Determination of Phosphorus in Edible Oils by Inductively Coupled Plasma–Atomic Emission Spectrometry and Oil-in-Water Emulsion of Sample Introduction

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ABSTRACT: The determination of phosphorus was carried out using emulsion sample preparation followed by analysis by inductively coupled plasma–atomic emission spectrometry. The optimal oil-in-water (o/w) emulsion and surfactant concentrations were found by applying response surface methodology. Two phosphorus emission lines were tested. Ethoxynonylphenol was a good choice for the emulsion preparation. The optimal concentration for the oil in the o/w emulsion and the surfactant concentrations were *ca.* 8.0–37% w/w and *ca.* 1.0–10% w/w ethoxynonylphenol, respectively, for the 213.620 nm line; and an oil phase emulsion concentration between *ca.* 18–32% w/w and *ca.* 3–8% w/w ethoxynonylphenol for the 214.911 nm emission line. Good agreement was found between calibration curves for emulsified aqueous standards solutions and o/w emulsions. The use of aqueous standards for calibration purposes is a good addition in the analysis of this type of oil sample where certified standards are not available. Recoveries ranged from 98 to105% and from 102 to 116% for the 213 and 214 nm lines, respectively with relative standard deviations lower than 7%.

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Information on the total phosphorus content as phospholipids in edible oils is useful to the manufacturer to optimize the refining process. Phospholipids cause the loss of neutral oil during neutralization, and their presence leads to oil discoloration during deodorization and steam distillation. The total phosphorus content of oils is monitored to give an indication of phospholipid removal. The method based on the colorimetric determination of phosphorus as molybdovanado-phosphate is recommended by the AOAC (1). This method is not widely used because it is relatively lengthy. Alternative procedures (2,3) are often chosen because of greater speed or smaller sample size than the official method. A faster graphite furnace atomic absorption spectrometric method (4,5) is available. However, this method is single-element and has a limited linear working range.

Inductively coupled plasma (ICP) can be an extremely useful tool for analysis of phosphorus and other trace elemental contaminants present in edible oils as low as 1 ppm. This technique offers the advantage of simultaneously analyzing for the elements of interest in oil samples as small as 30 mL. Techniques including dry and acid digestions, extraction and sample dilution with a suitable organic solvent are time-consuming and prone to contamination. Systems, such as emulsions, have been used as an optional and promising method of sample introduction into the plasma (6) and have the advantage of using aqueous standards for calibration purposes. The use of surfactants to enhance the sample transport process into the plasma has been reported (7–9). The benefits of using surfactants for emulsifying an oil system have been described recently (10).

Dijkstra and Meert (11) analyzed phosphorus in edible oils by direct current plasma emission spectroscopy, using Conostan metallo-organic standards. Oil samples for analysis were diluted with kerosene, which lowered the detection limit due to the high background signal produced by this solvent. Limited work presented by Kassa (12) reported that techniques including sample dilution, solvent selection, standardization, and the use of a cobalt internal standard were useful for phosphorus and trace metal analysis in edible oils by ICP. Von Piechowski *et al.* (13) carried out the determination of phosphorus, among other elements in oils and fatty acids, by ICP–atomic emission spectrometry (AES). Samples were dissolved in formic acid and dioxane. For the phosphorus determination, they used lecithin dissolved in refined peanut oil for their standard solutions. Few details on sample preparation were given. Abe *et al.* (14) reported the simultaneous multielement analysis of some foods by ICP spectroscopy. Phosphorus in vegetable oil, olive oil, wheat germ oil, and safflower oil was extracted with nitric acid and then measured. A list of the concentrations of several elements found in these oils is reported.

The phosphorus content (in the form of phosphatides) of edible oils is an important parameter of the refining process, particularly in regard to the degumming step, a physicochemical process by which phosphatides and miscella are removed from edible oils. Improvements in phosphorus de-

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termination deserve some attention, mainly when routine analysis involves a considerable number of samples, as in the industry.

This work reports the use of oil-in-water (o/w) emulsions in the determination of phosphorus in edible oils by ICP–AES.

EXPERIMENTAL PROCECURES

Instrumentation. A PerkinElmer (Norwalk, CT) Model ICP Optima 3000 ICP emission spectrometer equipped with a PerkinElmer Model AS 90 autosampler was used. The instrumental and operational parameters are as follows: radio frequency (RF) generator, 40 MHz; operating power, 1400 W; nebulizer, Meinhard; spray chamber, Scott type; sample delivery, peristaltic pump; pump uptake rate, 1.0 mL/min; nebulizer flow rate, 0.50 L/min; plasma gas flow, 15 L/min; auxiliary gas flow, 1.7 L/min; observation height above RF coil, 5 mm; background correction, Automatic; integration time, 200 ms; working wavelength: P (I), 213.620 nm; P (II), 214.911 nm. A standard demountable type quartz plasma torch was used throughout. The i.d. of the alumina injector was 1.5 mm. A 10-roller peristaltic pump was used to feed the nebulizer with the sample solution. All functions of the plasma were computer-controlled.

Reagents. All reagents were of the highest available purity. Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA). Standard solution was freshly prepared as required from potassium dihydrogen phosphate, min. 99.5% (Riedel-De Haën AG, Seelze, Germany). Working standard solutions used for calibration purposes were prepared by suitable dilution of the stock standard solution. All glassware was cleaned in nitric acid prior to use. High-purity (99.95%) argon was utilized. Ethoxynonylphenol was obtained from Etoxyl (Maracaibo, Venezuela).

Sample. A commercial sunflower oil sample was used in this work.

Emulsion preparation. The o/w emulsions were prepared weighing the sunflower oil samples together with the surfactant according to the experimental design. They were left to stand overnight and then mechanically shaken for half an hour prior to analysis. The emulsified oil samples showed good homogeneity after manual shaking.

Optimization of ICP conditions. In order to evaluate the influence of the operating parameters on the intensity ratio of the Mg II 280.270 nm line to the Mg I 285.213 nm line, a two-level and a five-factor factorial study was designed. The five factors considered were: power, nebulizer argon gas flow rate, intermediate argon gas flow, solution flow rate, and viewing height. The optimal conditions found are listed in the Instrumentation paragraph above.

Design. A five-level design (15) was chosen. Two factors were examined: oil concentration (from 0–50% w/w) and surfactant concentration (from 0–10% w/w). The response chosen was the emission intensity of the analyte under study. The concentration range chosen for both factors was based on the literature references. This design is orthogonal.

RESULTS AND DISCUSSION

Optimal surfactant and o/w emulsion concentrations were found through the application of response surface methodology. This was based on finding values of these parameters which yield a maximum for a specific response, in this case the emission intensity. Both atomic lines 213.620 and 214.911 nm for phosphorus were used in order to give more possibilities for this particular analysis. The 213.620 nm line is more intense but is more prone to interference. So, if sensitivity is not a requirement for a particular application, the 214.911 nm line can be used as an optional line. Figures 1 and 2 show three-dimensional representations of the response as a function of the two factors for the 213.620 and the 214.911 nm emission lines, respectively. As it can be seen from Figure 1, phosphorus determined in the emulsion formed with ethoxynonylphenol surfactant presents maximal emission intensities when the composition of the o/w emulsion falls between *ca.* 8.0 and 37% w/w with *ca.* 1.0–10% w/w ethoxynonylphenol. The results obtained for the 214.911 nm emission line and represented in Figure 2 show a signal maximum at an o/w emulsion concentration between *ca.* 18–32% w/w and *ca.* 3–8% w/w ethoxynonylphenol. These optimal conditions were used throught the present work.

The surfactant Tween 80 previously (10) was reported to have the potential to improve emulsion stability but had significant problems of contamination. The surfactants Tween 60 (HLB 14.9, similar to that of Tween 80), Triton X-100 and Corexit, a commercial product, used for decontamination purposes by the oil industry in oil spills, were also examined and also contained phosphorus contamination. Different trade names of some of these products were also examined, but unsuccessfully. Among the list of surfactants potentially useful regarding emulsion stability, ethoxynonylphenol, a nonionic

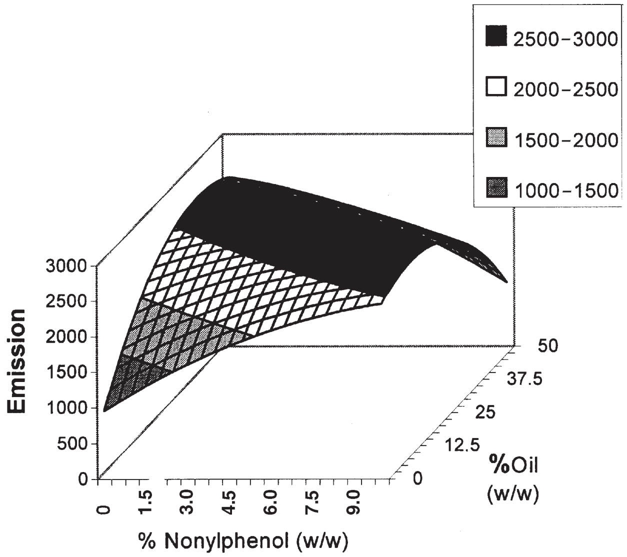

FIG. 1. Three-dimensional representation of the emission intensity for the phosphorus 213.620 nm line as a function of the emulsion and surfactant concentrations.

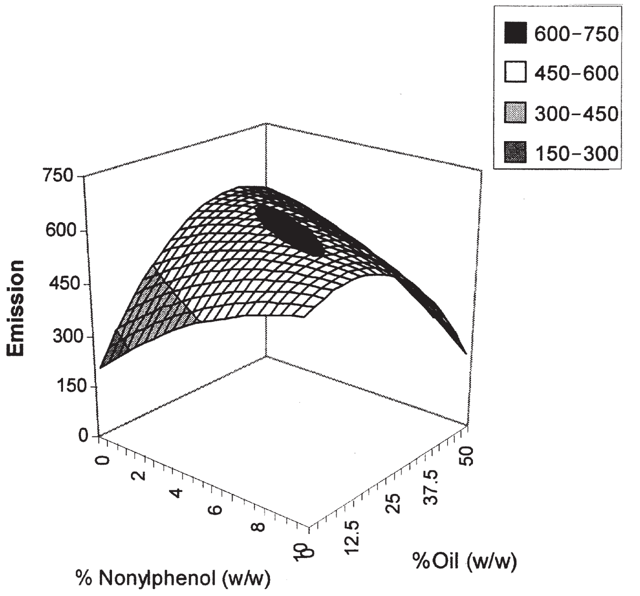

FIG. 2. Three-dimensional representation of the emission intensity for the P214.911 nm line as a function of the emulsion and surfactant concentrations. See Figure 1 for abbreviation.

surfactant (16), did not present a contamination problem and was the only one found that could be used to emulsify the samples.

Calibration. The possibility of using aqueous calibration solutions to quantify edible oil samples is one of the goals in this work. The accuracy of this new methodology has to be established experimentally. For this purpose, the estimation of the best o/w and surfactant concentrations (results from the response surface methodology) was used to prepare several sets of calibration curves: calibration solutions based on the surfactant in an aqueous matrix and calibration solutions based on the surfactant in an oil matrix. The average concentration was calculated on five replicate oil samples and quantified from the two calibration modes. The result of the calibration mode was 815 ± 54 $ng \cdot g^{-1}$ with a relative standard deviation of 7%, and the result of the standard addition method was 812 ± 20 $ng \cdot g^{-1}$ with a relative standard deviation of 2%. There is no significant difference between the results ($P < 0.05$). An attempt was made to use x-ray fluorescence spectrometry as a comparative technique to quantify the samples, but the detection limit of this technique was too great.

Accuracy of the proposed method. The accuracy of the proposed method was tested for phosphorus by spike-and-recovery experiments at 100, 200, and 300 ng g^{-1}. These tests were carried out at the two emission lines. The results are given in Table 1. The values reported are the average recoveries for four replicate samples. Better precision was reached with the 213 nm line. This line is more sensitive than the 214 nm emission line. Recoveries are in the range of 98–105% and 102–116% for the 213 and 214 nm lines, respectively. No significant difference was found by comparison of the average recovery for both lines, with 100% of recovery at the 95% confidence level (17).

TABLE 1
Recoveries (relative standard deviations) for Spiked Sunflower Oil (%)

Element	Spiked concentration		
	100 $ng \cdot g^{-1}$	200 $ng \cdot g^{-1}$	300 $ng \cdot g^{-1}$
P (213 nm)	98 (6)	105 (4)	101 (3)
P (214 nm)	116 (7)	102 (4)	112 (3)

Phosphorus analysis of emulsified edible oils by ICP–AES is a good alternative to the existing methods. The use of aqueous standards for calibration purposes, as shown in this work, is a good addition to the analysis of this type of oil sample, where certified standards are not available.

A benefit of this method is the fact that the laborious and lengthy sample digestion procedures are avoided with the use of sample emulsion with a suitable surfactant. In this particular case, it is necessary to use surfactants free of phosphorus, since the phosphorus contamination problem limited the use of several surfactants with excellent emulsion stability properties, such as Tween 80 and Tween 60. However, emulsions with ethoxynonylphenol had sufficient stability for 48 h after emulsion preparation, during which time, phosphorus can be measured. Accurate and precise results were obtained.

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