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Sonochemical reduction of the antioxidant activity of olive mill wastewater

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Abstract

The use of ultrasonic irradiation to reduce the antioxidant activity of olive oil mill wastewaters (OMWs) originating from two-phase and three-phase decanters was examined. Sonication of diluted OMW samples was conducted at ultrasonic frequencies of 24 and 80 kHz, an applied power varying between 75 and 150 W, and liquid bulk temperatures varying between 25 and 60 °C. At the conditions in question, the reduction in antioxidant activity was found to increase with decreasing temperature and increasing power and frequency. Addition of NaCl in the samples also appeared to enhance reduction. Antioxidant activity of OMW samples was assessed using the recently developed Co(II)/ethylenediaminetetraacetic acid (EDTA)-induced luminol chemiluminescence analytical protocol, while the total phenolic load was measured according to the Folin–Ciocalteu method.

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1. Introduction

Wastewaters from olive oil mill wastewaters (OMWs) constitute an important environmental issue in the Mediterranean countries. More than 30 million m³ of liquid and solid wastes are produced in the short harvesting season lasting between December and February containing a highly polluting organic load including sugars, tannins, polyphenols, polyalcohols, pectins, and lipids (Lesage-Meesen et al., 2001). Most frequently, OMWs are pumped and discharged into evaporation ponds, or directly dumped in rivers, or spread on soil. The environmental concern is mainly due to the compounds of phenolic nature, which contribute to the antimicrobial and phytotoxic effects of OMWs, thus limiting its microbial degradability (Borja et al., 1997).

Because of the associated environmental problems and potential hazards, several OMW treatment technologies have

been developed aiming at the removal of the main toxic organic compounds prior to discharge. Of these, there is little doubt that anaerobic digestion is the most environmentally compatible and least expensive wastewater treatment method, which may also overcome the problem of seasonal OMW production (Ergüder et al., 2000). Nonetheless, some bacteria (e.g., methanogens) are particularly sensitive to the excess of inhibitory and/or toxic compounds such as the phenolic ones, thus limiting the performance of anaerobic digestion (Capasso et al., 1992; Benitez et al., 1997). Consequently, several other treatments have been investigated for OMW treatment. These include advanced oxidation processes (AOPs) such as ozonation (Benitez et al., 1997; Andreozzi et al., 1998; Amat et al., 2003), photocatalysis (Vigo and Cagliari, 1999), hydrogen peroxide/ferrous iron oxidation (the so-called Fenton's reagent) (Rivas et al., 2001), and wet air oxidation (Debellesfontaine et al., 1994; Mantzavinos et al., 1996). Given the specific treatment objectives, AOPs may be used either for the complete mineralization of all pollutants to carbon dioxide,

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water, and mineral salts, or for the selective removal of the more bioresistant pollutants and their conversion to biodegradable intermediates. In the latter case, AOPs are usually coupled with biological posttreatment.

In recent years, ultrasound irradiation has received increasing attention for the destruction of organic pollutants in waters and wastewaters. The process comprises cyclic formation, growth and subsequent collapse of microbubbles occurring in extremely small intervals of time, and release of large quantities of energy over a small location. Sonochemical degradation in aqueous phase involves several reaction pathways and zones such as pyrolysis inside the bubble and/or at the bubble–liquid interface and hydroxyl radical-mediated reactions at the bubble–liquid interface and/or in the liquid bulk (Thompson and Doraiswamy, 1999). The relative importance of the various mechanisms involved primarily depends on the physicochemical properties of the pollutants in question; the process is more selective towards hydrophobic and volatile species that can be degraded easily via pyrolytic reactions, while hydrophilic and less volatile compounds are degraded slowly via hydroxyl radical-induced reactions. Various investigations report successful removal of a wide range of organic pollutants from relatively dilute aqueous solution, and it appears that the applications of this novel means of reaction in environmental remediation and pollution prevention is unlimited (Adewuyi, 2001; Psillakis et al., 2004; Vassilakis et al., 2004).

The aim of this work was to study the sonochemical degradation of OMWs with emphasis on the effect of various operating parameters on the reduction of antioxidant activity and associated phenolic compounds. Antioxidant activity was assessed using the recently developed Co(II)/ethylenediaminetetraacetic acid (EDTA)-induced luminol chemiluminescence analytical method, while the total phenolic load was measured according to the Folin–Ciocalteu method.

2. Experimental and analytical

2.1. Materials

EDTA, luminol (3-aminophthalhydrazine) (>97% pure), caffeic acid (>99% pure), and boric acid (>99.5% pure) were obtained from Sigma-Aldrich (Germany). Cobalt(II) chloride hexahydrate, perhydrol 30% H₂O₂, sodium hydroxide, and sodium chloride (>99.5% pure) were from Merck (Germany). All chemicals were used without further purification. All solutions used for the experiments were prepared with water purified by an EASYpureRF water purification system supplied by Barnstead/Thermolyne.

2.2. Chemiluminescence method

Chemiluminescence measurements were carried out on a JENWAYTM 6200 fluorimeter (Jenway Gransmore Green

Felsted, Dunmow, Essex, UK), keeping the light off and using only the photomultiplier of the apparatus. The following solutions were used for all chemiluminescent measurements and were prepared each day:

- (1) Boric acid buffer (0.05 M): pH of the solution was adjusted to 9 using a NaOH solution (1 M).
- (2) Luminol solution: luminol, 100 µg/l (5.6×10^{-4} M), in the above borate buffer (pH=9).
- (3) Co(II)/EDTA in borate buffer: 10 mg of CoCl₂ · 6H₂O (8.4×10^{-4} M) was weighed and mixed with 50 mg of EDTA (2.63×10^{-3} M) and then dissolved into 50 ml of borate buffer.
- (4) Hydrogen peroxide (5.4×10^{-3} M): prepared by diluting the 30% stock solution accordingly. The concentration of the final solution was measured using the reflectometric peroxide test (code 16974; Merck).

The fluorimeter signal assigned as 100 was measured each day according to published guidelines (Atanassova et al., 2004). For sample analysis, 2 ml of Co(II)/EDTA was first mixed with 200 ml of luminol solution in a test tube and vortexed for 15 s. Fifty microliters of H₂O₂ aqueous solution was mixed with 50 µl of the sample (for all calibration runs, caffeic acid solutions were used instead) in a second test tube and vortexed for 10 s. The luminol buffer cocktail was then added into the second test tube using precision pipettes and mixed thoroughly. Then the mixture was rapidly transferred into a glass cuvette and the chemiluminescence measurement was taken.

2.3. Phenol content determination

The total phenol content was determined colorimetrically using the Folin–Ciocalteu reagent (Folin and Ciocalteu, 1927). A diluted sample or phenolic standard was mixed with the Folin–Ciocalteu reagent and 1 ml of a sodium carbonate-saturated solution. The final solution was left in the dark for 1 h, after which the absorbance of the solution was measured at 725 nm and compared against a blank prepared following the same protocol but without any sample. The standard curve was prepared using 0, 20, 40, 80, 120, 160, and 200 mg/l solution of caffeic acid in methanol:water. Total phenol values were expressed as caffeic acid equivalents (µg/ml).

2.4. Chemical oxygen demand (COD)

COD was measured using the closed reflux colorimetric method. The appropriate amount of sample was introduced into commercially available digestion solution (Hach Europe, Belgium) and the mixture was then incubated for 120 min at 150 °C in a COD reactor (Model 45600; Hach, USA). COD concentrations were measured colorimetrically using a DR/2010 spectrophotometer (Hach).

2.5. High-performance liquid chromatography (HPLC)

An HPLC system, manufactured by Shimadzu, comprising a two-solvent delivery pump, diode array (DAD), and fluorescence detectors, was used. OMW samples were analysed on a Superspher RP-18, 125×2-mm, 4- μ m column (Macherey-Nage, Germany), which was protected by a guard column packed with the same material and maintained at 40 °C. The injection volume was 10 μ l and detection was performed at 278 and 325 nm. The elution gradient program was as follows: from 0 to 20 min, 10:90 methanol:2.5% acetic acid in water at 0.3 ml/min; from 20 to 35 min, 40:60 methanol:2.5% acetic acid in water at 0.3 ml/min; and, finally, from 35 to 45 min, 100:0 methanol:2.5% acetic acid in water at 0.3 ml/min.

2.6. OMW samples

Olive oil residues were collected during the 2003–2004 harvest season in the Chania region, West Crete, from mills using either the classical three-phase centrifugation system or the new centrifugation system with a horizontal centrifugal two-phase decanter. In all cases, fresh olive oil residues from both systems were filtered and kept at 4 °C in the dark.

The average COD values for the two-phase and three-phase OMW samples were 2930 and 33,300 mg/l respectively, while the antioxidant activity was 110 and 1150 μ g/ml caffeic acid equivalents, respectively. The total phenolic load of the two-phase and three-phase OMW samples was 161 and 1617 μ g/ml caffeic acid equivalents, respectively.

For all sonication experiments, the two-phase OMW samples were diluted 1:10 with water and the three-phase ones were diluted 1:100 with water. Therefore, all OMW samples used in this study had a nearly common initial organic matter concentration.

2.7. Sonication experiments

An Ultrason 250 (LabPlant, UK) horn-type sonicator operating at a fixed frequency of 80 kHz and a variable electric power output up to 150 W was used for most sonication experiments. For those experiments carried out at a frequency of 24 kHz, a UP 400S (Dr Hielscher, Germany) horn-type sonicator operating at a variable electric power output up to 300 W was used. In either case, a titanium probe with a 7-mm-diameter tip was used to deliver the ultrasound energy to the liquid medium. Reactions were carried out in a cylindrical all-glass reaction vessel, which was closed during ultrasonic irradiation. The vessel was immersed in a water bath and the temperature was monitored with a Pt100 temperature sensor housed inside the water bath and connected to a temperature control unit (Polystat cc2 model; Huber, Germany), keeping a constant water bath temperature. In all cases, 195 ml of diluted OMW samples was prepared daily and subjected to ultra-

sonic irradiation. Experiments were discontinued periodically to remove 5-ml samples that were analysed by means of Co(II)/EDTA-induced chemiluminescence and Folin–Ciocalteu protocol.

3. Results and discussion

3.1. Sonochemical degradation of OMWs

In preliminary runs, diluted OMW samples were subject to ultrasonic irradiation for 240 min at 80 kHz ultrasound frequency, 150 W power output, and 25 °C water bath temperature. Figs. 1 and 2 show the HPLC chromatograms taken at 278 and 325 nm of the two-phase and three-phase OMW samples, respectively, before and after 240 min of ultrasonic treatment. These wavelengths were chosen as they are characteristic of the phenolic fractions typically found in OMWs. Irradiation for 240 min was capable of reducing the concentration of phenolic compounds since the height of the majority of peaks present in the untreated samples has substantially been reduced following treatment. This is consistent with the results of the Folin–Ciocalteu analysis showing a reduction in the phenolic content of 14% and 24% in the sonicated two-phase and three-phase samples, respectively. Fig. 3 shows the normalised antioxidant activity–time profile for the aforementioned preliminary runs; as seen, sonication for 240 min resulted in about 15% antioxidant activity reduction for both two-phase and three-phase OMW samples. The relatively low reduction of antioxidant activity even after prolonged irradiation times is possibly due to the fact that the sonochemical degradation of hydrophilic phenolics is likely to occur slowly through hydroxyl radical reactions in the liquid bulk (Vassilakis et al., 2004).

It should be pointed out that both antioxidant activity and total phenolic content are expressed in terms of equivalent caffeic acid concentrations, a model compound used for calibration purposes. The antioxidant activity as well as the phenolic content of the untreated two-phase and three-phase samples following dilution would correspond to about 16 mg/l caffeic acid. Therefore, it was decided to investigate the sonochemical degradation of an aqueous solution containing 16 mg/l caffeic acid at 80 kHz ultrasound frequency, 150 W power output, and 25 °C water bath temperature, and the results are also shown in Fig. 3. As seen, the rate of antioxidant activity reduction for the model compound in the absence of a complex matrix (such as the one in OMW samples) is substantially higher than that for the actual OMW samples reaching a value of 78% after 240 min; this was also accompanied by a 52% decrease in the total phenolic content. The sonochemical degradation of actual OMW samples is likely to occur through complex reaction pathways involving the formation of numerous intermediates competing for the available ultrasound energy; moreover, several of these intermediates may be

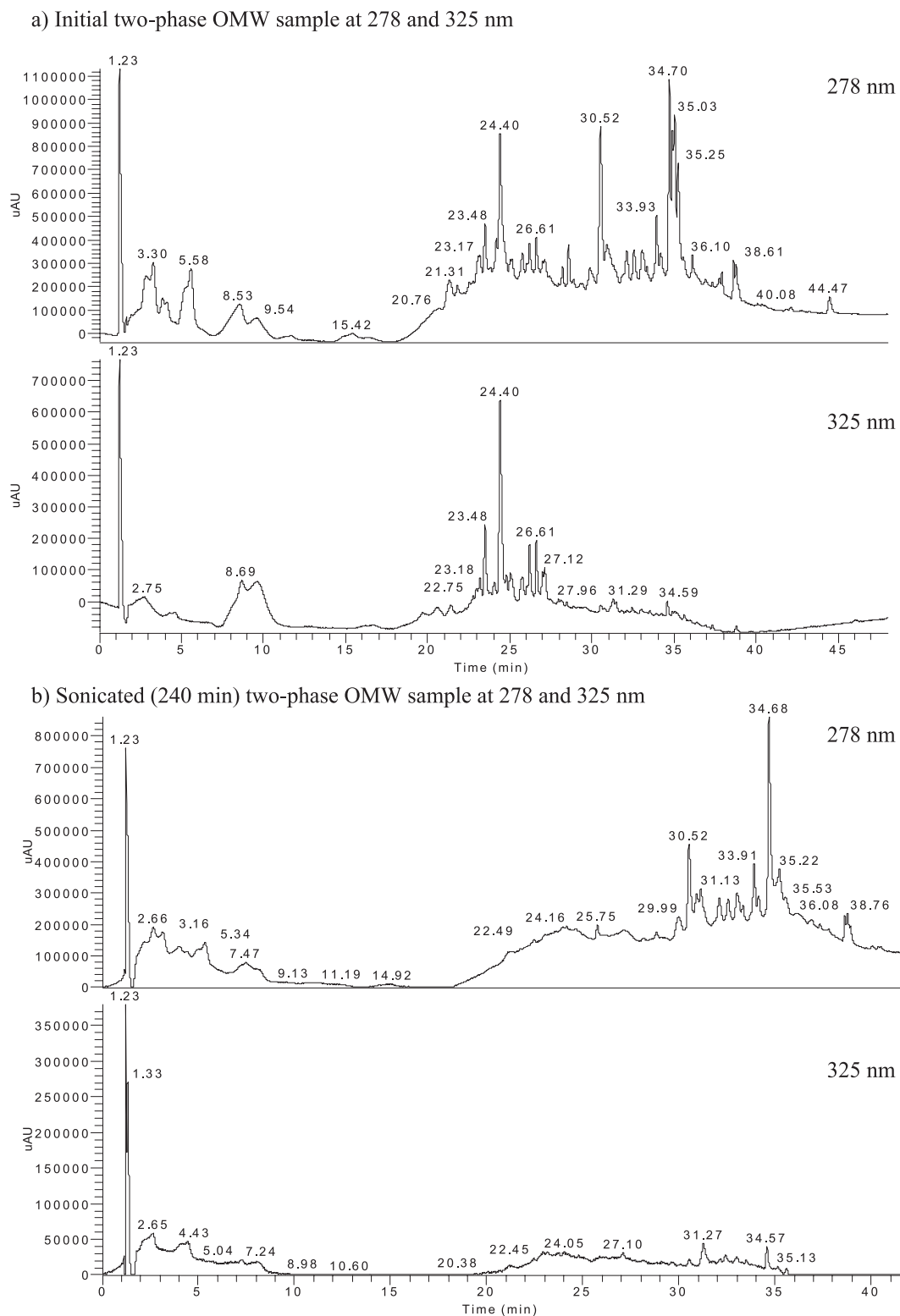


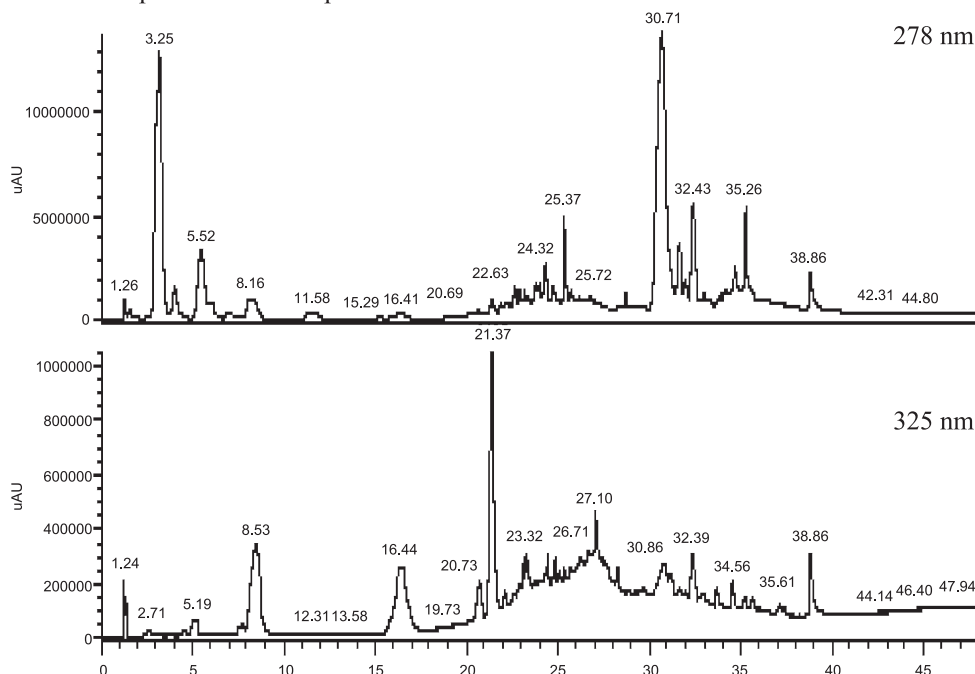
Fig. 1. HPLC chromatograms of a two-phase OMW sample at 278 and 325 nm before (a) and after (b) sonication for 240 min.

of phenolic nature, thus contributing towards the sample's antioxidant activity. On the other hand, with caffeic acid as the starting molecule, fewer reactions and intermediates are likely to occur and this would explain the enhanced performance of sonochemical degradation.

3.2. Effect of power and ultrasound frequency on the sonochemical degradation of OMWs

Fig. 4 shows the normalised antioxidant activity–time profile during the sonochemical degradation of diluted two-

a) Initial three-phase OMW sample at 278 and 325 nm



b) Sonicated (240 min) three-phase OMW samples at 278 and 325 nm

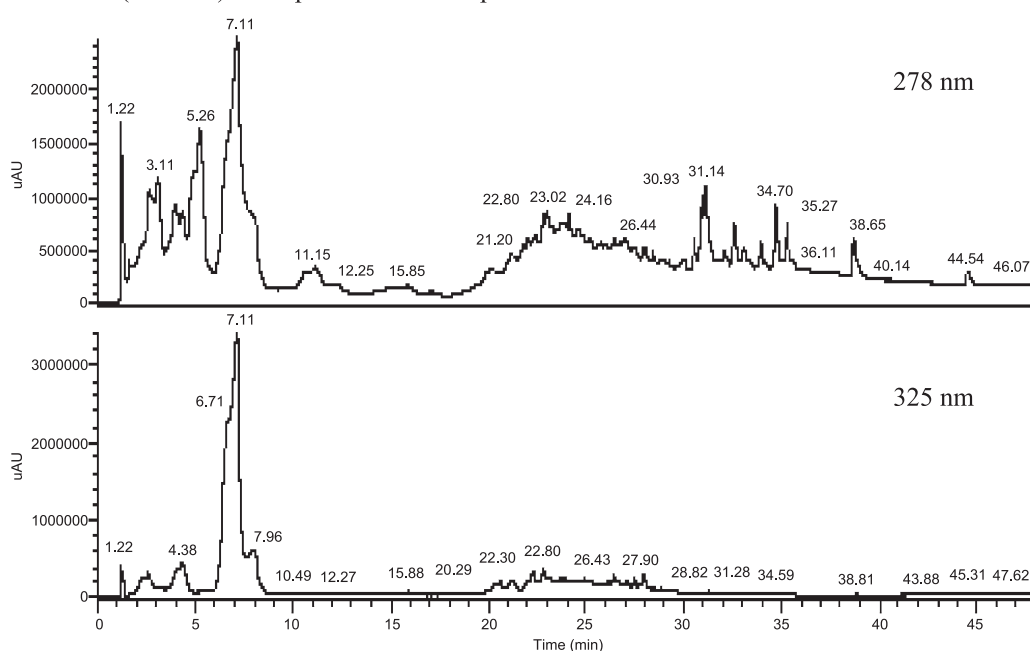


Fig. 2. HPLC chromatograms of a three-phase OMW sample at 278 and 325 nm before (a) and after (b) sonication for 240 min.

phase and three-phase OMW samples at 25 °C as a function of power output and frequency. As expected, antioxidant activity reduction is enhanced with increasing power and ultrasound frequency and this is also consistent with the reduction of total phenolic content measured after 240 min of irradiation. In the case of two-phase samples a 6% and 14% degradation of the total phenolics was recorded at 75 and 150 W, respectively (runs at 80 kHz), while the respective values for the three-phase samples were 15% and 24%.

Under the present experimental conditions, sonication at 24 kHz had practically no effect on OMW antioxidant activity. Sonication for 240 min resulted in only about 3% reduction for the two-phase and three-phase samples, while the total phenolic concentration was reduced by only about 1%. Low frequencies are associated with delayed bubble growth and long collapse duration, leading to radical scavenging and recombination reactions, thus inhibiting mass transfer of hydroxyl and other reactive species into

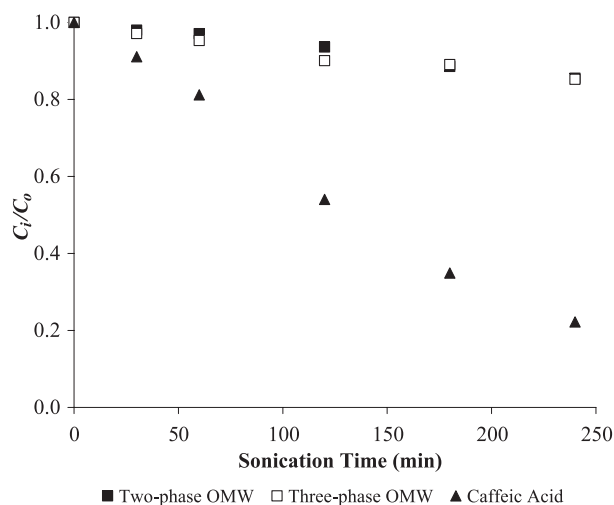


Fig. 3. Normalised antioxidant activity–time profiles during sonication (80 kHz of ultrasound frequency, 150 W of electric power output, and 25 °C water bath temperature) of diluted two-phase and three-phase OMW samples and a 16-mg/l aqueous solution of caffeic acid.

solution and consequently reducing degradation rates (Manousaki et al., 2004).

3.3. Effect of temperature on the sonochemical degradation of OMWs

Fig. 5 shows the normalised antioxidant activity–time profile during the sonochemical degradation of two-phase and three-phase OMW samples at 80 kHz, 150 W, and 25 °C as a function of liquid temperature. As seen, increasing the temperature from 25 to 40 to 60 °C suppressed the reduction of antioxidant activity. Similar results were obtained for the total phenolic content whose degradation also decreased with increasing temperature. The extent of total phenols reduction after 240 min of irradiation was 14% at 25 °C, 6% at 40 °C,

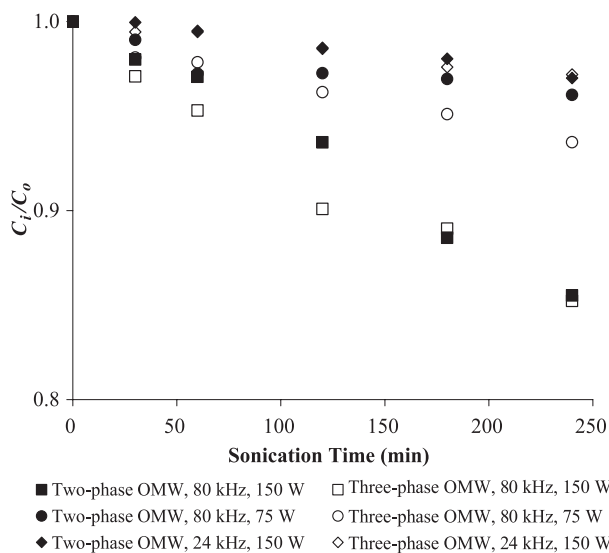


Fig. 4. Effect of electric power and frequency on the sonochemical degradation of diluted two-phase and three-phase OMW samples.

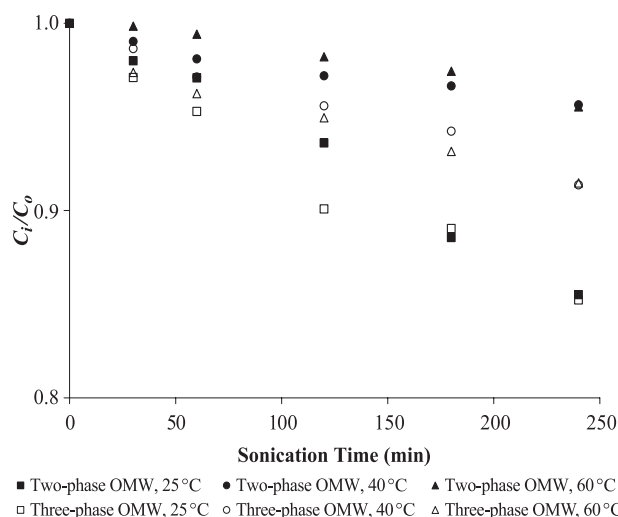


Fig. 5. Effect of temperature on the sonochemical degradation of diluted two-phase and three-phase OMW samples.

and practically no conversion at 60 °C for the two-phase OMW samples, with the respective values being 24%, 13%, and no conversion for the three-phase OMW samples. It is generally believed that although increased temperatures are likely to facilitate bubble formation due to an increase of the equilibrium vapour pressure, the sonochemical effect of such bubbles may be reduced. This is due to the fact that bubbles contain more vapour, which cushions the implosion growth as well as use enthalpy for condensation (Thompson and Doraiswamy, 1999).

3.4. Effect of dissolved species on the sonochemical degradation of OMWs

Fig. 6 shows the antioxidant activity–time profile during the sonochemical degradation of two-phase and three-phase OMW samples at 80 kHz, 25 °C, and 150 W, and in the

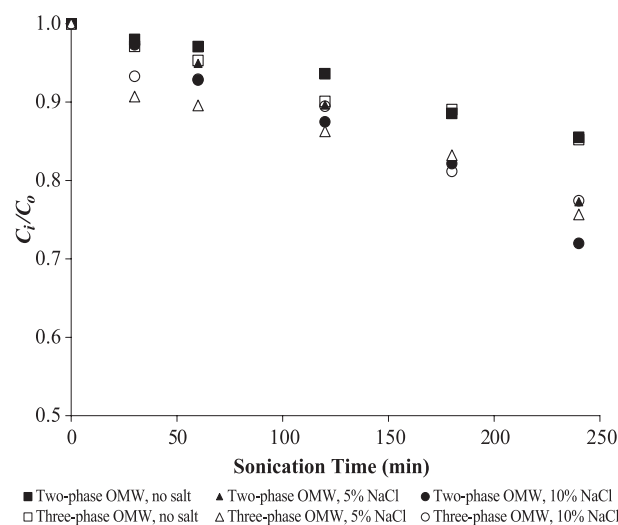


Fig. 6. Effect of NaCl on the sonochemical degradation of diluted two-phase and three-phase OMW samples.

presence of 5% and 10% NaCl (% wt/vol, NaCl:water). Addition of NaCl enhanced antioxidant activity reduction and this can be attributed to an increase in the partitioning in the presence of NaCl, leading to accumulation of the reactant species at the cavitation implosion sites. This is due to the salting-out effect where fewer water molecules are available for dissolving the analyte molecules, preferably forming hydration spheres around the salt ions (Psillakis and Kalogerakis, 2003). It should be mentioned here that on top of the partitioning enhancement, adding salt results in reduced vapour pressure and increased surface tension (Seymour and Gupta, 1997), thus promoting a more violent collapse of the cavitation bubble.

Interestingly, the reduction in total phenolic load was not so pronounced; after 240 min of irradiation, the extent of total phenol reduction for the two-phase samples was 14%, 19%, and 15% at 0%, 5%, and 10% NaCl, respectively. The respective values for the three-phase samples were 24%, 31%, and 28%. The enhancement of sonochemical performance in the presence of salt is of particular interest due to the necessity of diluting the original OMW samples with significant amounts of water. The possibility of using seawater for dilution of these samples may be an appealing option.

In a final set of experiments at 80 kHz, 25 °C, and 150 W, it was attempted to investigate the effect of KBr and *n*-butanol on the sonochemical degradation of two-phase and three-phase OMW samples as these compounds are typical radical reaction inhibitors (scavengers). The rationale behind this was to check for the major reaction mechanisms responsible for OMW antioxidant activity reduction; a decrease in degradation rates would confirm the contribution of radical reactions towards degradation. Unfortunately, these experiments failed to reach safe conclusions since both scavengers heavily interfered with the chemiluminescence method.

4. Conclusions

In the present study, low-frequency ultrasonic irradiation was found capable of reducing the antioxidant activity of OMWs originating from two-phase and three-phase decanters. Although the complex matrix of the OMW samples affected treatment performance, maximisation could be achieved by choosing optimal operating conditions.

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