyses using standard mangiferin. The experimental profiles are fitted into a two-parameter modified first-order kinetic model and a three-parameter modified logistic model and checked using the goodness-of-fit criterion. The *Curcuma amada* retained its antioxidant activity after MAE treatment and the antioxidant activity of mangiferin obtained after extraction using DPPH free radical scavenging assay is studied.

Keywords: Microwave-assisted extraction, Mangiferin, Antioxidant activity, ANOVA, Mathematical modeling

1 Introduction

Traditional plant spices, similar to fruits and vegetables, are known to contain health promoting components such as vitamins, minerals, antioxidants and prebiotics (Omenn et al., 1996). In particular, plant spices are used in foods, because they impart desirable flavours and may fulfill more than the one function for which they are added. Extensive research is being conducted on traditional medicines, on different plant species and their therapeutic applications all over the world. *Curcuma amada*, commonly known as mango ginger, is an important member of the Zingiberaceae family. It has an Indo-Malayan origin and is distributed widely in the tropics from Asia to Africa and Australia (Sasikumar et al., 2005). *Curcuma amada* is named mango ginger because it is morphologically similar to ginger and imparts a mango flavour, and is typically used in the manufacture of pickles, culinary preparations and salads for flavour, candy and sauce (Shankaracharya, 1982). *Curcuma amada* has pharmacological significance for a variety of ailments. Therapeutically, mango ginger is used to treat a range of mood and medical disorders in traditional and *Ayurvedic* medicine. *Curcuma amada* is credited with diverse bioactive molecules demonstrating antibacterial, antifungal, anti-inflammatory, anti-hypercholesterolemic, insecticidal, aphrodisiac, antipyretic and antioxidant properties (Singh et al., 2010). Mangiferin is an important bioactive constituent of mango ginger containing xanthone-C-glycoside, which has numerous pharmacological properties and is an important phytochemical. It has antidiabetic, cardioprotective, immunomodulatory, antioxidant, antitumour, hepatoprotective and vasorelaxant properties and is useful in the treatment of biliousness, skin diseases, bronchitis, asthma and inflammation (Jatoi et al., 2007). Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents (Romanik et al., 2007). Keeping in mind the requirements such as shortened extraction time, reduced solvent

consumption, increased pollution prevention and the special care needed for thermolabile constituents, numerous extraction techniques have been developed for the purpose of obtaining pharmacologically active compounds from various plant sources such as supercritical fluid extraction (SCFE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and heat reflux extraction (HRE). However, because of several disadvantages with the traditional extraction techniques like sonication and Soxhlet extraction, non-conventional extraction techniques like supercritical fluid extraction, extraction by microwave and ultrasound sources have gained importance. The use of microwaves in analytical sciences is not new, the first reported analytical use for microwave oven was in 1986 for the extraction of organic compound (Dean, 2010). In recent years, microwave-assisted extraction (MAE) has attracted growing interest as it allows rapid extraction of solutes from solid matrices, with extraction efficiency comparable to that of the classical techniques (Camel, 2000). Heating occurs in a targeted and selective manner in MAE with practically no heat being lost to the environment, and the mechanism can significantly reduce the extraction time (Huie, 2002). This means it requires less solvent volume and is thus time conserving with improved product recovery. Further, the extraction solvent used is usually water or ethanol, which is inexpensive, nontoxic and environmentally benign (Ferguson et al., 2012). Samples pretreated with solvents with higher microwave absorbing capacity when coupled with extracting solvents like ethanol bring about heating by at least two competing mechanisms namely, direct heating from the interaction of microwaves with ethanol and heating from the diffusion of excess heat resulting from the interaction of the microwaves with the pretreated matrix (Mandal et al., 2007). In our previous study (Padmapriya et al., 2012), microwave-assisted extraction of mangiferin from *Curcuma amada* was studied using only two independent factors namely, microwave power and extraction (irradiation) time. However, it has been observed that several other extraction

variables such as solvent concentration, ethanol concentration and pre-leaching time could also be influential factors in the optimization of the extraction protocol of a bioactive compound, which may act dependently or independently (Dhobi et al., 2009). In the present study, therefore a more rigorous approach has been applied to understand the influence of these independent factors on mangiferin extraction using mathematical modeling. The presence of mangiferin in final *Curcuma amada* extract was confirmed using High Performance Liquid Chromatography (HPLC) using standard mangiferin and was further subjected to Fourier transform infrared spectroscopy (FTIR) analysis for identification of the functional groups. The antioxidant activity of mangiferin obtained after extraction using DPPH free radical scavenging assay has also been studied.

2 Materials and methods

2.1 Plant material

Fresh and healthy *Curcuma amada* (mango ginger) were purchased from the local market in Durgapur, West Bengal. The rhizomes were washed, peeled and cut into fine pieces and then dried in a hot air oven (OVFU) at 70°C until constant weight and was well blended. Mangiferin standard was purchased from Sigma Aldrich, USA.

2.2 Microwave-assisted Extraction (MAE)

Microwave-assisted extraction (MAE) was performed using a microwave apparatus (Samsung Trio, Model CE117ADV; 230V ~ 50Hz) in a closed vessel system. 2.5gm of dried *Curcuma amada* powder was extracted with 25ml solvent under different MAE conditions. After extraction, the vessels were allowed to cool at room temperature before opening. Microwave power (250W, 350W, 450W, 500W 550W and 900W), ethanol concentration (50-100%, v/v), extraction time (1-120 s, with an interval of 5s) and pre-leaching time (1-30

min, with an interval of 5min) were evaluated for the extraction of mangiferin from *Curcuma amada*. The extraction of mangiferin was carried out with the method of Padmapriya et al. (2012). The final extract was evaporated and dissolved in DMSO before UV-vis spectrophotometric (Techcomp, UV 2310) analysis. For the estimation of mangiferin, the method described by Joubert et al. (2008) was used and the absorbance was measured at 410nm.

2.3 High Performance Liquid Chromatography (HPLC) analysis

The final extract of *Curcuma amada* was analyzed by High Performance Liquid Chromatography (HPLC) (Waters 600) equipped with a UV-vis detector (Waters 2489) according to the method described by Muruganandan et al. (2002). Chromatographic separation was performed on a reverse-phase column (C18, 4.6 × 250 mm**,** Waters) with the temperature of the column being maintained at 25°C. The mobile phase was acetonitrile and 3% acetic acid in the ratio 16:84 at a flow rate of 0.5ml/min. The sample injection volume was 10µl. The peaks were evaluated based on their absorbance at 254nm. Retention time and concentration of the samples were compared with pure standard of mangiferin (Sigma Aldrich, USA).

2.4 Fourier Transform Infrared Spectroscopy (FTIR) analysis

The mangiferin extracted after MAE at 550W was further subjected to FTIR analysis for identification of the functional groups. Comparing the functional groups present in standard mangiferin, the damaged functional group of the extracted mangiferin can be identified. A known weight of the final sample extract was mixed with potassium bromide and loaded onto a Perkin Elmer instrument. The samples were scanned in model spectrum-100 system in

range of 400-4000 cm^{-1}. The spectral data obtained were compared with a standard mangiferin chart to identify the functional groups present in the sample.

2.5 DPPH radical scavenging activity

The DPPH assay was carried out according to the method reported by Ara and Nur (2009). DPPH solution (0.004% w/v) was prepared in 95% methanol. The stock solution was diluted to final concentration of 1µg/ml, 5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively. The freshly prepared DPPH solution was added in each of the test tubes containing the final concentrations of *Curcuma amada* methanolic extract, and after 10min of incubation the absorbance was taken at 517nm using a spectrophotometer. The scavenging effect (%) of DPPH free radical was measured using the following equation:

DPPH radical-inhibition scavenging effect (%) = [(absorbance of control – absorbance of test sample) / (absorbance of control)] × 100.

2.6 Statistical analysis

The screening of the variables has been done using one-factor-at-a-time (OFAT) analysis, which has several advantages such as run size economy, fewer level changes and providing protection against the risk of premature termination of experiments (Qu et al., 2005). It must be noted that although processes are commonly optimized in most industrial experiments using OFAT design approach, optimal conditions or interactions between variables cannot be predicted with this methodology (Wardhani et al, 2008). However, OFAT design allow to find out more rapidly whether a factor has any effect and is therefore a sequential learning process (Morgan et al., 1974). The statistical software Graphpad Prism v5.0.0.2 was used for the data analysis. A two way analysis of variance (ANOVA) was implemented to calculate the significance of the differences in the content of mangiferin. Means and coefficients of variance were

170 computed for all qualitative analysis and treatments with homogeneous means ranked using
171 the Newman-Keuls post-hoc test. The significance of the results was established at values
172 greater than 0.05 in all the experiments performed. The parameters of the empirical models
173 were fitted with a nonlinear least-squares (NLLS) Marquardt-Levenberg algorithm, using the
174 device-independent plotting program Gnuplot.
175
176 **3 Mathematical modeling of mangiferin extraction**
177 Kinetics of microwave-assisted extraction of mangiferin is performed at the experimental
178 design points for the three independent variables namely, microwave power, ethanol
179 concentration and pre-leaching time. In all these cases, the experimental data seem to follow
180 a sigmoidal curve for which a two-parameter modified first order kinetic model (Wardhani et
181 al., 2010) and a three-parameter delayed logistic model with a final asymptote (Yukalov et
182 al., 2009) are chosen to describe the evolution of microwave-assisted mangiferin extraction
183 given as:
184 $Y = Y_{\max}(1 - e^{-k_m \cdot t})$, and (1)
185 $Y = Y_{\max}/(1 + e^{-k_m(t-\tau)})$ (2)
186 where Y is the mangiferin content (mg/g) at time t, $Y_{\max}$ is the maximum mangiferin content
187 (mg/g) when time approaches infinity, k_m is the first-order mangiferin extraction constant or
188 the specific rate of mangiferin concentration (1/s), and τ is the time delay (s) else the value at
189 t=0 would always be half the value at $t=\infty$ (and there is no sufficient reason to assume such a
190 restriction).
191 A generalized expression to describe the dependency of both microwave power (P) and
192 ethanol concentration (E) on extraction time can be written using a slightly modified delayed
193 logistic model as follows:

194 $$Y = \frac{Y_{\max}\left(1 + \frac{\mathrm{P\ (or\ E)}}{\mathrm{P_{ref}\ (or\ E_{ref})}}\right)}{1 + e^{-k_m(t-\tau)}} \quad (3)$$

195 where P_{ref} (or E_{ref}) is the parameter related to microwave power (or ethanol concentration)
196 respectively and where the mangiferin yield Y is twice the initial value.
197 It is important to clarify that the extraction process is the result of an interaction between
198 *Curcuma amada* (mangiferin) and ethanol, causing the kinetic dependence to be of the
199 second order. During extraction, concentration of mangiferin (solute) increases and goes to
200 saturation although it is not yet extracted. However if excess ethanol (solvent) is added to the
201 solution, the extraction of mangiferin occurs. This reduces the apparent kinetic dependence
202 from a second order rate equation to a pseudo first order rate equation (see Eqns. 1 and 2).
203

204 **4 Results and Discussions**

205 Figures 1a and 1b show the chromatographic profile of the mangiferin from *Curcuma amada*
206 after MAE and standard mangiferin respectively. The retention time of 6.51min obtained
207 from the extract agreed well with the standard verifying the presence of mangiferin in
208 *Curcuma amada* extract.
209

210 **4.1 Effect of Microwave power**

211 Figures 2(a-e) show the effect of microwave power on mangiferin content with extraction
212 time; the symbols represent the experimental data while the continuous curves represent the
213 model fit. It can be clearly seen in these figures that there is a steady increase in mangiferin
214 content up to 50s at the power range from 250-550 W after which it reaches a threshold
215 value. However at 900W there is a significant decrease in the mangiferin content and the
216 yield decreases drastically, as was observed by the response at 900W (not shown). This is in
217 accordance with the observations of our previous study (Padmapriya et al., 2012) where a

similar response was obtained for 600W. The mangiferin content of *Curcuma amada* in the control sample i.e. before microwave-assisted extraction (MAE) was 0.0046mg/g. The mangiferin content at 250W and 550W after 50s of microwave extraction is found to be 0.0146mg/g and 0.7161mg/g respectively. This manifold increase in mangiferin content is maximum (more than 150 times higher than the control sample) at 550W after 50s of extraction compared to 250W (around 3 times higher than the control sample) for the same extraction time. This accelerated extraction of mangiferin by increasing microwave power can be correlated to the direct effects of microwave energy on molecules by ionic conduction and dipole rotation which result in power dissipated in volumetric basis inside the solvent and plant material which generate molecular movement and heating. Microwave irradiation energy disrupts the bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions. Microwave irradiation energy can enhance the penetration of solvent into the matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and thus offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted. The steep decrease in mangiferin content at 900W is due to the rapid degradation of mangiferin at higher microwave power range. As the experiments are conducted in dry matter, as is usually the case (Mandal et al., 2007), chances of degradation due to drying or evaporation at a higher microwave power intensity are ruled out. Similar results of decrease in extraction yield of astragalosides from *Radix astragali* at high power due to disorderly molecular interactions have been reported in the optimization study of microwave-assisted extraction of four main astragalosides in *Radix astragali* (Yan et al., 2010).

Results of a two-way analysis of variance (ANOVA) with extraction time and microwave power as independent variables are given in Table 1. The mangiferin content in *Curcuma amada* is significantly dependent on microwave power and extraction time as well as their

243 interaction. Newman-Keuls test suggest that the mangiferin content is significant at 550W,
244 validating our experimental results of extracting the highest mangiferin content at 550W from
245 *Curcuma amada*. Student's independent T-test further confirms that both microwave power
246 and extraction time have a significant effect on the mangiferin content.
247
248 **4.2 Effect of extraction time**
249 As seen in Figure 3, mangiferin content increases significantly with the increase in extraction
250 time from 1-50 s before reaching a steady state. The mangiferin content of *Curcuma amada*
251 kept in a pre-leaching time of 1min and extracted at 550W for 50s is found to be maximum
252 around 0.7121mg/g. Beyond 50s of extraction time, no significant increase in mangiferin
253 content is observed. Similar observations are also reported for microwave-assisted extraction
254 of artemisin in from *Artemisia annua* (Pan et al., 2007) and tanshinones from *Salvia*
255 *miltorrhiza* bunge (Hao et al., 2002).
256
257 **4.3 Effect of solvent concentration**
258 Preliminary screening experiments (not reported in this study) with different organic
259 extraction solvents such as acetone, acetonitrile, methanol and ethanol have been carried out
260 and it was observed that ethanol yielded significant mangiferin content. Ethanol undergoes
261 less microwave absorption than water due to its lower dielectric loss value but the overall
262 heating efficiency for the solvent will remain higher than water due to increased value of the
263 dissipation factor. Extraction with aqueous ethanol has been reported in earlier studies since it
264 has less restrictions in food applications (Wardhani et al., 2010; Wang et al., 2010;
265 Hemwimon et al., 2007). Microwave-assisted extraction of 2.5g of dry *Curcuma amada*
266 powder is carried out at microwave power of 550W, pre-leaching time of 1min and
267 irradiation time of 1-120 s with aqueous ethanol as solvent. The effect of aqueous ethanol

concentration on mangiferin content can be seen in Figures 4(a-f). The mangiferin content increases significantly with increase in ethanol concentration up to 80% ethanol concentration; beyond 80% ethanol concentration there is a decrease in mangiferin content. Dhobi et al. (2009) found similar results in their work related to optimization of microwave-assisted extraction of bioactive flavonolignan-silybinin. A maximum mangiferin content of 0.8864 mg/g is obtained in 80% ethanol concentration at extraction time of 50s. One possible reason for the increased efficiency with 80% ethanol might be due to the increase in swelling of plant material by presence of some amount of water, which increased the contact surface area between the plant matrix and the solvent. Presence of some amount of water can also increase the mass transfer process by increasing the relative polarity of the solvent thus improving its solubilizing capacity. Similar results were reported by Li et al. (2004) during microwave-assisted solvent extraction and HPLC determination of effective constituents in *Eucommia ulmoides* Oliv.

Statistical results indicate that the mangiferin is positively correlated but insignificant with the ethanol concentration but significant with extraction time. The results of analysis of variance (ANOVA) is given in Table 2. Both the ethanol concentration and extraction time along with their interaction are significant with respect to the mangiferin content. Newman-Keuls test show that the mangiferin content is significant at higher ethanol concentration of 70%, 80%, 90% and 100% with ethanol concentration of 80% yielding the highest mangiferin content.

4.4 Effect of pre-leaching time

Figures 5(a-g) show the effect of pre-leaching time on the yield of mangiferin content. Similar to Figures 2(a-e) and 4(a-f), the symbols represent the experimental data and the continuous curves represent the model fit. Pre-leaching time can be defined as the contact

time between sample matrix and extracting solvent before microwave extraction. Microwave-assisted extraction of 2.5g of dry *Curcuma amada* powder is carried out at microwave power of 550W, 80% ethanol concentration, irradiation time of 1-120 s for different pre-leaching time of 1-30 min. It is observed from these figures that with an increase in pre-leaching time from 1min to 20min, there is an increase in mangiferin content. Beyond a pre-leaching time of 20min, there is no noticeable increase in the yield of mangiferin content. It can be inferred that pre-leaching time of 20min allows sufficient swelling of the plant matrix. This increased hydrated status of plant material helps in the bursting of the cell wall due to internal thermal stress and enlargement of the cellular pores thus facilitating leaching of the target analyte. The results for analysis of variance for pre-leaching time and extraction time as independent factors are given in Table 3. The Newman-Keuls test indicates that the pre-leaching time is not a significant factor contributing to the mangiferin content.

Figure 6 shows the DPPH radical scavenging activity of mangiferin extracted from *Curcuma amada* by microwave-assisted extraction at the optimal condition of microwave power 550W, pre-leaching time 20min, extraction time 50s and ethanol concentration 80%. It is observed that the IC50 value for mangiferin extracted from *Curcuma amada* was 17.04μg/ml and the radical scavenging activity was directly proportional to the concentration of mangiferin with an inhibition of 97.65% at 100 μg/ml. From this observation, it is clear that mangiferin obtained from microwave-assisted extraction at 550W, pre-leaching time of 20 min, extraction time of 50s and 80% ethanol concentration retained its antioxidant property. It is important to note here that Stoilova et al. (2005) had earlier established the antioxidant properties of mangiferin standard using DPPH radical scavenging activity of mangiferin.

FTIR analysis has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts (Eberhardt et al., 2007; Hazra et al., 2007). Figure 7 shows the FTIR spectrum of

mangiferin extracted from *Curcuma amada* by MAE at 550W and mangiferin standard. Six functional groups were identified. FTIR spectrum results of mangiferin after microwave-assisted extraction showed peaks at 3399 cm^{-1} indicated presence of secondary OH^- bond, peak at 2917 cm^{-1} showed presence of C-H anti-symmetric stretching, peak at 1658.70 cm^{-1} indicated presence C-O stretching, peak at 1436.91 cm^{-1} indicated presence of CH-CH bending and peak at 1316.17 cm^{-1} indicated presence of C-O bond. Peak at 1023.22 cm^{-1} showed presence of C-C stretching in the mangiferin structure. Comparing the FTIR analysis of mangiferin extracted by MAE and mangiferin standard (see Table 4) revealed the similarity and variation in the functional group. The absorption spectra showed that the C-O bond and C-O-C stretching of the mangiferin were affected during the extraction process.

The results of the validation using the two-parameter first-order kinetic model (Eq. 1) and using the three-parameter logistic model (Eq. 2) for microwave power (Figures 2(a-e)), ethanol concentration (Figures 4(a-f)) and pre-leaching time (Figures 5(a-g)) are shown respectively. As the response for 900W was found to vary widely from the initial five responses (i.e. 250W, 350W, 450W, 500W and 550W) and did not follow a clear sequence, it was neglected while validating the kinetic model for microwave power with extraction time. To check the goodness of fit, the ratio of the root mean square (RMS) value to the maximum (limit) value of mangiferin content is considered. The optimized parameter set and the corresponding value of the statistical indicator Y_{RMS}/Y_{max} are summarized in Tables 5 and 6. The goodness of fit statistical indicator helps to determine how well the curve fits the data. The curve fits (based on Eq. 3) of the temporal evolution of yield on microwave power and ethanol concentration are shown in Figures 8 and 9 respectively. The best-fit parameter values for [P_{ref}, k_m, τ] are found to be [759.42, 0.14, 11.68] and [2315.22, 0.13, 10.91] using the NLLS Marquardt-Levenberg algorithm. The corresponding indicator Y_{RMS}/Y_{max} equals

342 0.054598 and 0.070991 respectively for microwave power and ethanol concentration,
343 indicating a good fit.

344 **5 Conclusions**

345 Mangiferin was extracted from *Curcuma amada* using microwave-assisted extraction
346 technique. Maximum mangiferin content of 1.1156mg/g was obtained at microwave power of
347 550W and extraction time of 50s with 80% ethanol as a solvent and pre-leaching time of 20
348 min, and retained its antioxidant properties. The experimental profiles fitted into a two-
349 parameter modified first-order kinetic model and a three-parameter modified logistic model
350 with sufficient accuracy. The microwave-assisted extraction of mangiferin from *Curcuma*
351 *amada* using ethanol can be safely employed in food and medicinal industries as it is efficient
352 not only from the industrial point of view, but also eco-friendly since it prevents
353 environmental hazards. This indicates the usefulness and significance of microwave-assisted
354 extraction as a novel extraction technique in biotechnological applications.
355

356 **Conflict of interest**

The authors declare that they have no conflict of interest.

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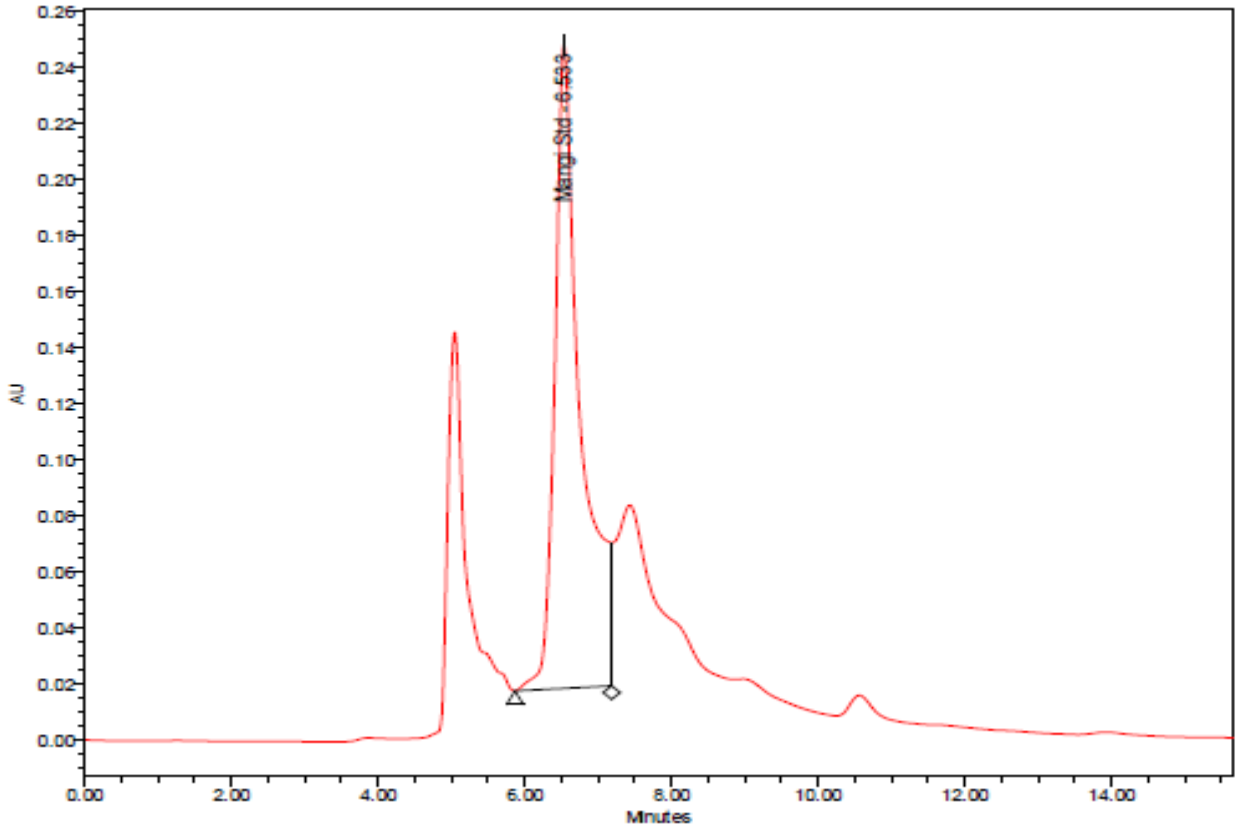


Figure 1a. HPLC chromatogram of mangiferin standard.

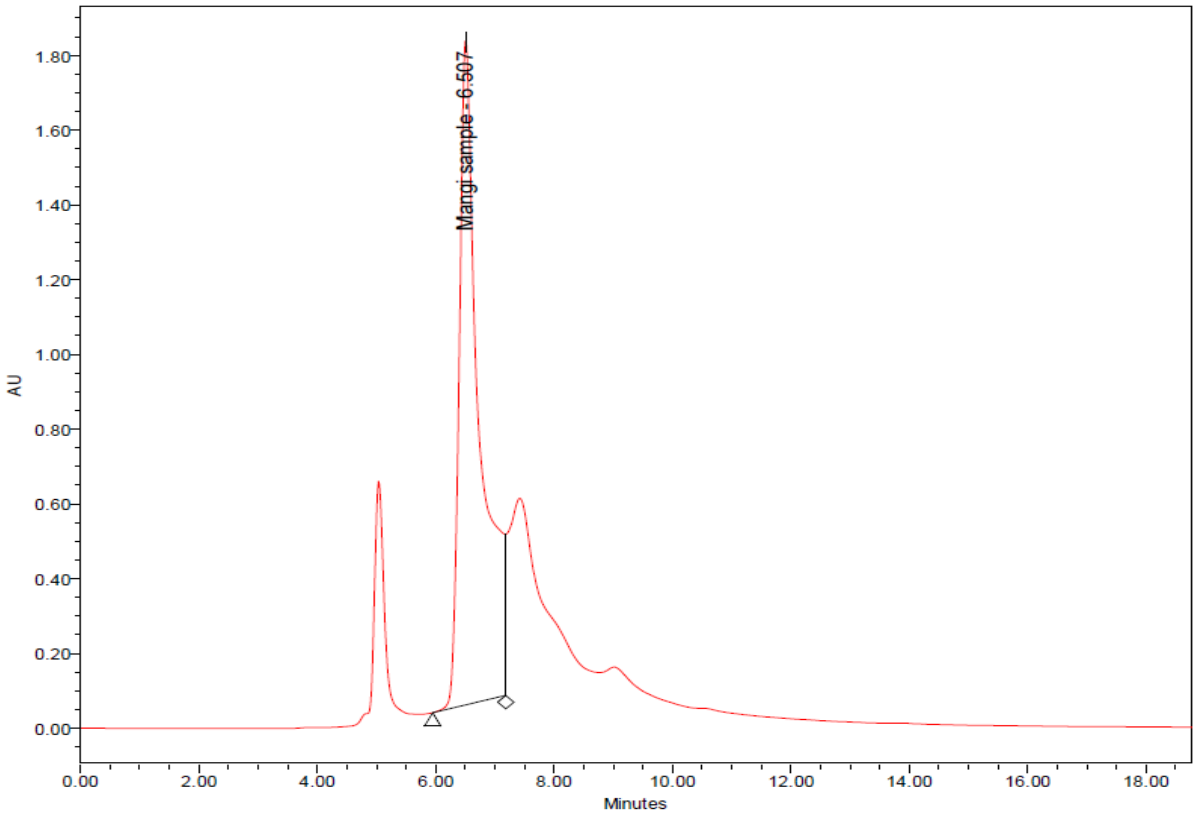
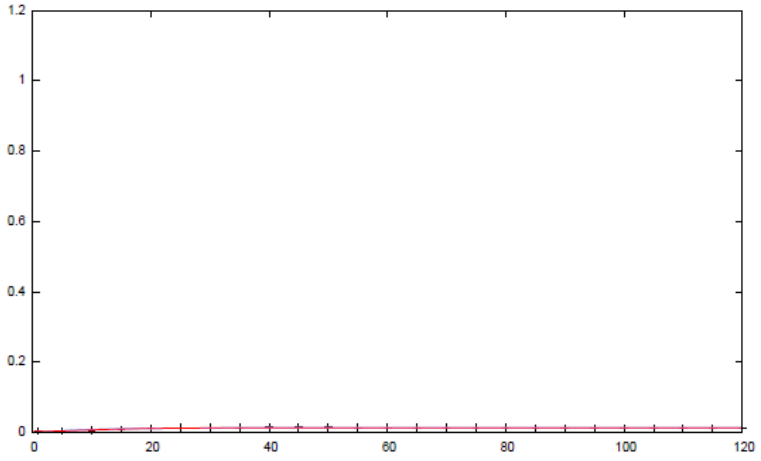


Figure 1b. HPLC chromatogram of mangiferin extracted from *Curcuma amada* by microwave assisted extraction (MAE).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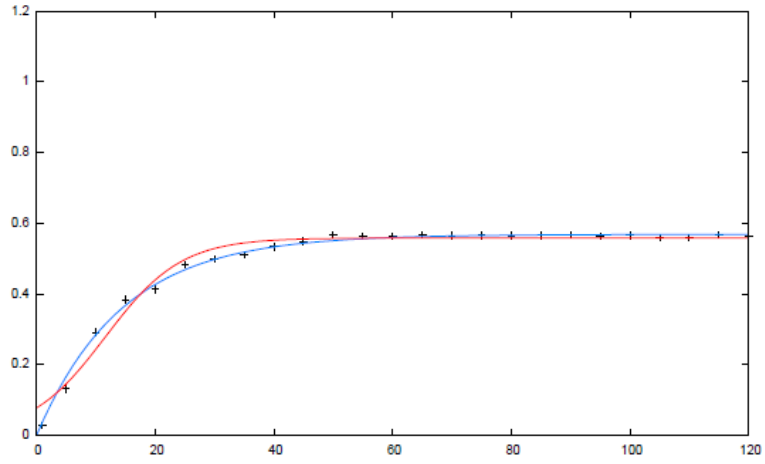
0.6
0.4
0.2
0
0
20
40
60
80
100
120

(a)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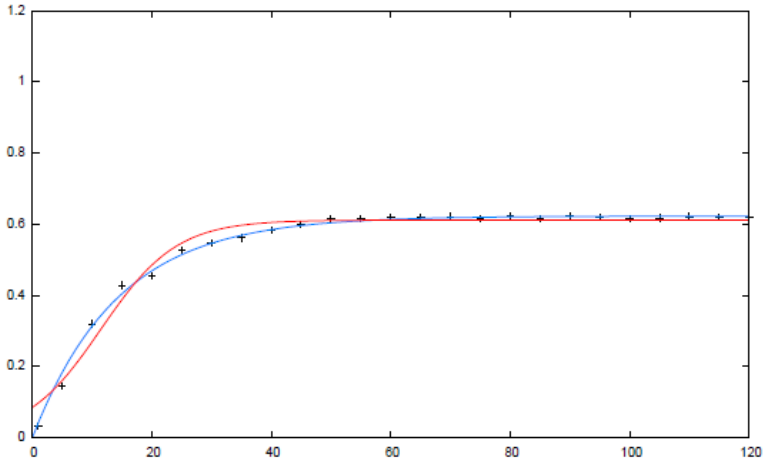
120

(b)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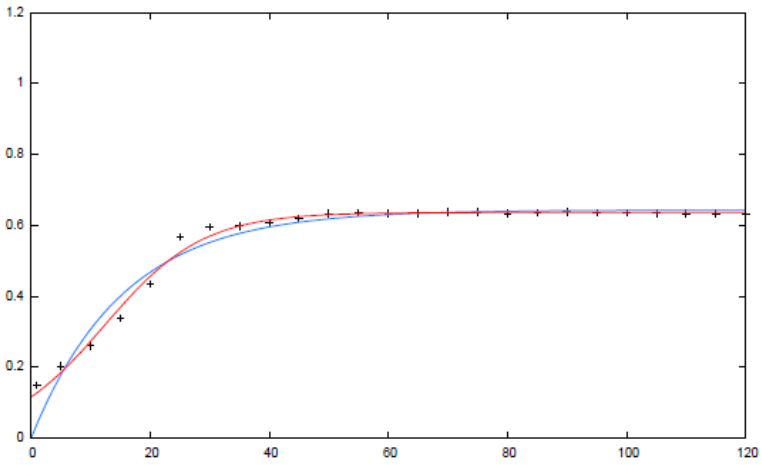
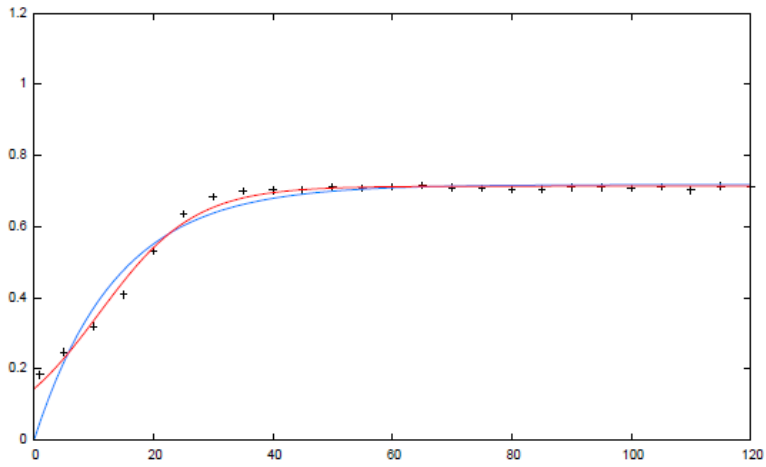
(c)



(d)



(e)

Figure 2. Temporal evolution of the effect of microwave power (as an independent variable) on the yield of mangiferin content extracted from *Curcuma amada* at various experimental design points (x-axis: time in s, y-axis: yield in mg/g). The experimental data (symbols) are fitted to the 2-parameter model (Eq. 1, blue) and 3-parameter logistic model (Eq. 2, red) are shown in Figs. 2(a-e) and for 250W, 350W, 450W, 500W and 550W respectively.

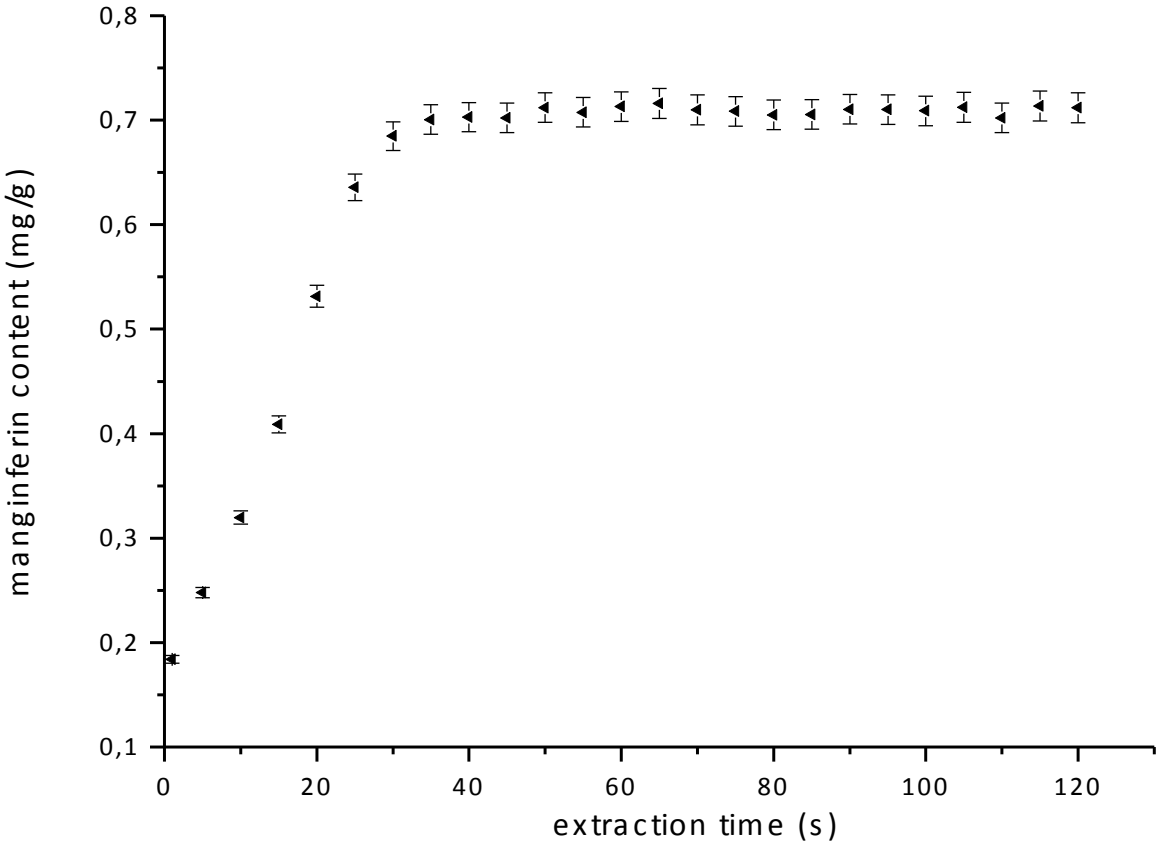
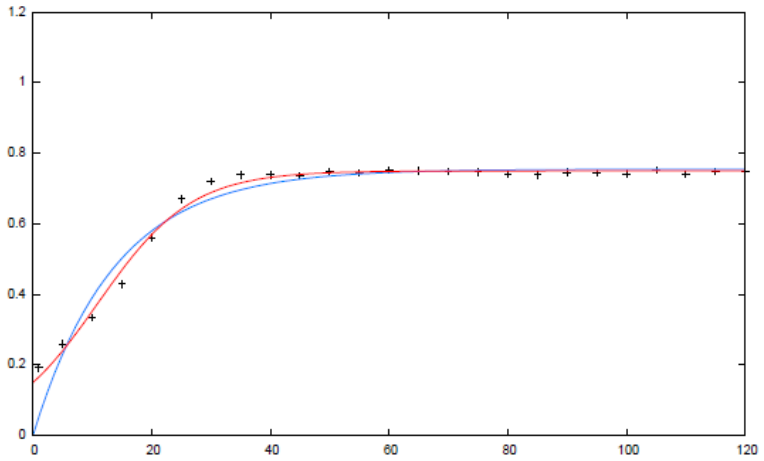


Figure 3. Influence of extraction time on the yield of mangiferin content. Extraction condition: pre-leaching time-1min, microwave power - 550W and ethanol concentration - 100%.The results are expressed as means of yield ± S.D.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60
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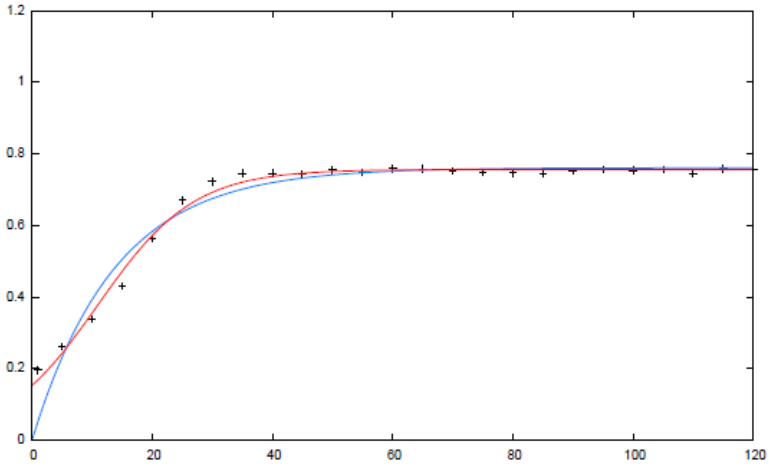
100
120

(a)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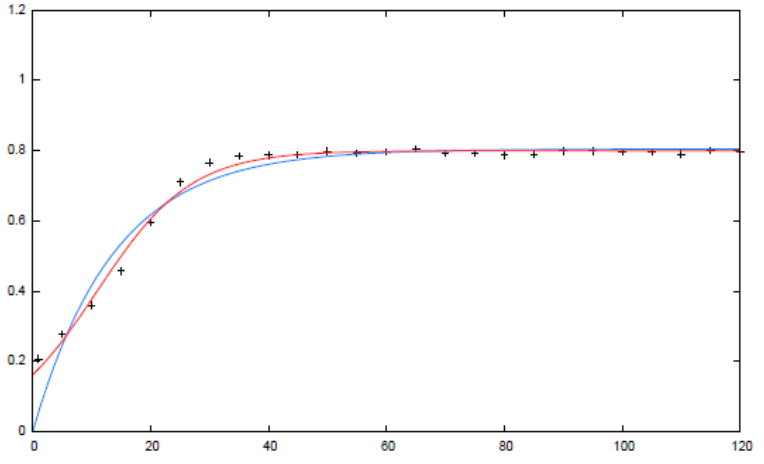
(b)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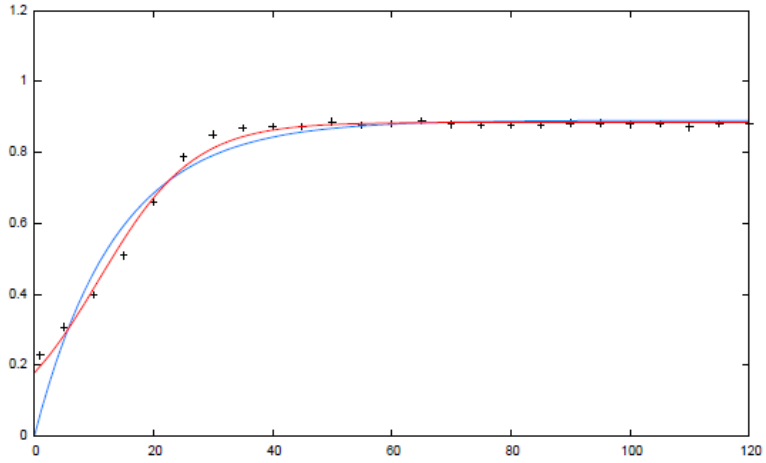
(c)



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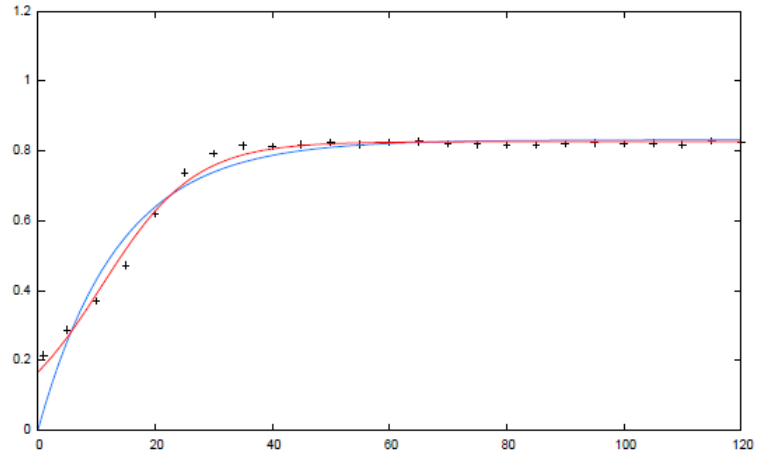

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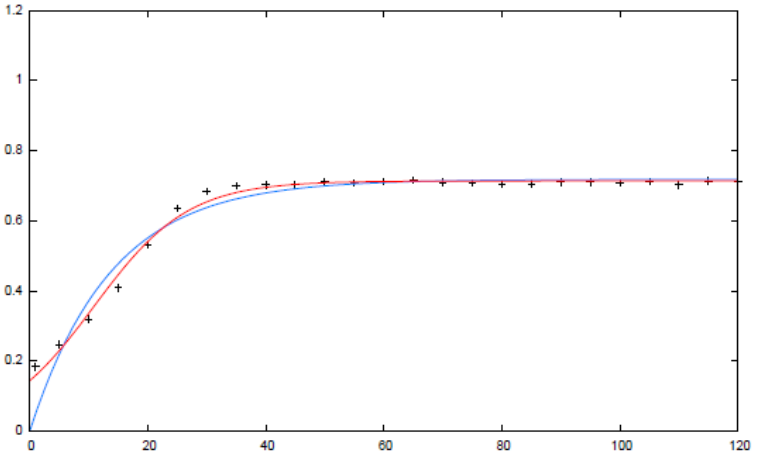
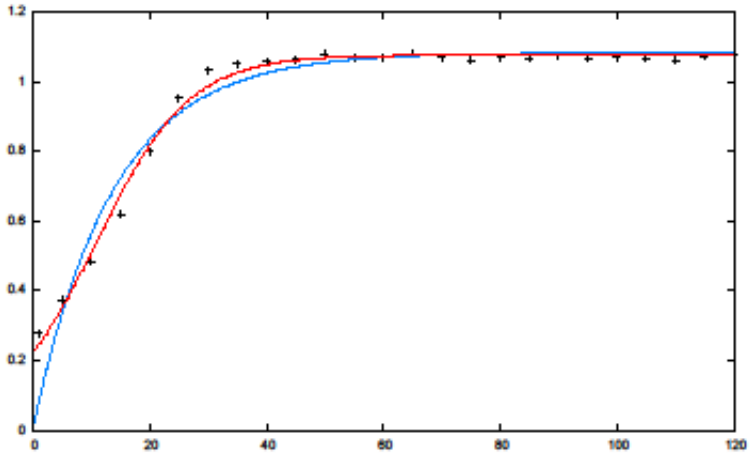


(f)

Figure 4. Temporal evolution of the effect of ethanol concentration (as an independent variable) on the yield of mangiferin content extracted from *Curcuma amada* at various experimental design points (x-axis: time in s, y-axis: yield in mg/g). The experimental data (symbols) are fitted to the 2-parameter model (Eq. 1, blue) and 3-parameter logistic model (Eq. 2, red) are shown in Figs. 4(a-f) for 50%, 60%, 70%, 80%, 90% and 100% respectively.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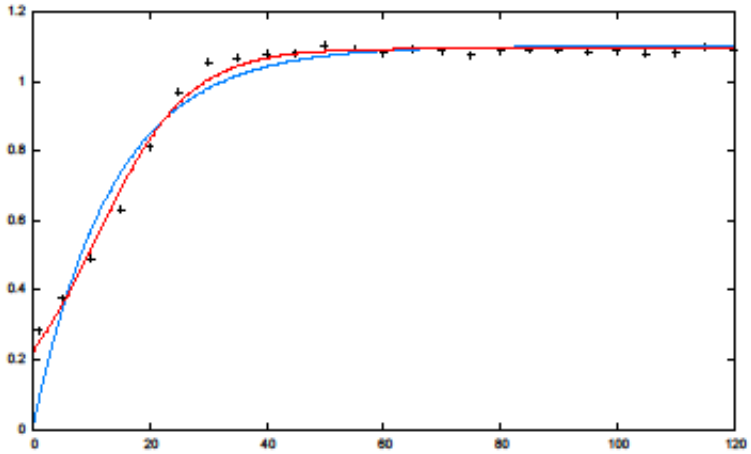
(a)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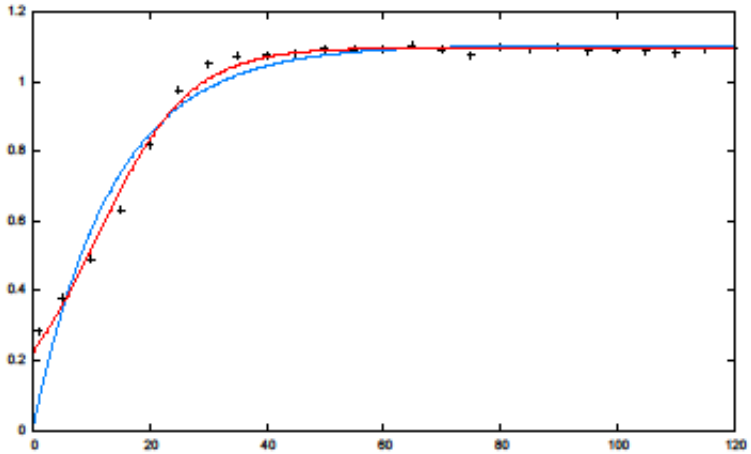
(b)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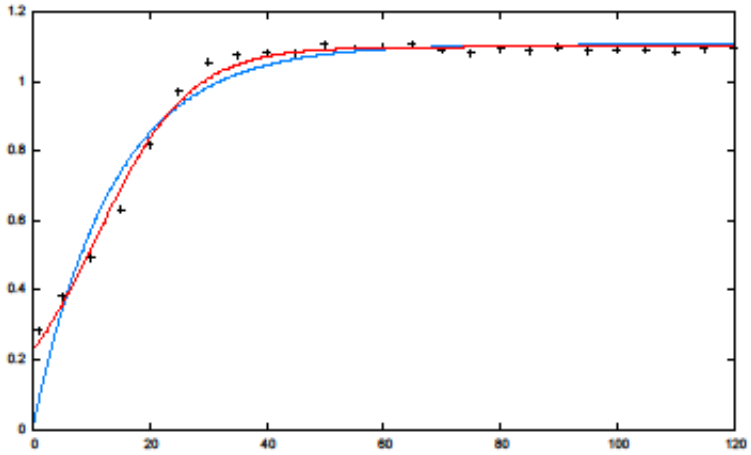
(c)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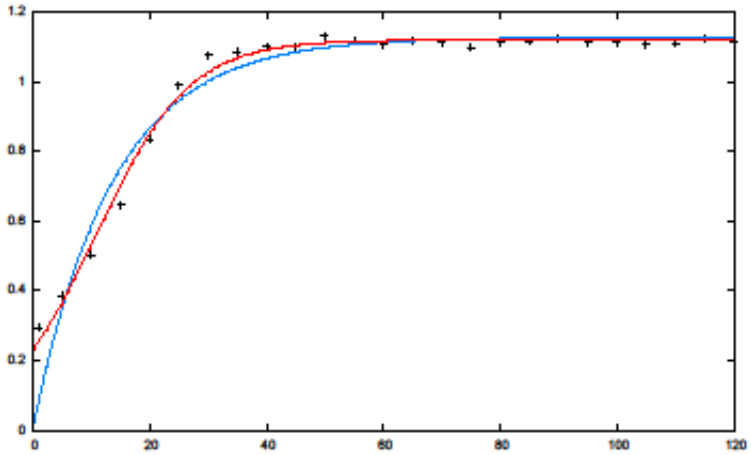
(d)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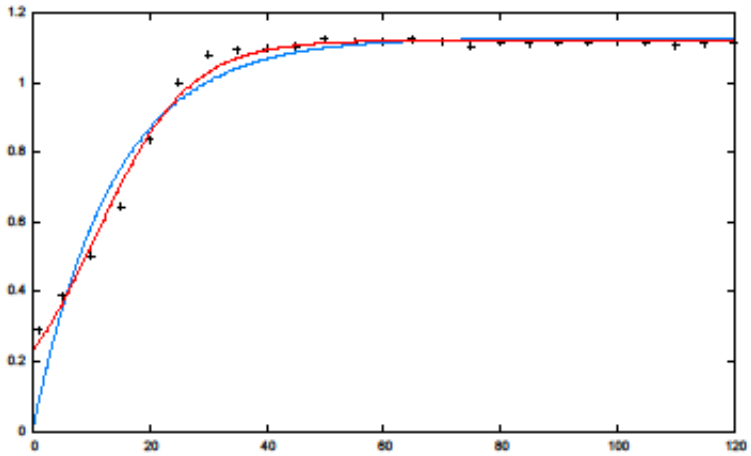
(e)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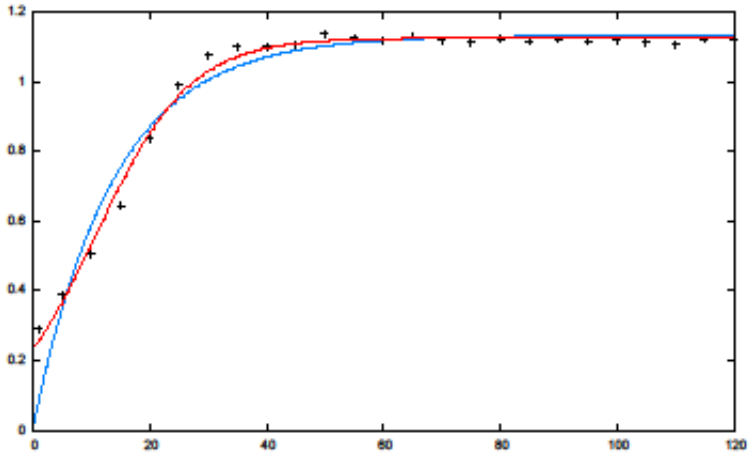
(f)



(g)

Figure 5. Temporal evolution of the effect of pre-leaching time (independent variable) on the yield of mangiferin content extracted from *Curcuma amada* at various experimental design points (x-axis: time in s, y-axis: yield in mg/g). The experimental data (symbols) are fitted to the 2-parameter model (Eq. 1, blue) and 3-parameter logistic model (Eq. 2, red) are shown in Figs. 5(a-g) for 1min, 5min, 10min, 15min, 20min, 25min and 30min respectively.

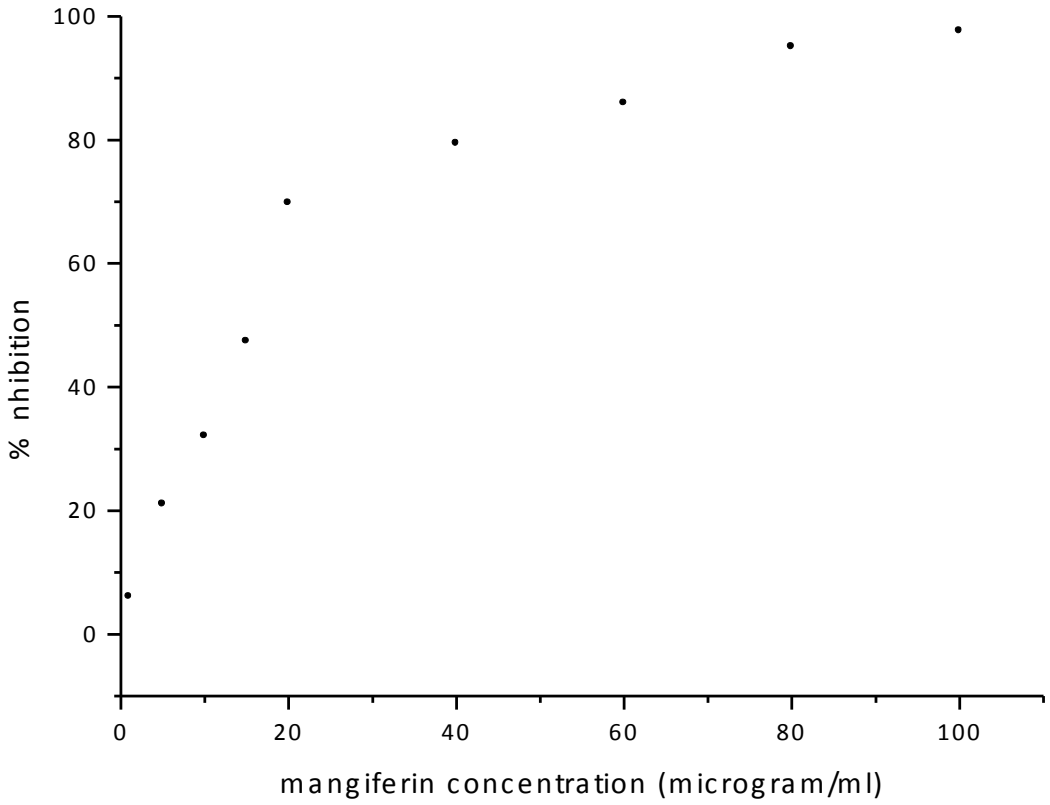
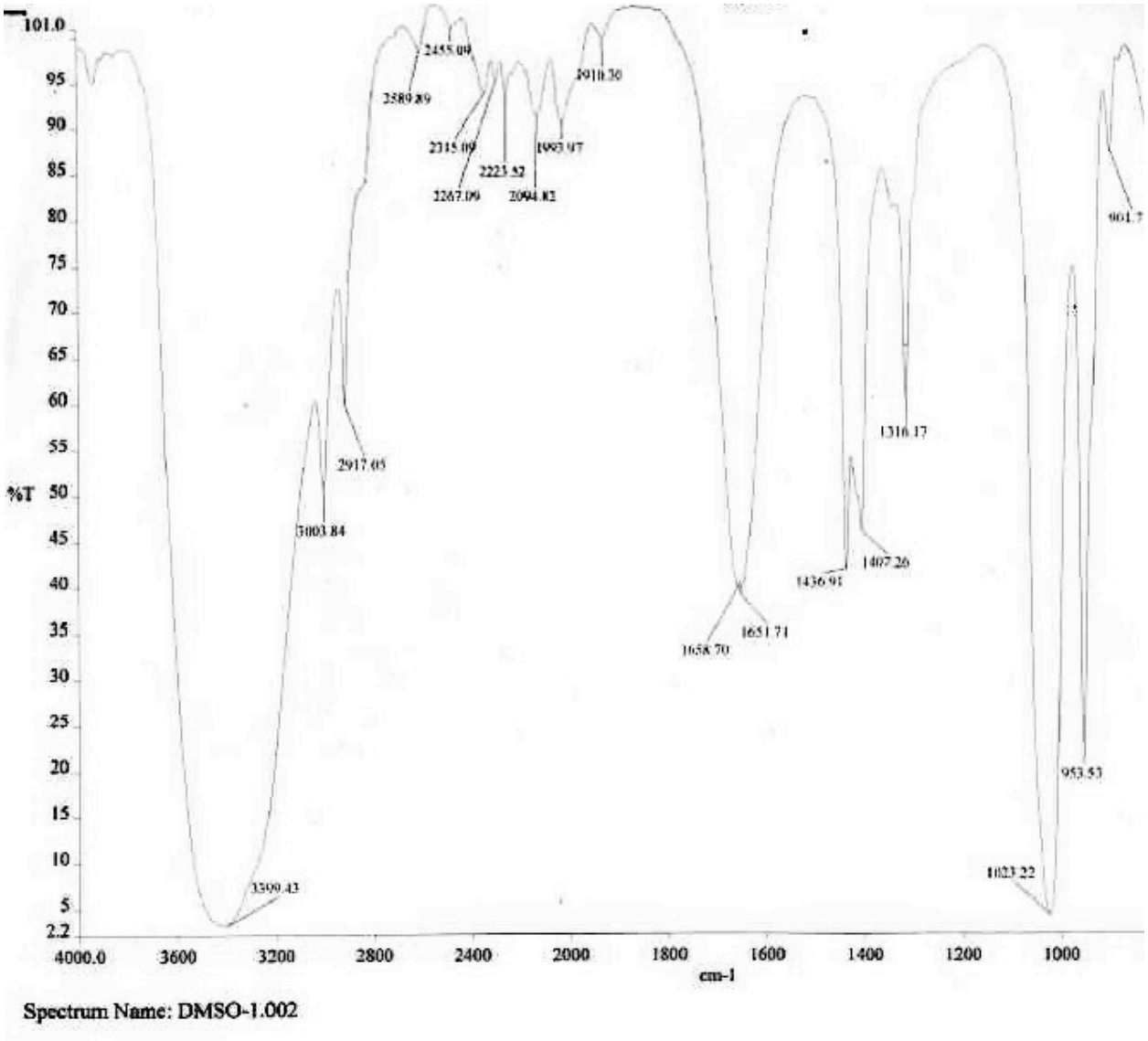


Figure 6. DPPH radical scavenging activity of mangiferin obtained from microwave assisted extraction of Curcuma amada at microwave power - 550W, pre-leaching time - 20min, extraction time - 50s and ethanol concentration - 80%.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10
5
2.2
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
cm-1
2455.09
2589.89
2315.09
2223.52
2267.09
2044.82
1993.97
2910.30
2917.05
3003.84
3309.43
1658.70
1651.71
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1407.26
1316.17
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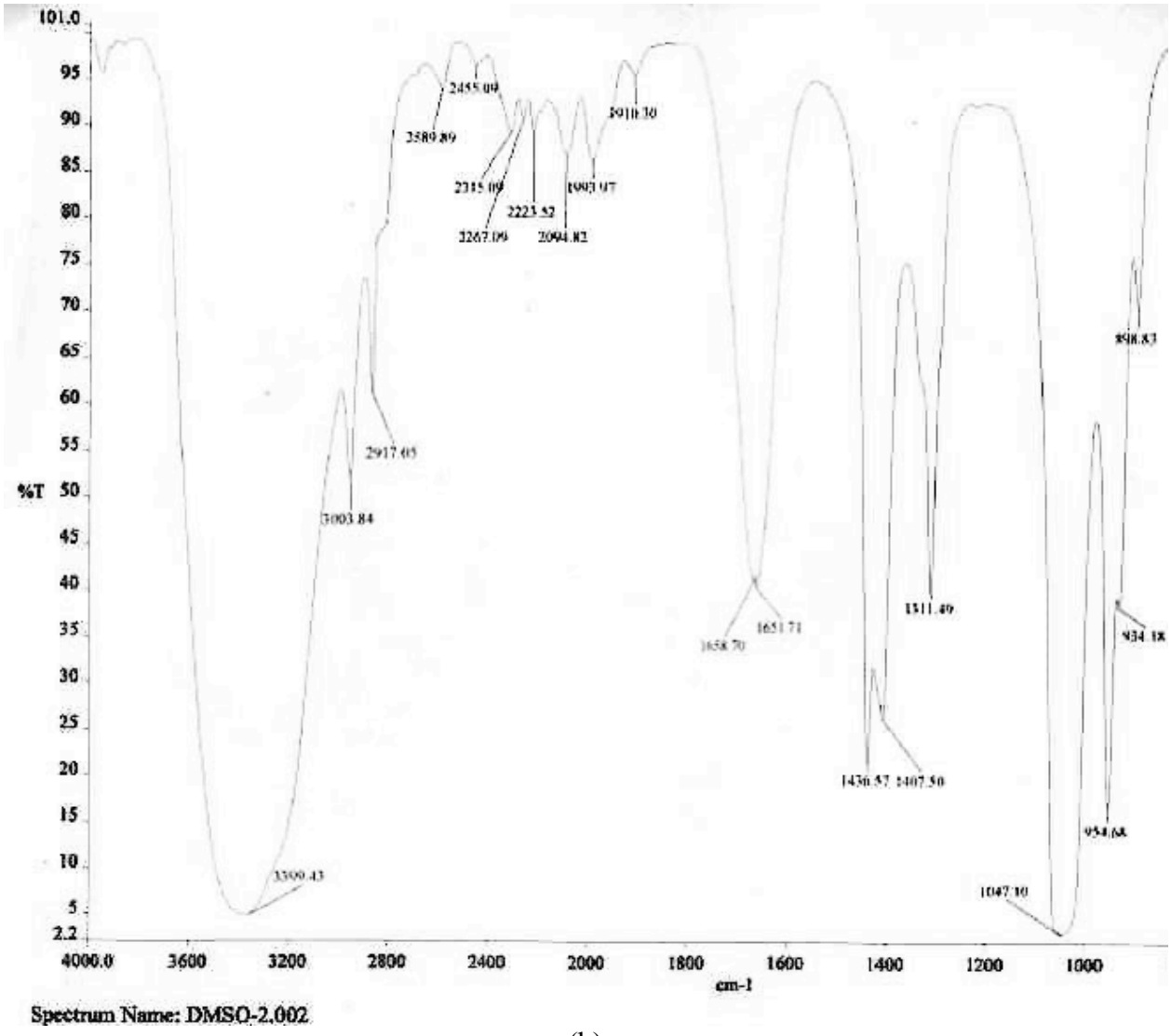
953.53
661.7
Spectrum Name: DMSO-1.002

(a)



(b)

Figure 7. FTIR spectrum of mangiferin extracted from (a) Curcuma amada by MAE at 550W and (b) mangiferin standard (for peak values refer Table 1).

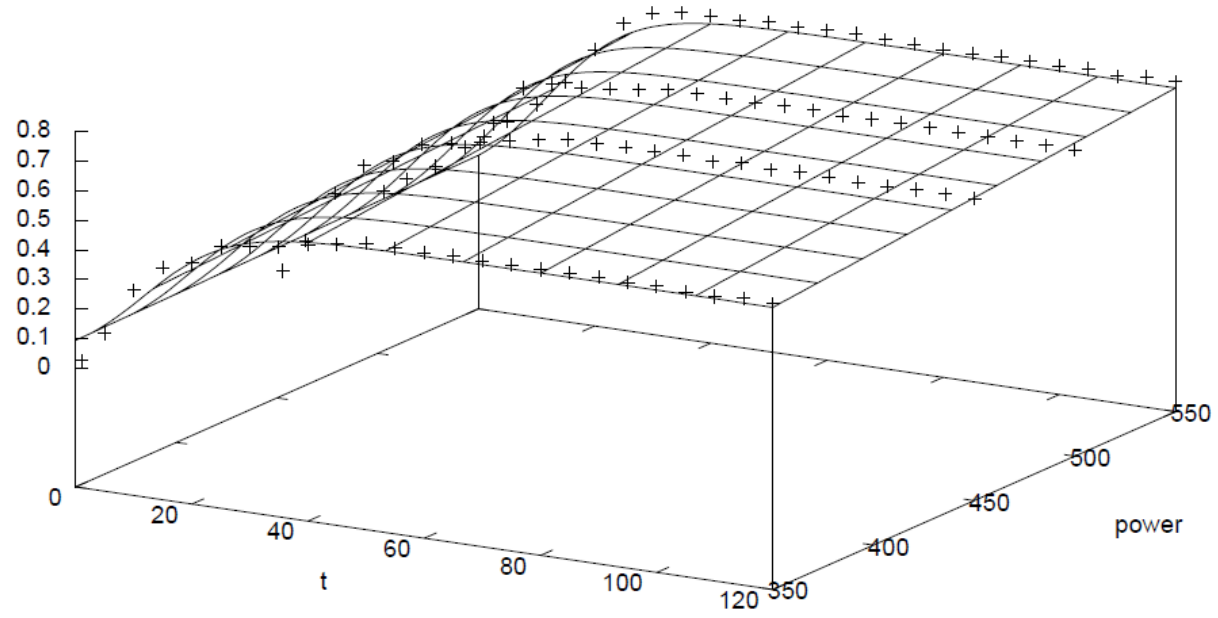


Figure 8. Temporal evolution of yield on microwave power during the extraction process (in accordance with a modified logistic expression; see Eq. 3) for all the design points used in the experimental setup.

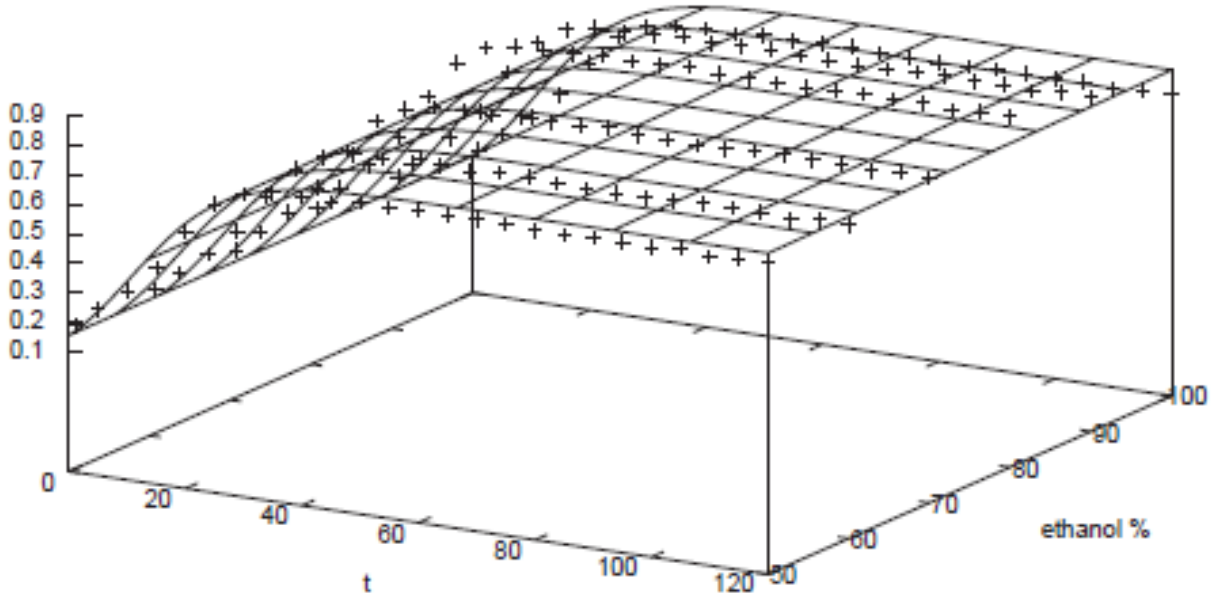


Figure 9. Temporal evolution of yield on the ethanol concentration during the extraction process (in accordance with a modified logistic expression; see Eq. 3) for all the design points used in the experimental setup.