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Electronic Supporting information

Magnetic separation of fatty acids with iron oxide nanoparticles and application to extractive deacidification of vegetable oils

Manuel Cano, Kamal Sbargoud, Emmanuel Allard and Chantal Larpent

E-mail address: Larpent@chimie.uvsq.fr

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1. XRD analysis of the MNPs

Figure S1 shows the X-ray powder diffraction pattern of the as synthesized MNPs.

The reflection 2θ peaks at 30°, 35.6°, 43°, 54.5°, 58° and 63° corresponding to the typical Miller indices of inverse spinel magnetite: (2 0 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 0 0) respectively. The patterns were found to coincide with the JCPDS database for magnetite (JCPDS file 19629; Joint Committee on Powder Diffraction: Swarthmore, PA).

The average particle size has been calculated from the peak width at half height maximum, which occurs at 35.3 degrees 2θ for magnetite (Miller indices, (3 1 1)), using the Debye-Scherrer equation. Average size was ~8 nm.

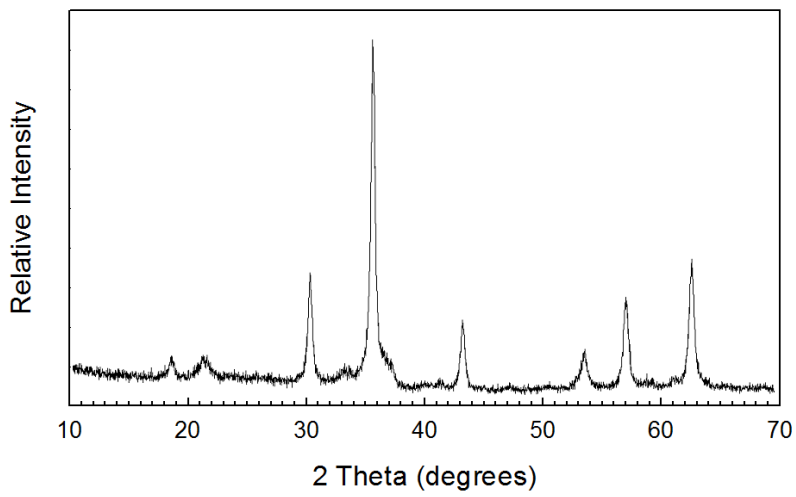

Figure S1. X-ray powder diffraction pattern of the MNPs used in this study.

2. Specific surface area of MNPs from AFM analysis and comparison of the adsorption capacity with other adsorbents

The AFM images (Figure 4 in the main text) were analyzed using WSxM software 5.0 develop 4.2:[S1] the flooded volume was calculated to estimate the average specific surface area of MNPs after adsorption experiments. Assuming a density of 5.15 $g \cdot mL^{-1}$ for magnetite, the calculated value of the average specific surface area of MNPs isolated after adsorption experiments was 105 $m^2 \cdot g^{-1}$.

Considering the average size of 8 nm deduced from DRX, the calculated specific surface area of the starting MNPs is equal to 144 $m^2 \cdot g^{-1}$.

Table S1 compares the maximum adsorption capacities (Q_{max}) of MNPs with other adsorbents reported in the literature.

Table S1. Maximum adsorption capacities (Q_{max}) for different adsorbents of FFA reported in the literature and comparison with our experimental data.

	Surface Area *(m^2/g)*	**Adsorption capacity per weight unit** *(mg/g)*	**Adsorption capacity per surface unit** *(mg/m^2)*
Ion exchange resins[16] soaked with methanol	unknown	270	-
Chitosan[18]	unknown	71.2	-
SiO_2/MgO[19]	301.7	185	0.6
Fe_3O_4 NPs (this work)	105-144	125	0.85-1.2

3. Effect of the MNPs load.

In addition to the influence of the initial concentration of oleic acid (for a fixed MNPs load, 30 mg) described in the main text, we investigated the influence of the adsorbent loading for a given concentration of oleic acid (250 mg·L^{-1}) in ethanol-hexane.

The variation of the amount of OA adsorbed for different MNPs loadings (in g) is shown in figure S2a. Figure S2b show the variations of the amount of OA adsorbed on MNPs (mg OA adsorbed /g NP) as a function of weight ratio of OA to MNPs introduced in the reaction mixture (mg OA_{init}/g NP). Similar results are obtained from experiments at variable concentrations of oleic acid and experiments performed at variable MNPs loadings thus suggesting that adsorption capacity is not affected by increasing the adsorbent load and hence that there is no decrease of the availability of the active sites (in good agreement with the observed absence of aggregation of NPs).

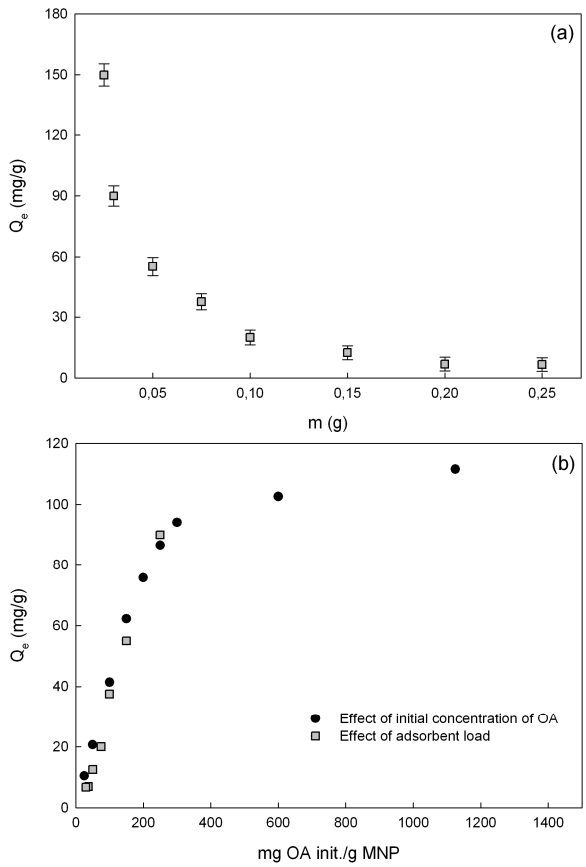

Figure S2. (a) Effect of adsorbent load on the OA adsorption in ethanol-hexane. (V = 30 mL, t = 120 min, T = 298 K, C_{ini} = 250 mg·L^{-1}). (b) Variations of the amount of OA adsorbed on MNPs (mg OA adsorbed/g NP) as a function of weight ratio of OA to MNPs introduced in the reaction mixture (mg OA_{init}/g NP).

4. FFA in sunflower and olive oils.

Typical FFA contents of sunflower and olive oils are given in Table S2.[S2]

Molecular weight, melting and boiling points for the most abundant fatty acids in sunflower and olive oils are given in Table S3.[S3]

Figure S4 shows the formulae of the most abundant saturated, monounsaturated and polyunsaturated fatty acids in sunflower and olive oils.

Table S2. Typical FFA content of sunflower and olive oils.[S2]

	Sunflower Oil	**Olive Oil**
Linoleic acid (C18:2ω-6)	47 %	14 %
Oleic acid (C18:1ω-9)	20 %	55 %
Stearic acid (C18:1)	4 %	3 %
Palmitic acid (C16:0)	6 %	13 %
Others	2 %	1 %

Table S3. Molecular weight, melting and boiling points for the most abundant fatty acids present in sunflower and olive oils.[S3]

	Molecular Weight (g/mol)	***Melting Point (°C)***	***Boiling Point (°C)***
Palmitic Acid (C16:0)	256.42	61-62.5	351[a]
Stearic Acid (C18:0)	284.48	67-72	376.1[a]
Oleic Acid (C18:1ω-9)	282.46	13-14	360[a]
Linoleic Acid (C18:2ω-6)	280.45	-5	230[b]

(a) under atmospheric pressure; (b) under reduced pressure: 16 mmHg.

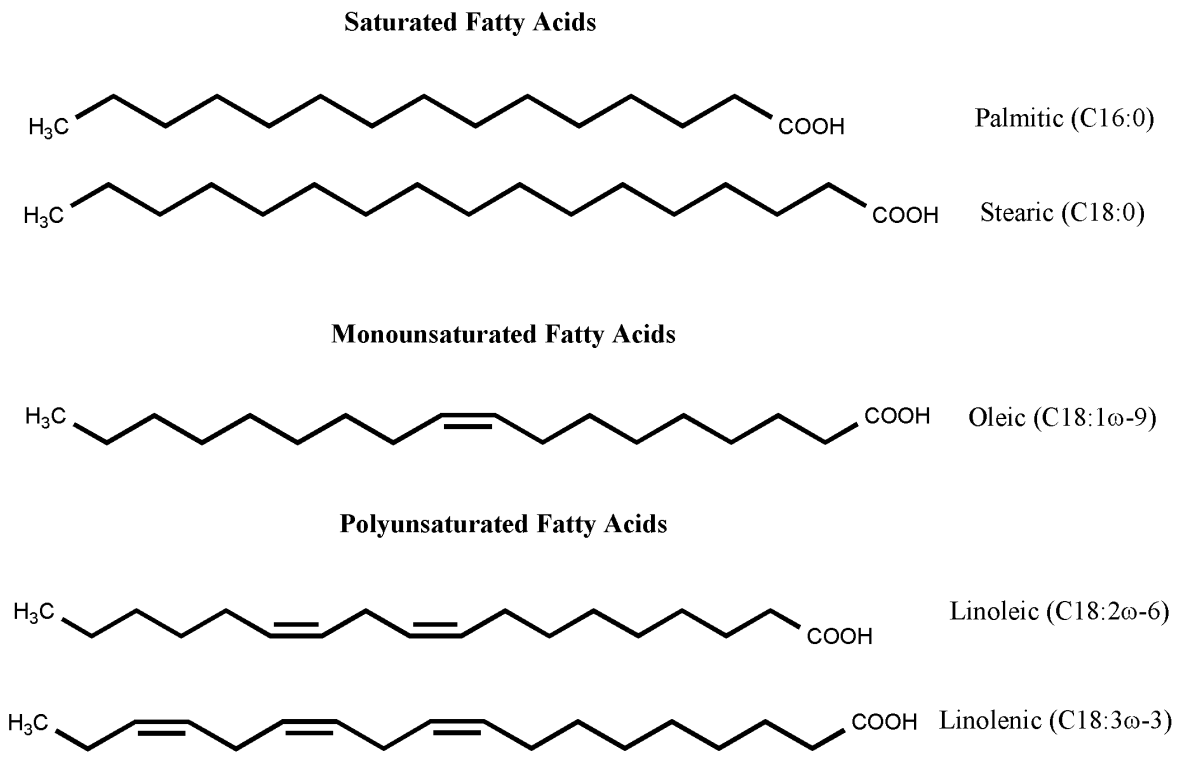

Figure S3. Structures of the most abundant saturated, monounsaturated and polyunsaturated fatty acids in sunflower and olive oils.

5. DT-TGA curves for FA coated MNPs

Figure S4 shows the DT-TGA obtained for PA, SA, OA and LA coated MNPs. It demonstrates that each FA coated MNPs have slightly different maximum decomposition peak temperatures which vary in the same order as the boiling points of the fatty acids (Table S4).

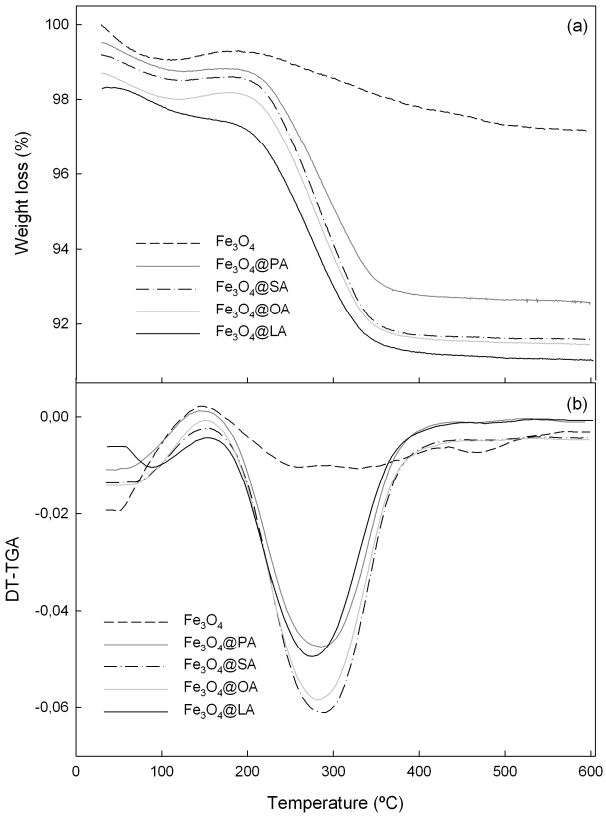

Figure S4. Thermogravimetric analyses of the starting MNPs (Fe_3O_4), stearic acid-coated MNPs (Fe_3O_4@SA), palmitic acid-coated MNPs (Fe_3O_4@PA), oleic acid-coated MNPs (Fe_3O_4@OA), and linoleic acid-coated MNPs (Fe_3O_4@LA) prepared at saturated adsorption conditions.

(a) TGA curves (figure 5a in main text).

(b) DT-TGA: Representation of first derivate of the weight loss with respect to the temperature plotted against the temperature.

Table S4: Characteristics of fatty acid coated MNPs deduced from TGA

Fatty acid	Weight loss (%)[a]	FA content (mg/g)[a]	FA content (mmol/g)[a]	T max (°C)[b]	Boiling point (°C)[c,d]
Palmitic acid (PA)	7.1	71	0.27	283	351[c]
Stearic acid (PA)	7.61	76	0.27	288	376[c]
Oleic acid (OA)	7.36	74	0.26	284	360[c]
Linoleic acid (LA)	7.27	73	0.26	274	230[d]

(a) from TGA curves; (b) maximum decomposition peak temperature from DT-TGA; (c,d) boiling point of the acid:[S3] (c) under atmospheric pressure, (d) under reduced pressure : 16 mmHg.

6. FTIR spectra of Olive and Sunflower oils.

The IR spectra of the olive and sunflower oils used in this study show the intense C=O stretching band for the triglyceride ester groups at 1746 cm^{-1} and a very weak band at 1710 cm^{-1} for the C=O stretching band of the carboxylic acid group of FFA.

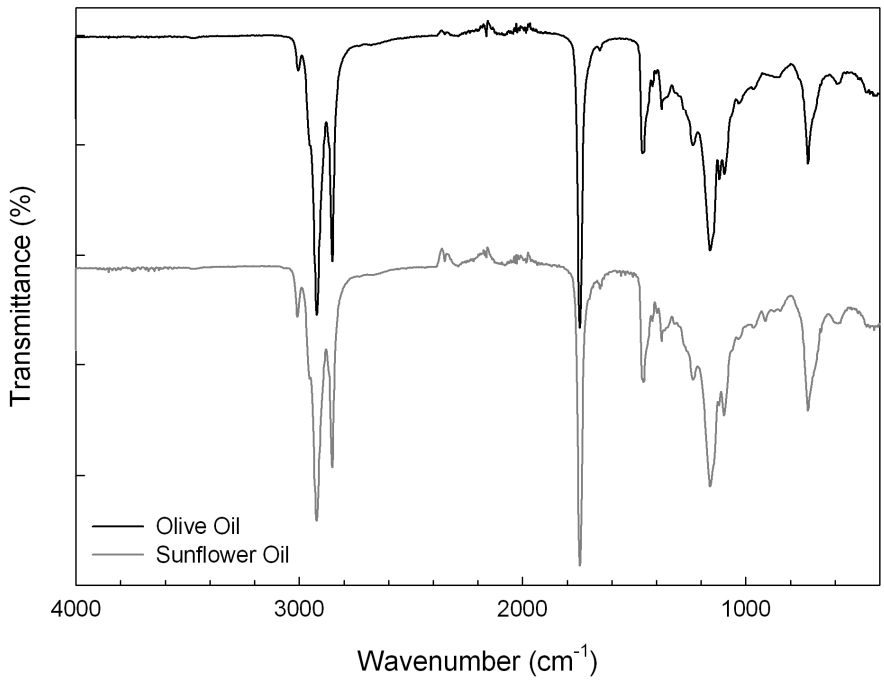

Figure S5. ATR-FTIR spectra of the olive and sunflower oil samples used in this study.

7. Elemental analyses of the FFA loaded MNPs magnetically separated after adsorption experiments.

The percentages of C and H, determined by elemental analyses, in MNPs before and after adsorption of OA in hexane-ethanol (Fe_3O_4@OA) and FFA in crude olive and sunflower oils (Fe_3O_4@FFA) are given in Table S4. These results are in good agreement with the percentage of weight loss obtained by TGA.

Table S5. Elemental Analysis (%) for the MNPs, before (Fe_3O_4) and after the adsorption at saturated conditions in synthetic sample (Fe_3O_4@OA), sunflower and olive oils (Fe_3O_4@FFA).

	% C[a]	*% H*[a]	**FFA load**[b] **(mmol/g)**	**FFA load**[b] **(mg/g)**	**% wt from TGA**
Fe_3O_4 starting particles	0.62	0.58	-	-	2.44
Fe_3O_4@OA from organic solution	6.37	1.31	0.295 ± 0.02	83 ± 7	7.36
Fe_3O_4@FFA from Olive oil	7.59	1.45	0.351 ± 0.02	99 ± 7	8.92
Fe_3O_4@FFA from Sunflower oil	5.10	1.14	0.236 ± 0.02	66 ± 7	7.14

(a) experimental error: ± 0.5% ; (b) calculated as OA load.

8. Determination of iron in oils before and after treatment with MNPs by fluorescence using 1,10-Phenanthroline (Phen) as fluorescent probe

1) Introduction

This analytic method is based on the quenching of the fluorescence of Phen (emission at 365 nm with an excitation at 266 nm) upon complexation with Fe^{2+}.[S4] The emission intensity ratio F_0/F (without and with iron) increases linearly with the increase of iron concentration. The linear range is from 30 ppb to 1 ppm with a detection limit of 1.35 ppb, at neutral pH. For the determination of iron, regardless of the oxidation state, the samples are prepared in the presence of hydroxylamine to reduce Fe^{III} to Fe^{II}.[S4]
In our experiments we used the standard addition calibration method to determine the iron concentration.

2) Experimental procedure

- Reagents: Hydrochloric acid (aqueous solution 37 % wt) and hydroxylamine hydrochloride (99%) were obtained from Alfa Aesar, $FeSO_4 \cdot 7H_2O$ (>99.5%) from Merck and 1,10-Phenanthroline (Phen, 99%) from Sigma.
The stock solution of Fe^{2+} (20 ppm) was prepared by dissolving $FeSO_4 \cdot 7H_2O$ in 0.25 M HCl. The stock solution of Phen (50 µM) were prepared by dissolving Phen in aqueous ethanol (50%). The ammonium acetate buffer solution (1 M, pH 7.0) was obtained by dissolving ammonium acetate (19.3 g) in water (250 mL).
Millipore water was used throughout the experiments.

- Fluorescence measurements: Steady state fluorescence emission spectra were recorded on a spectrofluorimeter FluoroMax-3 equipped with 150W Xenon Lamp and a slit width of 5 nm. 1x1 cm PMMA cells were used.

- Acidic extraction of iron from oil (according to ref. S5)
3 g of oil and 3 g of 5M hydrochloric acid were weighted in a test tube. The tube was capped and the mixture heated overnight at 50°C in a water bath with vigorous magnetic stirring. After cooling to room temperature the mixture was centrifuged at 5000 rpm for 10 min and the lower acid aqueous layer was withdrawn with a polypropylene pipette. For iron determination by fluorescence, the aqueous phase was diluted 5 times in water ("working solution").
These experiments were performed on olive oil and sunflower oil before and after treatment with MNPs (with 10 % wt adsorbent load).

- Determination of iron by fluorescence:
Working solution (0.25 mL), Phen solution (0.4 mL), buffer solution (0.8 mL), 1M ammonia solution (0.25 mL) and 5%wt hydroxylamine hydrochloride solution (0.2 mL) were added sequentially in a 4 mL disposable PMMA fluorometry cuvette. The pH of the solution was close to 7. The mixed solution was heated up to 80 °C in water-bath for 10 min and cooled for 20 min to room temperature. The solution was completed to 4mL with water. The fluorescence spectrum upon excitation at 266 nm was recorded at 25°C and the emission intensity at 365 nm was measured.

For addition calibration 10, 20, 30µL aliquots of the stock Fe^{2+} solution (corresponding to 50-150 ppb Fe) were added.
The reference spectra, Phen alone and without Phen, were obtained by substituting the working solution and the Phen solution by water, respectively.

3) Results

- <u>Olive oil</u>

Figures S6 show the fluorescence spectra of the reference solutions (Phen alone and without Phen) and of the olive oil extracted samples alone and with calibrated additions of iron before (a) and after (b) treatment with MNPs for FFA removal.
Figure S7 shows the addition calibration curves for olive oil before and after treatment with MNPs. It can be seen that the variation of F_0/F versus iron concentration displays a straight line with quite similar values before and after treatment with MNPs indicating that the concentrations of iron in olive oil are quite similar before and after treatment with MNPs.
The calculated values of iron concentrations are respectively 2.8 ± 0.4 ppm before and 2 ± 0.4 ppm after treatment with MNPs.

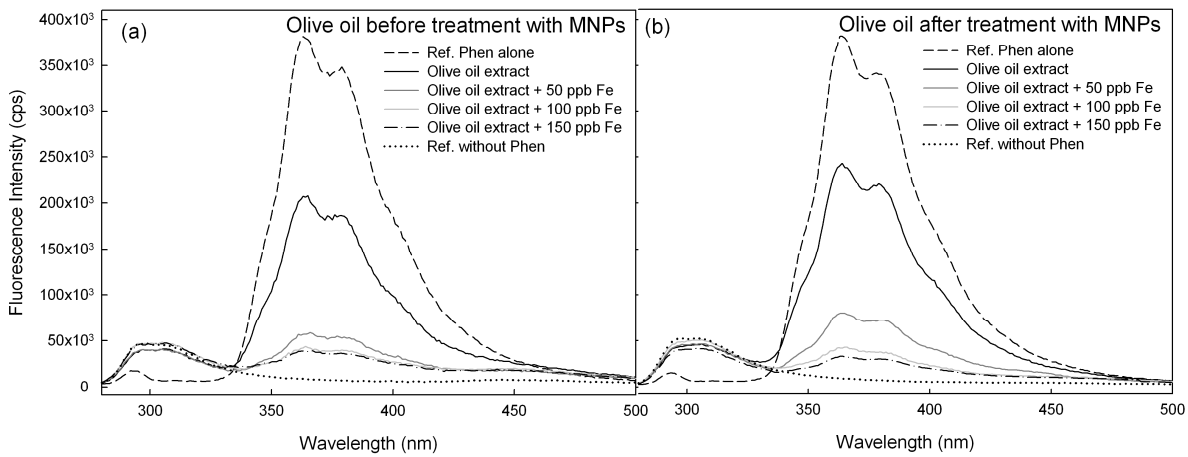

Figure S6. Fluorescence spectra for olive oil extract, olive oil extract with addition of 50, 100, 150 ppb of Fe^{2+} for calibration and reference samples with Phen alone and without Phen.

a) Olive oil before treatment with MNPs.

b) Olive oil after treatment with MNPs for FFA removal.

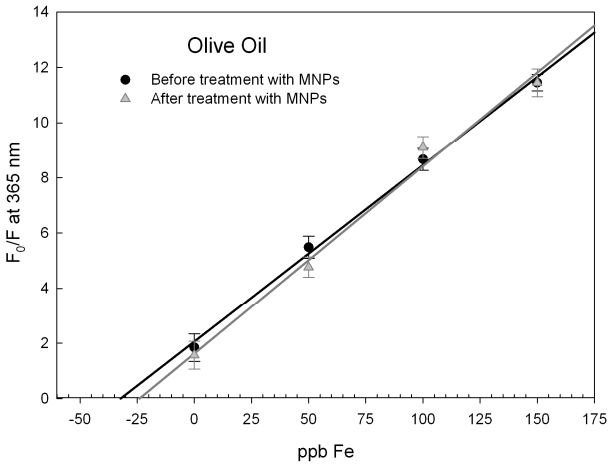

Figure S7. Standard addition curves obtained for olive oil before and after treatment with MNPs.

- <u>Sunflower oil</u>

Figure S8 shows the fluorescence spectra obtained for sunflower oil extracts before and after treatment with MNPs. The fluorescence spectra are quite similar thus indicating that the iron concentration remained unchanged after removal of FFA with MNPs.
Figure S9 shows the fluorescence spectra of the sunflower oil extract after treatment with MNPs, alone and with calibrated additions of iron.
Figure S10 shows the addition calibration curve for sunflower oil extract after treatment with MNPs.
The calculated values of iron concentrations are 1.6 ± 0.4 ppm before and after treatment with MNPs.

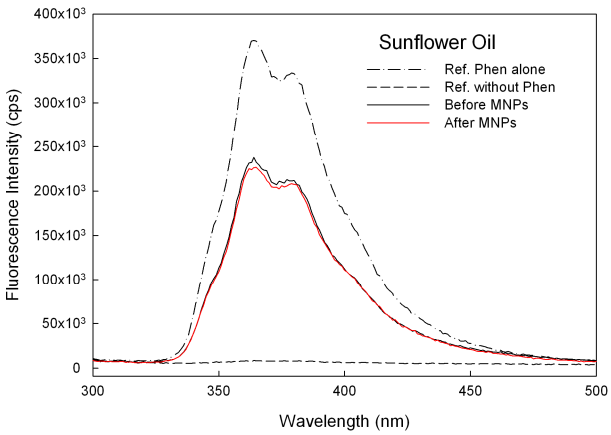

Figure S8. Fluorescence spectra for the sunflower oil extracts before and after treatment with MNPs and reference samples with Phen alone and without Phen.

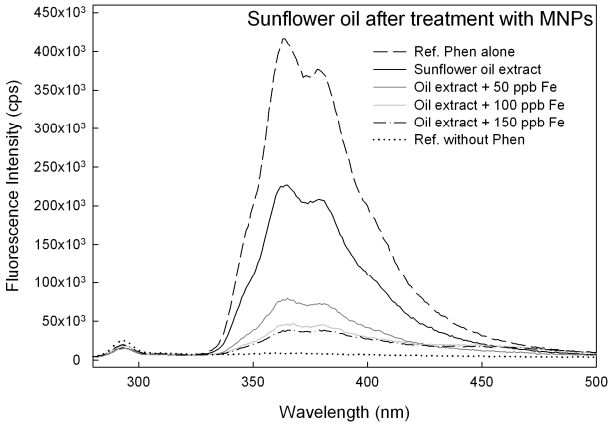

Figure S9. Fluorescence spectra of the reference solutions (Phen alone and without Phen) and of the sunflower oil extract after treatment with MNPs alone and with calibrated additions of iron.

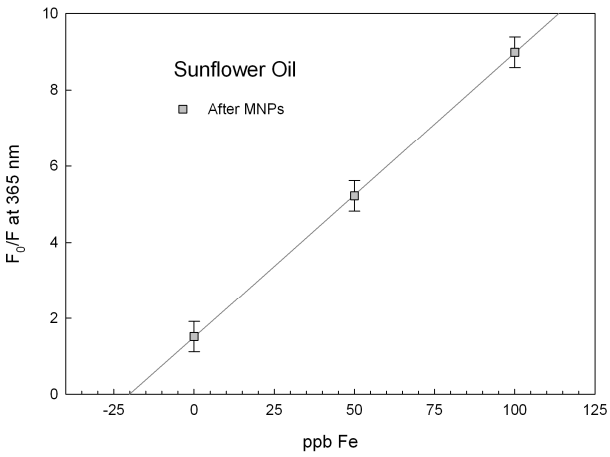

Figure S10. Standard addition curve obtained for sunflower oil after treatment with MNPs.

References:

S1 I. Horcas, R. Fernandez, J.M. Gomez-Rodriguez, J. Colchero, J. Gomez-Herrero and A.M. Baro, *Rev. Sci. Instrum.*, 2007, **78**, 013705.

S2 R. Kowalski, *Acta Chromatogr.*, 2007, 18, 15-23.

S3 M. M. Prieto, J. C. Bada Gancedo, M. I. Lombardía Prendes and E. Graciani, *Grasas Aceites*, 1998, 49, 151-158.

S4 G.-H. Zhu, H.-X. Ju and B.-F. Ye, *Chinese J. Chem.*, 2002, **20**, 301-304.

S5 A. De Leonardis, V. Macciola and M. De Felice, *Int. J. Food Sci. Technol.*, 2000, **35**, 371-375.